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# *Identification of microbial patterns for plant protection in a microbiota context*

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## Summary

All multicellular organisms are colonized by a diverse range of microorganism, called the microbiota. The host-associated microbiota influences and contributes to host phenotypes, for example, by alleviating abiotic and biotic stresses. The microbiota contributes to resistance against pathogen invasion but can be altered to a dysbiotic state that facilitates pathogen growth. Plants constitute the largest biomass of terrestrial ecosystems and are an important source of food for humans. In the past, research on disease development in plants was focused on uncovering the plant immune system involved in pathogen recognition – and how pathogens evade detection. However, recent studies offer a more holistic view of potential functions beyond plant-pathogen interactions, and provide first insights into the relevance of the host-associated microbiota in order to limit pathogens. Individual members of the plant-associated microbiota were shown to harbour plant protection capacity. However, their protective effects may depend on the context of the microbiota. Higher order interactions within the microbiota may lead to different outcomes compared to when studied individually. This highlights the importance of considering the interactions of the entire microbiota on disease suppression.

In this thesis, synthetic communities built from a collection of 224 bacterial strains – called the At-LSPHERE – were investigated for their protection potential against a foliar pathogen *Pseudomonas syringae* pathovar *tomato* in the model plant *Arabidopsis thaliana* in a gnotobiotic system. In a first step presented in Chapter II, the stability of microbiomes was investigated through passaging of the synthetic community over subsequent plant populations. During the first passage, a loss of species richness and diversity was observed, which was accompanied by a loss in protection. After the first passaging, the microbiota was stable and pathogen colonization remained at a comparable low level of colonization. The extent to which an even more proficient health-associated microbiota could be selected for by challenging with the pathogen in each passage was also investigated. A significant and reproducible distinction in microbiota composition within microbiota passaged based on opposite extremes of plant phenotypes was found – that is healthy versus diseased. However, it proved difficult to propagate differences in microbiota composition trough passaging from plant to plant to achieve consistent plant phenotypes across plant generations.

In Chapter III, pathogen colonization was linked to changes in strain abundance and presence. The synthetic community establishment was resilient against perturbation of initial strain abundances and pathogen invasion. Drop-out experiments of the main bacterial phyla of the phyllosphere revealed that the capacity to prevent pathogen colonization was most pronounced in the Proteobacteria. Synthetic community experiments, in which strains were replaced for others, showed that synthetic communities can be altered towards different disease outcomes. A limited number of microbiota members appeared to be crucial for plant protection. In addition, strains with inherently higher levels of protection were found to be generally more abundant *in planta* compared to strains with lower levels of protection.

In Chapter IV, plant protection was investigated using 35 bacterial strains combined in 136 randomly composed synthetic communities of five strains each. Through classification and regression analyses, three strains were identified as the most important predictors of pathogen colonization outcomes. The prediction accuracy of microbiota-mediated protection was 87-93% of correctly predicted protection, while a random classifier correctly predicted 51-56%. The *in silico* pathogen prediction was confirmed by validation experiments. The three most important strains conferred higher protection in combination. A refined data analysis revealed another strain combination to be important for plant protection, while individually, they were intermediate to non-protective.

In conclusion, the presence of a diverse set of beneficial microbes from a model microbiota contributes to plant protection. The microbiota harbours diverse and redundant mechanisms to limit pathogen invasion and growth. In some groups, particularly the Proteobacteria, these mechanisms were enriched. Our results also suggest that protection is conferred by a limited number of members of the microbiota, and these members are likely to be competitive colonizers. The approach presented here allows the identification of microbial patterns important for protection in a community context. It can be adapted to identify features relevant for microbiota function in other biological systems.

# Zusammenfassung

Alle multizellulären Organismen werden von einer Vielzahl von Mikroorganismen besiedelt – kollektiv als Mikrobiota bezeichnet. Die Wirt-assoziierte Mikrobiota beeinflusst den Phänotypen des Wirts, wie zum Beispiel durch Linderung von abiotischen und biotischen Stressoren. Die Mikrobiota trägt zur Resistenz gegen Kolonisierung von Krankheitserregern bei, kann jedoch in einen dysbiotischen Zustand geraten, der das Wachstum von Krankheitserregern begünstigt. Pflanzen machen den grössten Anteil der terrestrischen Biomasse aus und sind eine wichtige Nahrungsquelle für den Menschen. In der Vergangenheit konzentrierte sich die Forschung zur Krankheitsentstehung bei Pflanzen auf die Erforschung des pflanzlichen Immunsystems, welches Teile der Krankheitserreger erkennen, und darauf wie diese Krankheitserreger der Erkennung entgegenwirken. Jüngere Studien bieten eine ganzheitlichere Sicht auf mögliche Funktionen, die über die Interaktion zwischen Pflanzen und Krankheitserreger hinausgehen, und liefern Erkenntnisse über die Bedeutung der wirtsassoziierter Mikrobiota für die Eindämmung von Krankheitserregern. Es wurde gezeigt, dass einzelne Mitglieder der Mikrobiota die Pflanze gegen Krankheit schützen können. Ihre Schutzwirkung kann jedoch von Wechselbeziehungen mit der übrigen Mikrobiota abhängen. Im Zusammenhang mit der Mikrobiota führe die Schutzwirkung einzelner Mikroben zu verschiedenen, manchmal widersprüchlichen Ergebnissen, als wenn sie alleine untersucht wurden. Dies zeigt, wie wichtig es ist, die Wechselwirkungen der gesamten Mikrobiota bei der Unterdrückung von Krankheiten zu berücksichtigen.

In dieser Arbeit, wurden synthetische Mikrobiota, die aus einer Sammlung von 224 Bakterienstämmen – der *At*-LSPHERE genannt – gebildet und auf ihre Schutzwirkung gegen den Krankheitserreger *Pseudomonas syringae* pathovar *tomato* in einem gnotobiotischen System mit der Modellpflanze *Arabidopsis thaliana* untersucht. In einem ersten Schritt, der in Kapitel 2 beschrieben ist, wurde die Stabilität der Mikrobiota über mehrere aufeinanderfolgenden Zyklen untersucht. Während der ersten Passage wurde ein Verlust an Artenreichtum und Diversität beobachtet, der mit einem Verlust an Schutz vor dem Krankheitserreger einherging. Nach der ersten Passage war die Mikrobiota stabil, und die Besiedlung mit dem Krankheitserreger blieb auf einem vergleichbar niedrigen Niveau über die nächsten Zyklen hinweg. Es wurde auch untersucht, inwiefern auf eine noch besser schützende Mikrobiota selektieren werden konnte. Es wurde ein signifikanter und reproduzierbarer Unterschiede zwischen der Mikrobiota der beiden Extreme der Pflanzenphänotypen gefunden – das heisst gesund oder erkrankt. Es erwies sich jedoch als schwierig, Unterschiede in der Mikrobiota-Zusammensetzung zu propagieren, um beständige Pflanzenphänotypen zu erlangen.

In Kapitel 3 wurde der Zusammenhang zwischen der Besiedelung der Krankheitserreger und der Präsenz und Dichte der bakteriellen Stämme untersucht. Die Mikrobiota erwies sich als widerstandsfähig gegenüber Störungen von Unterschieden in der ursprünglichen Zusammensetzung der Bakterien und der Invasion von

Krankheitserregern. Das Weglassen von Bakterien der wichtigsten Phyla offenbarte, dass die Fähigkeit, die Besiedlung von Krankheitserregern zu verhindern, bei den Proteobakterien am ausgeprägtesten war. Experimente mit synthetischen bakteriellen Gemeinschaften, bei denen Stämme durch andere ersetzt wurden, zeigten, dass der Schutzeffekt der Mikrobiota geändert werden kann. Zusätzlich zeigten diese Experimente, dass der Schutzeffekt wahrscheinlich von einer limitierten Zahl von Bakterien abhängig ist. Stämme, die alleine einen höheren Schutzeffekt zeigten, wiesen eine höhere Besiedlung der Pflanze auf.

Im vierten Kapitel wurde der Schutzeffekt von 35 Bakterienstämmen untersucht, die in 136 zufällig zusammengestellten Gemeinschaften von jeweils fünf Bakterien auf die Pflanze gegeben wurden. Drei bakterielle Stämme waren die wichtigsten Vorhersager des Schutzeffekts der Gemeinschaften in Klassifikations- und Regressionsanalysen. The Analysen konnten in 94-100% der Fälle den Schutzeffekt korrekt einordnen, während nicht trainierte Analysen eine korrekte Einschätzung in 32% der Fälle hatten. Der Schutzeffekt der drei bakteriellen Stämme, die die wichtigsten Vorhersager des Schutzeffekts waren, wurde in Validierungsexperimenten bestätigt. Die drei wichtigsten Stämme boten in Kombination einen höheren Schutzeffekt. Durch eine verfeinerte Datenanalyse konnte ein weiteres Paar von Stämmen identifiziert werden, die zusammen einen Schutzeffekt zeigen, während sie einzeln nur mittelmäßig bis gar nicht schützend wirkten.

Zusammenfassend konnte gezeigt werden, dass das Vorhandensein von verschiedenen und diversen Mikroben für den Schutzeffekt der Mikrobiota wichtig ist. Die Mikrobiota besitzt verschiedene und überlappende Mechanismen um die Besiedelung und das Wachstums des Krankheitserregers zu limitieren. Diese Mechanismen sind in gewissen Gruppen, wie den Proteobakterien, besonders ausgeprägt. Unsere Ergebnisse weisen darauf hin, dass der Schutzeffekt der Mikrobiota von einer limitierten Zahl von Mikroben abhängig ist, und diese Mikroben konkurrenzfähig auf der Pflanze sind. Der hier vorgestellte Ansatz ermöglicht die Identifikation von mikrobiellen Mustern, die für den Schutzeffekt der Mikrobiota wichtig sind. Das aufgezeigte Vorgehen kann auf andere biologische Systeme ausgeweitet werden.

# Chapter I

# Introduction

**The host-associated microbiota has an intimate relationship with its host**

Virtually all multicellular organisms are colonized by diverse microbes - bacteria, fungi and viruses - collectively called the microbiota. In animals, microbes can colonize skin, mucosal tissue, and are most numerous in the gut, which has been the focus of most studies so far [1,2]. Research has shown that these host-associated microbial communities are important for the development and health of their host. For instance, the gut microbiota of healthy individuals confers some level of protection against pathogens [3-6], also referred to as colonization resistance [7], aids in the training of the immune system [8-10], and is crucial for the digestion of food [11,12]. The composition of the early-life gut microbiota was even associated with long-term health and disease outcome. Imbalances in the gut microbiota, called dysbiosis, can lead to asthma, diabetes, allergies and cardiovascular diseases [13,14]. A dysbiosis in the microbiota is also caused by inflammation or antibiotic use, which open up niches for pathogens to invade and infect the host, while they are not able to colonize in a healthy gut microbiome [15]. While antibiotic treatment often resolve infection by pathogens, they do not address the underlying dysbiosis issue [16]. Clinically, faecal microbiota transplantation has been used successfully to treat recurrent infection with *C. difficile* [17,18], demonstrating that a healthy microbiome is important for a functioning gut in humans and animals.

**Importance of plants and its associated microbiota**

Like animals, plants are colonized by diverse microbes that contribute to its fitness, and *vice versa* [19]. The microbiota affects plant growth, productivity, health, adaptation, physiology and germination, and has contributed to diversification within the plant kingdom [20,21]. Like in the human gut, the plant-associated microbiota is the first barrier of defence against pathogens [22]. Similarly, as was found in humans, a dysbiosis state can lead to increase incidence of disease in plants [23,24:Erlacher, 2014 #354]. The importance of the plant-associated microbiota is one reason to further our understanding of it. On the other hand, plants form the fundamental basis of food chains and for ecosystem functions in nearly all terrestrial ecosystems, highlighting the importance of plants as a host in research [25]. The phyllosphere – compromising all above-ground parts of the plant including leaves, stems and flowers – is estimated to have a surface of more than $10^8$ km$^2$, making up a vast environment to host upwards of $10^{26}$ bacterial cells [26]. The phyllosphere is less diverse than the underground compartment, the rhizosphere, but in both environments, these plant-associated bacterial communities establish in a stable manner consistent in their taxonomic composition, even in annual plants [27]. The emergence of next-generation sequencing and cultivation-independent analyses allowed deep insights into the community composition of various hosts, including the model plant *Arabidopsis thaliana* [27-29], close relatives [30], as well as tree species [31,32], and various crop plants including barley [33], rice [34], grapevine [35], tomato [36] and soybean[27]. These studies consistently revealed that the bacterial communities are composed of only a few phyla, dominated by Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes [27,28]. The leaf-associated communities were dominated by Proteobacteria,

with the most abundant genera being *Methylobacterium*, *Pseudomonas*, and *Sphingomonas* [37]. The consistency of the plant-associated microbiota hints at a deterministic assembly, and it was shown that the plant can attract microbes, i.e. through root exudates [38,39].

**Disease establishment dogma revisited – the microbiota as an important factor**

In classical plant pathology literature, the emergence of disease was viewed as the equation of three factors forming a triangle– a susceptible host, a virulent pathogen, and favourable environmental factors [40] (Figure 1AB). A susceptible host's immune system might not recognize the pathogen through its pattern recognition receptors (PRR), while a resistant host will mount a suitable immune response protecting it from the pathogen [22]. The presence of appropriate virulence genes in the pathogen can determine whether it can evade and suppress the host's immune response [22]. Environmental factors, like humidity, temperature, and wind, will contribute to the pathogen's dispersal and survival during the infection period, creating favourable conditions for infection [41]. The interaction of the factors of these three players will determine the chance of successful infection and disease establishment (Figure 1AB). However, research of the past decade has shown that studying this so-called genotype-environment (GE) model is incomplete and must also include the microbiota, as mentioned above. At least part of the plant immune system is also involved in cooperative interactions between plants and microbes and influences the colonization of beneficial microbial communities [22]. Therefore, it was suggested to widen the dogma that plant health is determined by the host genotype (G), the environment (E) and the microbes (M) and their respective interactions (GEM) [42] (Figure 1C). By doing so, we will transform the disease triangle into a pyramid, with the four sides influencing each other. It was shown that different plant genotypes exhibit a distinct microbiota [39,43-45] and microbiota-plant interactions can be genotype-dependent [46], while abiotic factors can influence the microbiota-plant interaction as well [44,47,48].

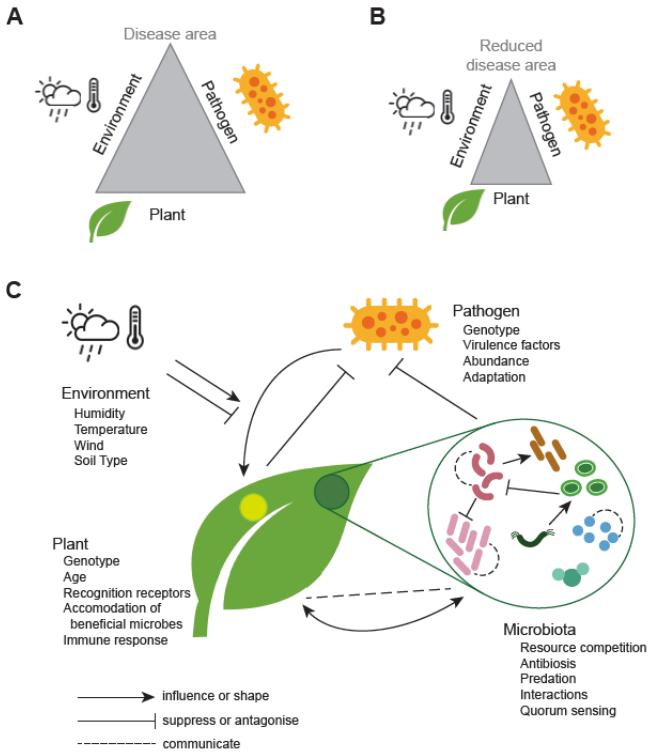


**Figure 1: Disease triangle adjusted to include the microbiota as an important factor of plant protection.** A. The traditional disease triangle takes into account the plant's susceptibility and the pathogen's virulence, and whether the environment is favourable to infection. B. When the plant is more resistant, the pathogen less virulent or the environment less favourable to infection, the disease area - i.e. the chance of development of disease – is reduced. C. The disease pyramid is schematically depicted by the four sides – environment, plant, pathogen and microbiota. Their interactions are shown by arrows and important factors influencing each part of the disease triangle are presented below their names. The figure is adapted from Brader et al. [41], Zhan et al. [49] and Hacquard et al. [22].

## Plant-associated microbes confer protection against pathogens

The plant-associated microbiota has been shown to be crucial for limiting pathogen colonization as a first line of defence [50-52] (Figure 1C). Historically, the importance and effect of the microbes on plant health was studied in a one-on-one relationship, with a limited selection of beneficial microbes being studied for their plant protection against a few pathogens [19]. Using culture-independent methods, it was possible to infer the composition of the microbiota that is associated with plant-protection, though these correlations cannot provide information about the specific underlying mechanisms driving observed patterns of relative abundance [53]. For this purpose, bacterial strains were isolated from natural communities, and analysed for their protection potential [54-58]. Not all members of the microbiota confer the same level of protection when tested individually *in planta*, some have a neutral effect on the host and do not confer protection, this variation of protectiveness can vary within the same genus [59,60]. Mechanisms found to be important for microbe-conferred protection include direct antibiosis of the pathogen [54,56,57], competition for resources[56,61] and activation of plant immune responses, where both induced systemic resistance or system acquired

resistance genes were found to be upregulated, priming the plant against pathogen attacks[62,63]. Formation of biofilms seems to be a feature of protective strains as well, as it might form a physical barrier that is impenetrable by the pathogen [58,64,65].

**Higher order interactions affect protection potential of microbiota**

While plant protection was found to be conferred by a few individual members, an increase in protection potential could be shown when a consortia of strains was applied [51,66]. In general, higher diversity of the microbiota was often associated with reduced invasion of a specific species or pathogen [56,67-69] (Figure 2). When an environment is colonized by a low diverse microbiota, only a few resources will be used by the resident microbes, leaving a repository of resources for the invader to grow on. However, higher diverse microbes will use up a broader range of resources and even break down resources through interactions and crossfeeding [70,71]. It was shown that a community with more overlapping carbon source spectrum as *Ralstonia solanacearum*, a soil-borne pathogen, reduced the pathogen more than a community with less overlap [72]. Another study found a correlation between higher microbial biomass and less diversity in commensal microbiome with higher pathogen load [73]. Introducing more diverse beneficial microbes thus seems to be an efficient way of ensuring plant protection.

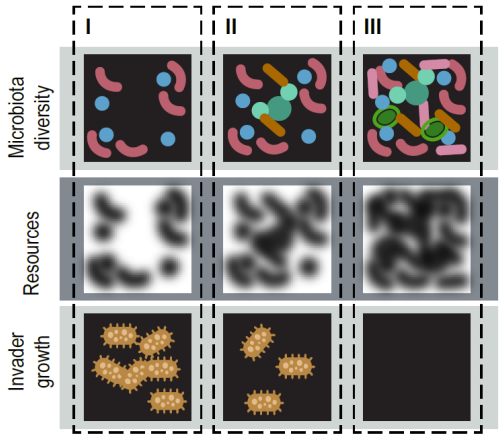


**Figure 2: Diversity of resident microbiota on invader success and growth is dependent on resource depletion.** A diverse microbiota will deplete the resources of an environment more efficiently, leaving less resources for the invader. In I, a microbiota of low diversity will deplete only a few resources, leaving a niche for invaders, which can grow to high abundance. In II, a more diverse community will deplete resource more efficiently, leaving a few for invader to grow on. Very diverse microbiota (III) deplete the resources of their environment, helped by their interactions, and exclude invaders from the environment. The Figure is adapted from Mallon et al. [71].

While some studies found that the consortium of strains reduced pathogens further than individual strains on their own [51,56,60]. Two of these studies also found a synergistic formation of biofilm production, adding a mechanism of protection to the consortia that was absent in individual strains [51,56]. It could also be shown that a strain that did not confer protection individually was the main contributor to protection conferred

when it was applied to the plant in a small community [60]. A study showed that the consortia of strains had no improvement over individual strains [54]. One reason for consortia showing no improvement over individual strain could be the lack of compatibility of protective mechanisms, as was shown in another study where individually protective strain inhibited each other's mechanism of protection when applied in combination with each other [74]. The various outcomes of protection upon combining microbes with known individual traits, highlights the importance of studying the protection potential of strains within a community context.

**Application of biocontrol strains in agriculture to increase sustainability**

The traditional treatment and management of pathogens, not only in plants (i.e. agriculture), but also in human and animal medicine, have focused on administering antimicrobials [75], with little regards for the maintenance of the beneficial members of the microbiome itself. Due to warming temperature that accompany climate change and human activities, pathogens re-emerge and spread to new regions [76]. Additionally, pollution increases antibiotic resistances found in microbial communities, suggesting that current chemical treatment of pathogens will cease to be efficient soon [77,78]. To reduce pathogens more sustainably, possible biocontrol strains and the application of beneficial microorganism are more thoroughly researched, and now represent a fast-growing sector in agronomy [79]. The majority of these probiotic formulations comprise strains that are native to the plants [80]. However, while pathogen emergence and load increase, we are also faced with loss of biodiversity [25,79], accompanied with a loss of beneficial microbes [81,82]. This makes fundamental research important to understand the mechanisms of how beneficial microbes persist within the community and counteract pathogens. We are limited in our understanding of how to retain or restore a healthy microbiome [83]. Investigating microbiome members that are important for plant health could be harnessed to rescue threatened host species or ecosystems will improve our understanding going forward [79].

**Synthetic community experiments – bridging gaps in fundamental understandings**

Identification and characterization of mechanisms in complex host-associated communities is challenging, but often require and depend on high throughput sequencing, which has opened up new possibilities to study microbial communities without relying on cultivation of microbes [19]. Despite the new insights from description of microbiome structure, the conclusions drawn from observation of solely culture-independent methods often depend on correlation and co-occurrence network. These analysis may be insufficient for interpretation of underlying mechanisms [84]. Synthetic microbial communities (SynCom) offer the opportunity to test hypotheses through targeted manipulation in gnotobiotic systems [19]. To investigate the fundamental drivers of i.e. plant protection, SynCom experiments allow both the deconstruction of communities top-down and to build them from bottom-up. Through this, keystone strains can be sought out. This was highlighted recently through targeted removal and addition of parts of the community to establish

causal relationships in community assembly [85]. Another recent study showed the underlying cause of dysbiosis in a plant mutant by removing a single strain from the SynCom [86].

One prerequisite to conduct SynCom experiments is the availability of a representative strain collection. For plants, several representative collections have been established from various plant species [28,87-89]. The *At*-LSPHERE is a leaf-associated bacterial culture collection of wild *Arabidopsis thaliana* plants native to Switzerland and Southern Germany [28]. The 224 genome-sequenced bacterial isolates cover 54 % of the taxonomic diversity found by culture-independent methods. In addition to the microbial collection, a gnotobiotic growth system is required to control for environmental conditions and prevent contamination. For *Arabidopsis thaliana*, several of these growth-systems were developed and include systems based on agar [60,88,90], hydroponic [91], inert inorganic matter like calcinated clay [28,85,86,92] or sterilized peat [93]. In addition to the growth system, infection protocol have been established [63], disease assessment through pathogen growth [60] and disease severity scoring [94] of the well-studied foliar pathogen *Pseudomonas syringae* pathovar *tomato* DC3000 (*Pst*) [95]. This pathogen model bacterium was modified to carry the luminescence operon *luxCDABE* [96], enabling the visualization of growth and activity of the pathogen during the plant growth period. Though the *At*-LSPHERE is extensive, it might still lack microbes harbouring particular functions, thus, a gnotobiotic experiment will not provide a complete understanding of the entire natural ecosystem. However, the ability to investigate causal relationships between traits conferred by the microbiota, such as plant protection, provides a valuable basis for uncovering the principles underlying microbiota function. The findings of the gnotobiotic experiments can be translated to more natural systems through investigation of metatranscriptomic and metagenomic approaches [19].

**Aim and scope of this thesis**

The phyllosphere harbours a diverse microbiota that is dominated by bacteria [26]. The structure of the microbiota is not random, but establishes in a dynamic, but reproducible manner each growth season and between geographic locations [97]. The plant-associated microbiota can protect the plants against infection of pathogens that are detrimental to their health and productivity [62]. However, the discovery of the beneficial members and their trait consistency within the community are little studied, and found to be inconsistent when applied [98].

The aim and scope of this thesis was to investigate factors that contribute to a healthy leaf-associated microbiota in terms of protection from a foliar bacterial pathogen, and which members are important for plant protection of the microbiota. In order to so, we took advantage of a gnotobiotic growth system [85,92] and a bacterial culture collection, the *At*-LSPHERE [28] to conduct synthetic community experiments to link microbiota makeup to plant health outcomes.

In Chapter II, the stability of the microbiota over plant passages is described, and the influence of pathogen challenge on its composition. Additionally, the extent to which a plant health phenotype is

impacted by microbiota-conferred protection and microbiota composition was investigated. In Chapter III, the correlation of plant health phenotype and the microbiota composition was analysed, through a bottom-up approach. The microbiota inocula were perturbed by changing strain abundances and presence. In Chapter IV, a randomly composed community screen was conducted with machine learning algorithms to find microbiota patterns associated with pathogen colonization outcomes, and ultimately to identity of strains, that contribute to pathogen reduction.

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# Chapter II

# Successive passaging of plant-associated synthetic microbiota reveals stable establishment of the communities with the potential to select for plant-health associated communities

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B.E. and J.A.V. designed the study. B.E. performed experimental laboratory work. M.B.M., B.A.M. and C.I.C. contributed to the plant experiments. B.E. performed the data analysis. B.E. and J.A.V. wrote the manuscript.

# Abstract

Host-associated microbiota influence and contribute to host phenotypes such as resistance to biotic and abiotic stresses. Microbiomes can contribute to resistance to pathogen invasion, but also be altered towards a dysbiotic state. We investigated the stability of microbiomes through successive passaging of a synthetic community of *Arabidopsis thaliana* leaf-associated bacteria. We also evaluated the extent to which we can select for a health-associated microbiota by challenging the plants with the model foliar pathogen *Pseudomonas syringae* DC3000 at each passage. We observed a loss of species richness and diversity in the in the first passaging event, accompanied with a loss of protection. Pathogen infection had a small but significant effect on microbiota composition (PERMANOVA effect size 5.62 %, p-value 0.0099). Microbiota passaging with selection based on opposite extremes plant- phenotypes, i.e. healthy versus diseased, revealed a reproducible distinction in microbiota composition, while consistent health phenotypes proved difficult to achieve. Together, we show that the microbiomes changes within the first passage and is maintained over successive passages.


# Introduction

Multicellular organisms are hosts to a diverse range of microorganisms, which collectively are called the microbiota [1]. Understanding the influence of the microbiota on the host has become an important part of research [2,3]. The host-associated microbiota was shown to help with nutrient uptake [4,5], infer colonization resistance against pathogens [6,7], crosstalk with immunity and help with its development [8,9], as well as influence other traits such as time of flowering in plants [10].

One challenge of the current days and near future will be to produce enough food for a growing human population, while facing increased temperatures, drought, pathogen abundance and disease occurrence [3]. Research proposed and showed that biodiversity loss not only affects multicellular species, but also the microbiota in host-associated and free-living environments [2]. Shifts in microbiota towards a so-called dysbiosis can increase abundance of pathogens and other unwanted invaders that can lead to chronic diseases in humans [11,12], re-emergence of prior controllable diseases, or spread to prior spared regions [13]. Traditional measures to control diseases used chemical intervention to counteract pathogens, often to the detriment of beneficial members of the microbiota [14]. However, knowing that the microbiota contributes to the first line of defense against pathogens [8,15], the importance understanding what composes a healthy microbiome and how to retain and restore it becomes apparent[16].

Studies on plant-pathogen interactions have often been limited to study genotype and environment interactions; however, the importance of the interplay with the host-associated microbiota has been highlighted [17]. Within the microbiota, specific microbes can determine plant health [15,18-20]. The aforementioned loss of biodiversity due to human activities also results in loss of beneficial microbes [21,22],

highlighting a need to restore a functional microbiome. So far, much research has been done to isolate microbes and test them in a one-on-one association with the host-pathogen interaction of interest to identify beneficial strains. Application of these microbial inoculants as biocontrol strains have been shown with inconsistent success [23]. To address the challenges of producing scalable beneficial microbial application, is has been suggested to produce "self-assembled communities" that are enriched for particular functions [23]. One study suggested that the relative abundance of the important taxa conferring the protective function was more important than their presence itself [15].

One way to select and enrich for members of the microbiota associated with certain traits is through passaging of the microbiota over several growth periods, while selecting for the host phenotype [24]. This method was shown to be applicable to a wide range of microbe-associated traits, like increasing plant biomass [25,26], degradation of a pollutant [27], influencing flowering time [28], adaption to salt stress [29], selection for less disease [30], and even evolving a mild pathogen to beneficial one [31]. However, most of these passaging studies looked into manipulations of the soil or rhizosphere microbiota, only a few investigated the phyllosphere microbiota [32,33]. The phyllosphere of the plant consists of all above-ground parts of the plants, including its leaves and fruits, which are often used as food supply in crops [34]. The composition of leaf-associated communities is not random, but establishes in similar patterns year after year [35,36], likely sources include soil, air and seed [37,38]. While the engineered soil microbiome was shown to affect the next cycle of plant growth in what was termed "soil legacy" [28,39], such a legacy would be interesting to investigate for foliar pathogens, but was not yet demonstrated.

This study aims to investigate in what manner the protection potential of a synthetic community composed of native bacterial strains from *Arabidopsis thaliana*, called the *At*- LSPHERE collection [40], can be maintained through successive microbiota passaging. We investigate both the stability of the microbiome through passaging from plant to plant as well as the impact of pathogen challenge on the progression of plant phenotypes.

# Results

To investigate the stability of the microbiota and consequences of pathogen challenge, we set up a successive passaging experiment (Figure 1). For this, we inoculated axenic seedlings with strains of the *At*-LSPHERE (210 strains, called SynCom-210) (Supplemental Table 1, [40]) (Figure 1I). The plants were grown for an additional 11 days, at which time they were either challenged with a pathogen (*Pst*) or left unchallenged (mock-infected) (Figure 1II). Disease progression was monitored (Figure 1 III) as plants grew until 5.5 weeks of age (38 d). Then the plants were harvested (with and without selection as described below, Figure 1V), the microbiota was washed off and inoculated onto new axenic set of axenic seedlings (Figure 1V,VI) (see methods for more details). The cycle of growing, selection, and wash-off was repeated five times (Figure 1VII).

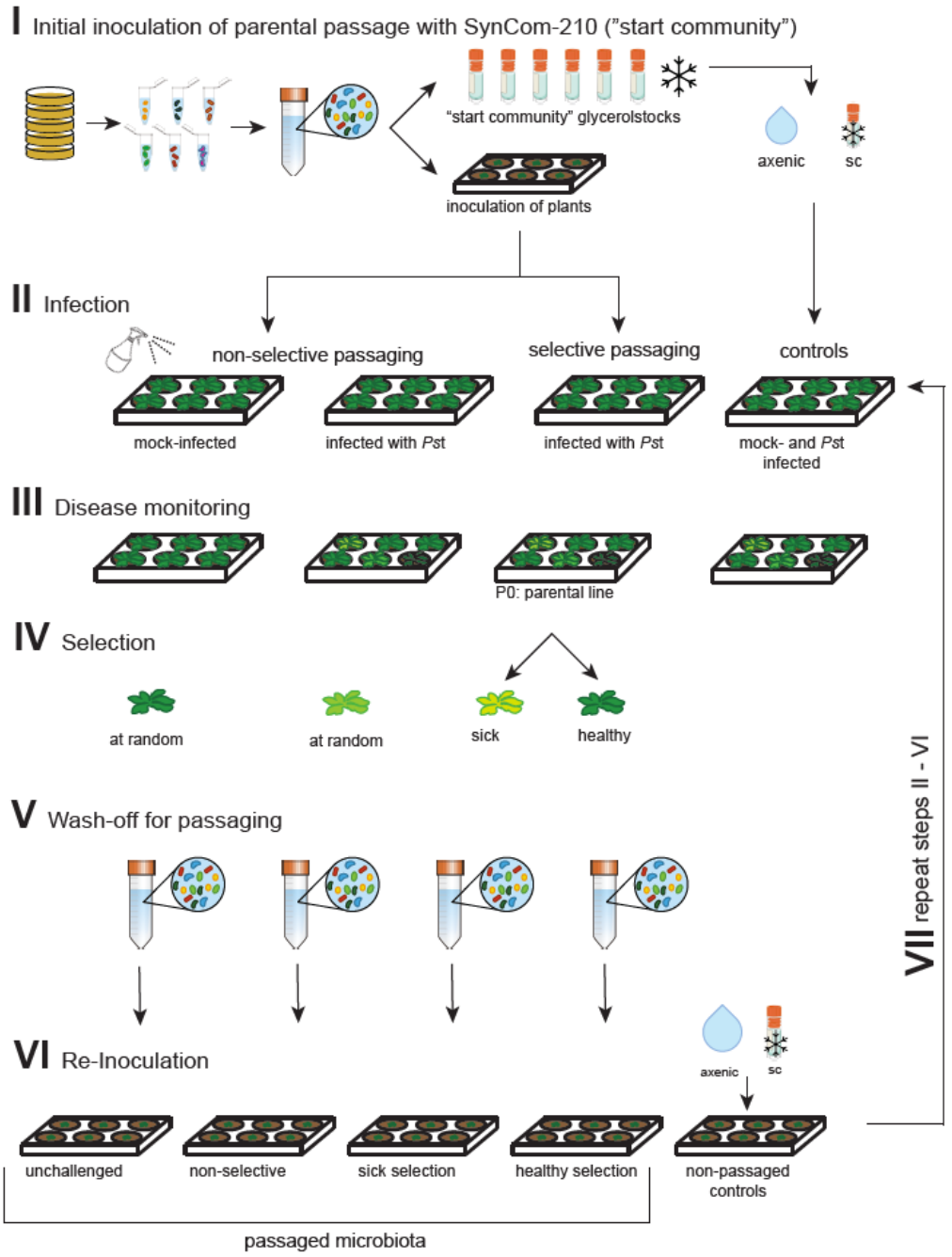


**Figure 1: Experimental design of the microbiota passaging experiment.** The figure summarises the experimental procedure performed to successively passage the microbiota. In step I, the 10 days old seedlings plants of the parental passage (P0) were inoculated with the microbiota (SynCom-210) (for details, see methods). After 11 days, plants were infected with pathogen (*Pst*) (II). The disease progression was monitored through pathogen luminescence 3 days post infection (dpi) and disease severity score at 7 and 14 dpi (III). At 14 dpi, the plants of each selection line were selected either randomly (unchallenged, non-selective) or based on plant phenotype (parental lines, sick and healthy selection) (IV). The community of the selected plants was washed-off (V) and used to re-inoculate a new set of plants (Passage x=1) (VI). The new set of plants was then cycled through steps II through V (infection to harvest) and another new set of plants was inoculated (Passage x+1). To control for disease establishment, four control conditions were included (two inculations, two infection types). The inoculation of plants with the frozen start community (see I) and buffer (axenic) were included in each passage, and were either infected with *Pst* or mock-infected.

To select the microbiota for changes of the disease phenotypes, we included passaging of plants selected at random that either were mock-infected (unchallenged) or infected (non-selective). To investigate selection for protection conferred by the microbiota and loss thereof, the microbiota was passaged from plants to plants and selected based on their disease phenotype (sick or healthy) (Figure 1IV, see methods for details). For each of the mentioned selection types (called "selection" henceforth), six replica lines were included, referred to as "selection lines" (see also [24]). Each selection line consisted of 18 plants that were distributed onto three 6-well-plates ("replica plates"). To have sufficient washed-off microbiota for inoculation of the next passage, creating glycerolstocks and for community profiling using 16S rRNA amplicon sequencing, we decided to select and pool four plants per selection line. At harvest, the selected plants per selection line were cut in half, one half was used directly for 16S rDNA analysis (pooled half plants), the other plant halves were used to wash-off of the microbiota (Figure 1V). The harvested suspensions of the selected plants were pooled, and aliquots were used for inoculation, determination of bacterial colonization (colony forming units (cfu)), 16S rRNA gene analysis and glycerol stocks (Figure 1VI). At every passage, the selection lines were kept separate throughout the entire experiment.

In each passage, including the parental passage (P0), mock-infected and infected axenic control conditions were included (ax_NI, ax_pst), as well as plants inoculated with a thawed aliquot of the start inoculum glycerol stock (referred to below as "start community (sc)") that were either mock-infected or infected with *Pst* (sc_NI, sc_pst) in each passage (Figure 1). These controls served to monitor whether the infection phenotypes were consistent through the passaging experiment since the pathogen titre was increased with each passage (Supplemental Table 2, see methods), and to allow a direct comparison to the parental passage.

**Non-passaged controls in each passage**

We first investigated non-passaged control conditions for similar community establishment, pathogen infection and disease progression in each passage. As a first parameter, commensal colonization of the mock-infected and *Pst* infected start community (sc) were compared (Figure 2A, Supplemental Table 4). In each passage, a median commensal colonization ranging between 5.6 x $10^7$ and 5.4 $10^8$ cfu g-1 fresh weight was reached (Supplemental Table 3). The only significant difference found was in the comparison of passage 2 to 4 in the mock-infected sc control (Supplemental Table 4). The similar commensal colonization in each passage suggested that carrying capacity of the plant was reached in each passage.

Pathogen luminescence at 3 days post infection (dpi) was similar in each passage for each of the control conditions (Figure 2B, Supplemental Table 5). The luminescence in start community inoculated plants differed significantly when comparing the parental passage (P0) to the last passage (P5). In each passage, except for P5, the axenic infected measurements were significantly different from the background (axenic mock-infected), but not from the start community controls. We saw that in passage 2, the background

luminescence was lower, though not significant, we still decided to normalize the luminescence of each passage to its background signal (axenic mock-infected) going forward (Figure 2B). We also noticed that the pathogen luminescence had a low range, the median luminescence of axenic infected (highest expected luminescence, averaged 1.3 x $10^5$ $ps^{-1}$) was only 1.4 times to 2.95 times higher compared to the background luminescence of axenic mock-infected (averaged 6.5 x $10^4$ $ps^{-1}$) (Supplemental Table 3).

Disease progression was monitored by scoring the disease symptoms on a scale from 1 to 5 at 7 and 14 dpi (Figure 2C, Supplemental Figure 1,2). For the non-passaged control conditions, we observed no disease symptoms in the mock-infected controls (ax_NI, sc_NI), few disease symptoms in the infected start community, while axenic infected plants had a high disease score in each passage (Figure 2C, see methods for details). Disease severity was comparable in the control conditions compared between passages at 7 dpi (Supplemental Table 6) and at 14 dpi (Supplemental Table 7).

Pathogen colonization at harvest (17 dpi) was similar for the infected control conditions in each passage (Figure 2D, Supplemental Table 4). Axenic infected plants had a high pathogen colonization, with a median colonization of 1.6 x $10^9$ cfu $g^{-1}$ fresh weight over all passages (Supplemental Table 3). The start community inoculated controls had a significantly lower pathogen colonization compared to axenic infected, with a median pathogen colonization of 2.3 x $10^6$ cfu $g^{-1}$ fresh weight throughout all passages. The consistency of the measurements meant that infection and disease progression was similar each passage.

In each passage, two samples of four pooled plants of start community inoculated plants were analysed with 16S rRNA amplicon sequencing to investigate community composition for both mock- and *Ps*t-infected conditions. PCA coupled with PERMANOVA analysis revealed that the establishment of the community differed in each passage (Figure 2E, Supplemental Figure 3). Differences in community composition was expected between experimental replicates, as was seen for replicate experiments (effect size of up to 13 %) [41].Here, the start community control had a size effect ranging from 10.5 % (P3 vs P4) to 33.9 % (P1 vs P5) (Supplemental Figure 3). The differences between passages were smaller than the difference of each passage to inoculum composition (Supplemental Figure 4A). As expected, the size effect of the difference of inoculum versus *in planta* communities was high, about 51 % (PERMANOVA, p-value 0.0099) (Supplemental Figure 4B), which was larger than the differences between the community after colonization in each passage.

The *in planta* community of mock-infected start community control was dominated by three ASVs having high abundances (> 10 %) (*Rhizobium* Leaf155, *Chryseobacterium* Leaf405, *Methylophilus* Leaf414) (Supplemental Figure 5A). 17 ASVs had relative abundances between 1-10 %, and after there was a tail of lower abundant ASVs. Namely, 33 ASVs lower than 1 %, and 55 ASVs lower than 0.1 %, while 21 of the 137 ASVs were not detected in any of 12 samples analysed (not shown due to readability of graph).

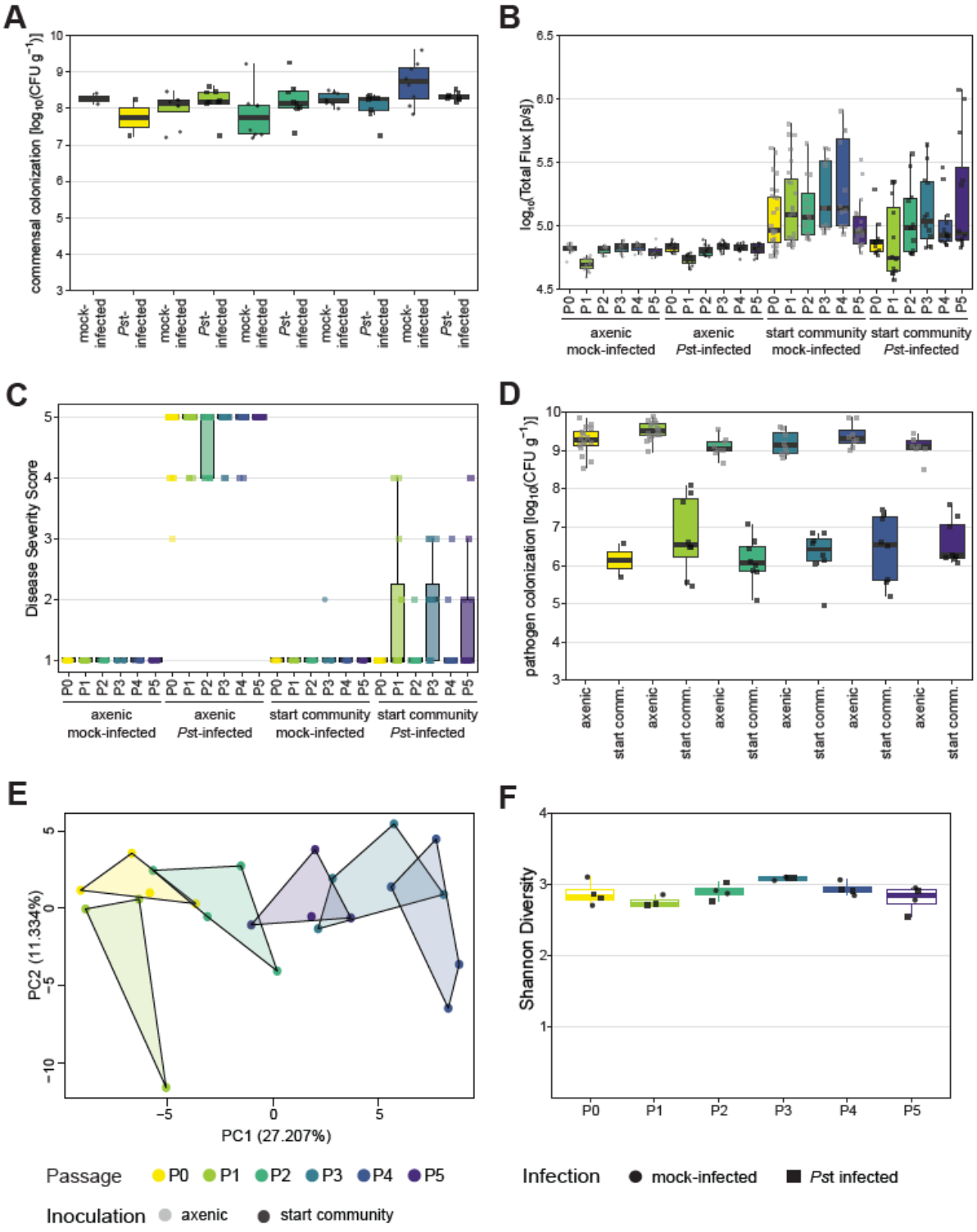


**Figure 2: Disease establishment and community composition in control conditions.** The similarity of disease establishment, colonization capacities and community establishments in each passage was controlled by inclusion of axenic (grey) and the start community (black) controls. A. Total commensal colonization in each passage in mock-infected (circles) and *Pst*-infected (squares) of start community controls. B. Pathogen luminescence at 3 dpi of the control conditions. C. Disease severity scores at 14 dpi for all control conditions in each passage. D. Pathogen colonization at 17 dpi of *Pst*-infected control conditions. E. Principal component analysis of start community controls in each passage. F. Shannon's diversity of start community controls in each passage.

The community composition of the inoculum had more even relative abudances of ASVs compared to established on plants (Supplemental Figure 5B). Most strains had a relative abundance between 1 % and 0.1 %. In total, 4 strains were non-detected in one or two samples (*Sphingomonas* Leaf10, *Acinetobacter* Leaf130, *Sphingomonas* Leaf22, *Bacillus* Leaf406) (not shown due to readability of graph). The composition reflects what was to be expected from the composition of the inoculum (roughly 1:1 ratio) [41,42].

Shannon's diversity was similar in each passage (Figure 2F), suggesting that we could also detect similar amounts of strains in each sample. The only significant difference detected was in the start communities at passage 1 (P1) versus 3 (P3) (Supplemental Table 8). Community evenness and richness were similar in each passage (Supplemental Figure 6, Supplemental Table 8). Overall, the control conditions in each passage were rather similar, and we considered them reliable controls for the passaging experiments.

Because the passaging not only relied on similar community established in each passage from the same start community as a control, but also on efficient wash-off of the microbiota off the plants. To ensure that the washed-off microbiota resembled the *in planta* microbiota, we compared the 16S rRNA analysis of the for the start community control samples of the different harvested microbiota (see methods for more details). The effect size of the PERMANOVA analysis was small, but significant (7.92 %, p-value 0.0099) (Supplemental Figure 7A). We conducted the same comparison for the unchallenged non-selective passaging in each passage, where comparisons were non-significant (Supplemental Figure 7B-E, size effects 3.37 – 12.7 %, p-values 0.208 – 0.95). This suggests that the washed-off microbiota was similar and representative of the *in planta* microbiota.

**Unchallenged passaging of SynCom-210**

To investigate the stability of the microbiota, we first analysed the data from the above-described experiment (Figure 1) in which the SynCom-210 was passaged without exposure to the pathogen (unchallenged) and in a non-selective manner, i.e. random plants. Passaged microbiota was able to colonize the plants to the carrying capacity and was not significantly changed compared to start community controls (sc_NI) (Supplemental Figure 8A, Supplemental Table 3,9).

When analysing the community composition over the passages, we observed a large shift from the parental passage (P0) to the first passage (P1), after which the passages (P2 to P5) clustered together (Figure 3A). PERMANOVA analysis of the first passaging event (P0 to P1) showed a size effect of 68.5 % (p-value 0.0099) (Supplemental Figure 9D), which was double the size effect of the biggest difference of start community control between passages (33.9 %) (Supplemental Figure 1). To put this into perspective, the shift from inoculum composition to the parental passage had a size effect of 75.5 % (p-value 0.0198) (Supplemental Figure 9B). This shift was higher than what was found for the comparison in start community controls (51 %, Supplemental Figure 4B), and might be the result of inoculating the start community from a frozen and thawed aliquot.

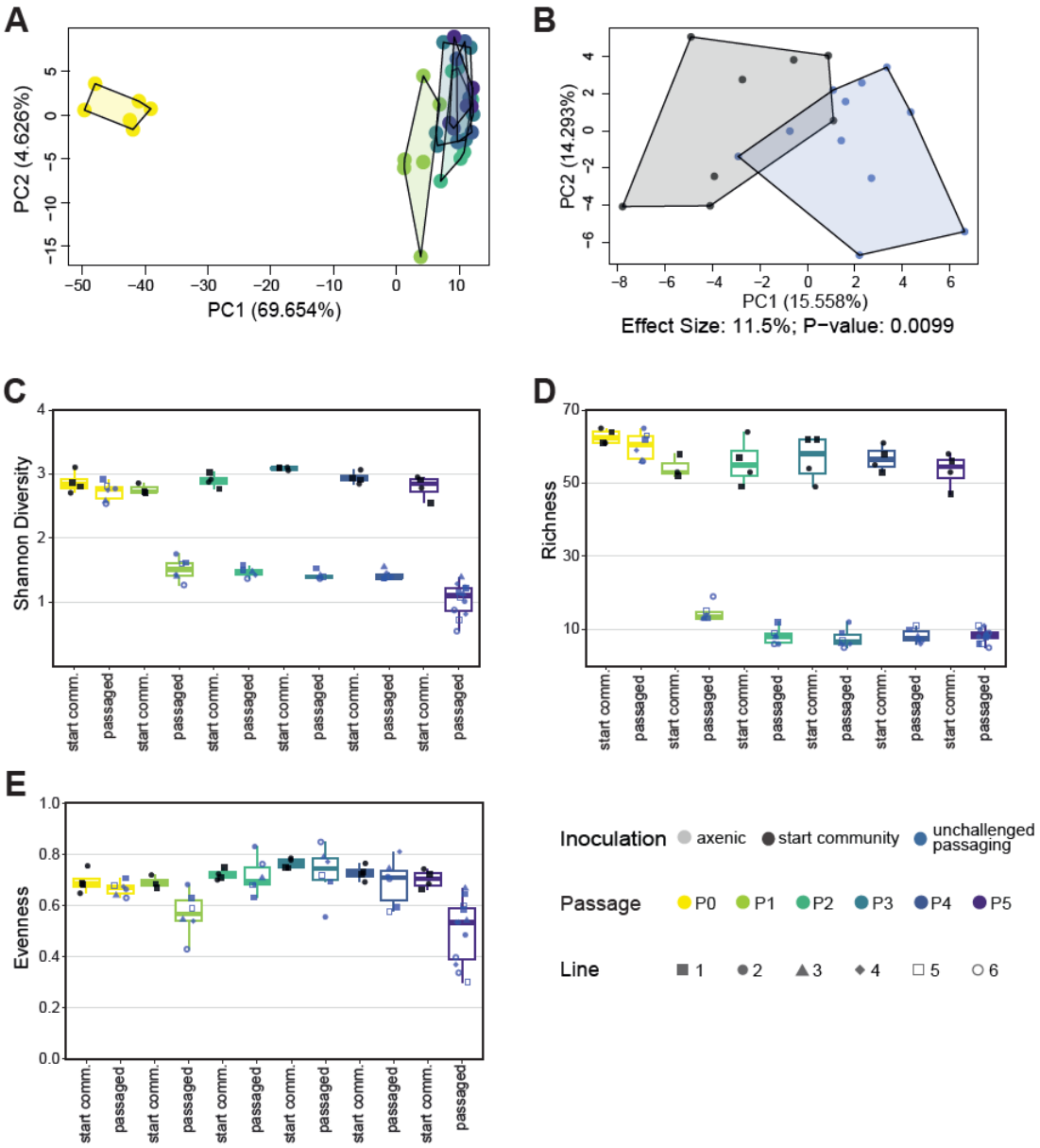


**Figure 3: Changes in community composition in the unchallenged passaging.** Analysis of the unchallenged (mock-infected) passaging of microbiota. A. Principal component analysis of community composition of each passage of the unchallenged passaging. B. PCA and PERMANOVA results of unchallenged passaging (blue) versus frozen start community control (black) in the parental passage (P0). C. Shannon's diversity of unchallenged passaging and start community controls. D. Species richness of unchallenged passaging and start community controls. E. Pielou's evenness of unchallenged passaging and start community controls.

The differences between *in planta* start community control and unchallenged passaging in parental passage was 11.5 % (p-value 0.0099, Figure 3B). This suggested that the difference from parental passage to passage 1 was more than what experimental variation would explain, almost as impactful than the *in planta* establishment of the community. After this first passaging event, the only other significant shift was from passage 4 to 5 (size effect 14.9 %, p-value 0.0495) (Supplemental Figure 9H). The size effect from passage

1 to passage 5 was 19.8 % with a p-value of 0.0198, which is a minor shift, mostly contributed to the passage event from P4 to P5 (Supplemental Figure 9C). In summary, after the first passage event, the community composition was rather stable.

To find the main explanatory factor for the shift in the first passage event, we calculated the community diversity, evenness, and richness of each passage. There was no difference in the mentioned parameters between start community controls and the unchallenged passaging in the parental passage (Figure 3C-E, Supplemental Table 10). However, the passaged communities (parental line to P1) had a lower diversity and species richness compared to parental passage and start community controls (Figure 3D,E). After the decrease in diversity and species richness in the first passaging event, both measurements remained stable. The evenness scores of the passaged communities were within a similar range through all passages compared to the start community controls, and the previous passage, except for the comparison from P4 to P5 (Figure 3E, Supplemental Table 10).

Next, we analysed microbiota changes at the level of ASV abundances. Since the largest shift in the overall community was observed between P0 and P1, we compared the community composition of the parental community to the first passage. In the parental passage, the community established in a similar way as discussed for the start community controls. A few ASVs had relative abundances of more than 10 %, with a long tail of ASVs with lower abundances (Supplemental Figure 10A). In contrast, the community composition of passage 1 had a faster decline in relative abundances, with more strains being non-detected in more samples, while the most abundant ASVs stay roughly the same (Supplemental Figure 10B). When we analysed ASV changes over the passages, 54 ASVs out of 137 ASVs had significant changes in relative abundances in the first passaging event (P0 versus P1), 28 of which were not detected in passage 1, and only one ASV (*Burkholderia* Leaf177) increased in abundance (fold change 2.31) (Supplemental Table 11). After the first passaging event, only four ASVs were changed from passage 1 to passage 5. Namely, *Arthrobacter* Leaf145, *Sphingomonas* Leaf231, *Methylobacterium* Leaf88 and *Methylobacterium* Leaf122 decreased in relative abundance, the last was not detected in passage 5 (Supplemental Table 11).

The loss in diversity from parental passage to passage 1, together with 25 % of ASVs decreasing in relative abundance in passage 1, while the most abundant strains remained abundant, suggested that the low abundant ASVs were lost during the first passaging event. Knowing that the washed off communities are similar to *in planta* communities (Supplemental Figure 7), this loss is not due to the wash-off itself, but likely due to dilution of the washed off microbiota prior to re-inoculation (see methods).

**Non-selective passaging of SynCom-210 with pathogen challenge**

To investigate, how pathogen infection affects the community composition over the passaging experiment, we next analysed the passaging of infected plants that were chosen at random, called non-selective passaging. The commensal colonization of non-selective passaging with pathogen challenge was not

affected and comparable to infected start community controls (sc_pst) over all passages (Supplemental Figure 8, Supplemental Table 3,9).

As seen for the unchallenged passaging, the community composition showed a major shift in the first passage event (P0 to P1). Unlike the unchallenged passaging, the comparisons of the community composition of the non-selective passaging between passages were not significantly different from each other, suggesting that the infection had a stabling effect on the community (Supplemental Figure 11). Fewer ASVs changed in abundance during passaging with pathogen challenge compared to the unchallenged passaging (Supplemental Table 12). In the first passaging event, 43 ASVs changed in abundance, all decreased in abundance. Of the 43 changed ASVs, 25 were undetected at passage 1. From passage 1 to passage 5, four ASVs changed in relative abundance, all decreased, sometimes to an undetected status. These were different ASVs compared to the unchallenged passaging, namely, *Brevundimonas* Leaf280, *Acidovorax* Leaf78, *Flavobacterium* Leaf82, and *Methylobacterium* Leaf91. Community parameters analysed, like Shannon's diversity, evenness and richness behaved the same as described for the unchallenged passaging, with a loss of diversity and richness in the first passaging event (Supplemental Figure 12, Supplemental Table 13).

The pathogen infection had a small influence on the community composition during passaging (Figure 4A, PERMANOVA size effect 5.62 %, p-value 0.0099). The difference of the comparison was only significant for passage 5 (PERMANOVA size effect 7.27 %, p-value 0.0396), but not in other passages (Supplemental Figure 13). Some ASVs benefitted from the infection (*Methylobacterium* Leaf106, *Xanthomonas* Leaf131, *Acidovorax* Leaf78, *Rhizobium* Leaf311), while others decreased in abundance in passaging with pathogen challenge (*Methylobacterium* Leaf86, *Methylobacterium* Leaf361, *Flavobacterium* Leaf82) (Supplemental Table 14).

**Establishment of disease over successive passaging**

To analyse how pathogen established disease in the passaging experiment, we compared the pathogen luminescence at 3 dpi, disease severity at 7 and 14 dpi, and pathogen colonization at 17 dpi of the non-selective passaging to control conditions (axenic and start community inoculated). As was shown above for the controls, disease established similarly each passage.

The low spread and high variation in pathogen luminescence at 3 dpi made interpretation of the pathogen colonization difficult (Figure 4B, Supplemental Figure 14). Hence, the t-test analysis showed significant differences for only two comparisons (non-selective line 3 and 5 versus axenic infected control at passage 1) (Supplemental Table 3,15). Disease severity at 7dpi remained comparable to start community infected plants but was significantly different to axenic infected in P0 and P1, and non-significant to axenic infected in following passages. 2 of 6 selection lines became significantly different to axenic infected in passage 5(selection lines 1 and 3) (Supplemental Table 16, Supplemental Figures 1, 2). At 14 dpi, the disease severity

increased on average in the non-selective passaging (Figure 4C), as did variation of the disease severity both at 7 and 14 dpi (Supplemental Figures 1, 2). At this timepoint, more selection lines remained significantly different to axenic infected controls, while consistently being comparable in disease severity to start community controls (Supplemental Table 17). This suggests, that while in non-protected controls the disease severity increased between 7 and 14 dpi, in protected treatments it tended not to. Taken together, the disease severity suggested that the passaged communities controlled the disease symptoms caused by the pathogen to a similar level as the start community control since the differences were not significant.

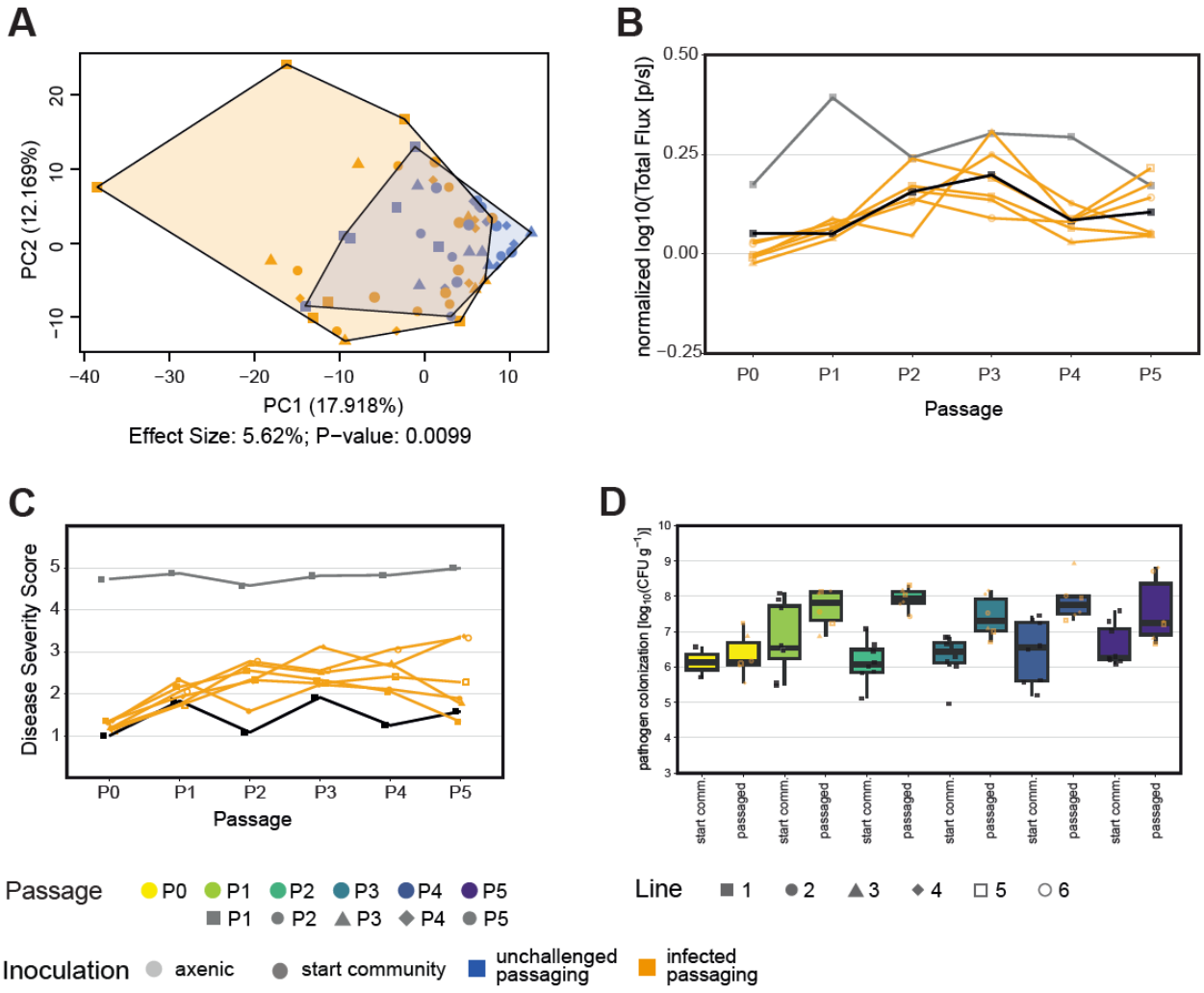


**Figure 4: Effect of pathogen infection on microbiota composition and disease establishment through passages.** A. Comparison of mock- (blue) versus *Pst*-infected (orange) passaging by PCA and PERMANOVA. The shapes correspond to the different passages P1 through P5. B. The mean of normalized pathogen luminescence at 3 dpi of non-selective passaging (orange) compared to axenic (grey) and start community controls (black). Standard deviation was omitted for readability purposes. C. Mean of disease severity score at 14 dpi of non-selective passaging (orange) compared to axenic (grey) and start community controls (black). Standard deviation was omitted for readability purposes. D. Pathogen colonization of non-selective passaging (orange) compared to start community controls (black). Data of control conditions is repeated from Figure 2.

The pathogen colonization increased in non-selective passaging from passage 0 to 1 (p-value 0.036) by a factor of 48 from 1.4 x $10^6$ to 6.6 x $10^7$ cfu $g^{-1}$ fresh weight (Figure 4D, Supplemental Table 3,9). When comparing the colonization in each passage to control conditions, we saw a trend that pathogen colonization went from being comparable to start community inoculated plants in passage 0 and 1 to being significantly different in passages 2 through 4. In the last passage, P5, the pathogen colonization was 1.8 x $10^7$ cfu $g^{-1}$ fresh weight was also higher than P0, but it was statistically non-significant to the pathogen colonization in passage 0 and 1. When looking at the data, the non-significant statistical results could be the results of the high variance seen in pathogen colonization in certain passages (control at P1 and P4, non-selective at P5), and the low sample number (n=2-6) (Figure 4D). The pathogen colonization of the non-selective passaged treatment remained significantly different to axenic controls in all passages (Supplemental Table 9). This suggested that the microbiota protects in all treatments, but protection was reduced after the first passaging experiment. We also saw that pathogen colonization between selection lines could vary, which had effects on the significance levels of the comparisons of the pathogen colonization especially in passage 5 (Supplemental Table 9).

All these measurements informed us that the first passaging event caused a decrease in protection conferred by the passaged communities, that remained at an unchanged intermediate level from passage 1 to 5, despite the pathogen challenge increasing in each passage (Supplemental Table 2).

**Selective passaging of SynCom-210 to drive disease phenotypes**

The last part of the passaging experiments consisted of the passaging of microbiota from plants selected of opposite disease phenotypes to investigate the progression of disease outcome conferred by the phyllosphere microbiota (Figure 1). As discussed before, the pathogen luminescence showed low range, but high variation within each line (Supplemental Figure 14). Comparing the median of pathogen luminescence of the selective passaging over passages revealed no visible differences (Figure 5A), only sick selection lines 1 and 3 had a significant increase in pathogen luminescence in passage 5 compared to the parental passage, the other lines were non-significant (Supplemental Table 18). Disease severity at 14 dpi over the passages revealed no visible distinction between healthy and sick selection as a whole (Figure 5B). There is a trend towards higher disease severity as seen before for non-selective passaging. Another trend is that sick selection lines seem to have a lower median disease severity at passage 5 than their healthy selection counterparts, which could be the case for lines 2, 4 and 6 (Supplemental Figure 2). However, the only significant difference was observed for healthy line 4 versus sick line 4 in passage 1 (Supplemental Table 20). The high variation in disease severity at both 7 and 14 dpi made analysis of comparisons difficult (Supplemental Figures 1,2).

Before analysing pathogen colonization, we investigated the pathogen abundance in passage 5 in greater depth, since we sampled more plants than just the selected ones for this endpoint of the passaging

experiment. There is a trend that the healthy selection had a higher and more variable pathogen colonization than either the non-selective or sick selection lines (Figure 5C). Healthy lines 3 and 5 had a low pathogen abundance, as did sick selection lines 1 and 2. On the contrasting end, healthy lines 4 and 6 had high pathogen abundance as well as a bigger variance. Due to the low sample number (n=3) and high variation, the differences were not significant (Supplemental Table 21). Overall, the pathogen abundance reflected the variation within selection and selection lines, as well as the inconsistency of disease establishment within selection.

The pathogen colonization of both healthy and sick selected plants remained stable over passages (based on significance) (Figure 5D, Supplemental Table 21). We found an increase in pathogen colonization for the non-selective passaging between passage 0 and 1, and a stabilization afterwards. This seemed not to be the case for the selective passaging. The healthy selection had a median pathogen colonization of $2.2 \times 10^6$ cfu $g^{-1}$ fresh weight, while the sick selection had a median of $1.6 \times 10^8$ cfu $g^{-1}$ fresh weight all passages combined (Supplemental Table 3).

The pathogen colonization differed significantly between healthy and sick selection in each passage, except for passage 4 (Supplemental Table 21). This suggested that we indeed select for the plants with either highest or lowest pathogen colonization. Compared to the control conditions, we saw that the pathogen colonization of both selections was significantly different to axenic infected controls, while being non-significantly different to both start community controls and non-selective passaging. In passage 1, the pathogen colonization of the sick selection became distinct from the start community control, while the one of the healthy selection was distinct to both sick selection and non-selective passaging, which had a higher pathogen colonization (Supplemental Figure 8, Supplemental Table 21). From passage 2 on, the pathogen colonization of the sick selection was non-significant to the one of axenic infected controls, suggesting a loss of protection in those selected plants. The pathogen colonization of the healthy selection remained unchanged to that of the start community, while also being significantly different to axenic infected controls. This suggested, that based on the selected plants, we saw differences between the selection types, be that non-selective, healthy or sick selection. However, the pathogen colonization only reflected the plants selected and chosen for microbiota passaging and did not reflect the entire population of the selection lines.

In summary, we saw that the selection lines within a selection had high variation in disease establishment. We could not see a distinction of the opposite selections over passages, likely due to high variation, but also inconsistency of disease establishment. There was a trend that sick selection lines had a lower disease severity score at 14 dpi in passage 5 (Supplemental Figure 2E,F). We did however, select plant that had the opposite disease phenotype and pathogen colonization differed between healthy and sick selection in all but one passage (passage 4).

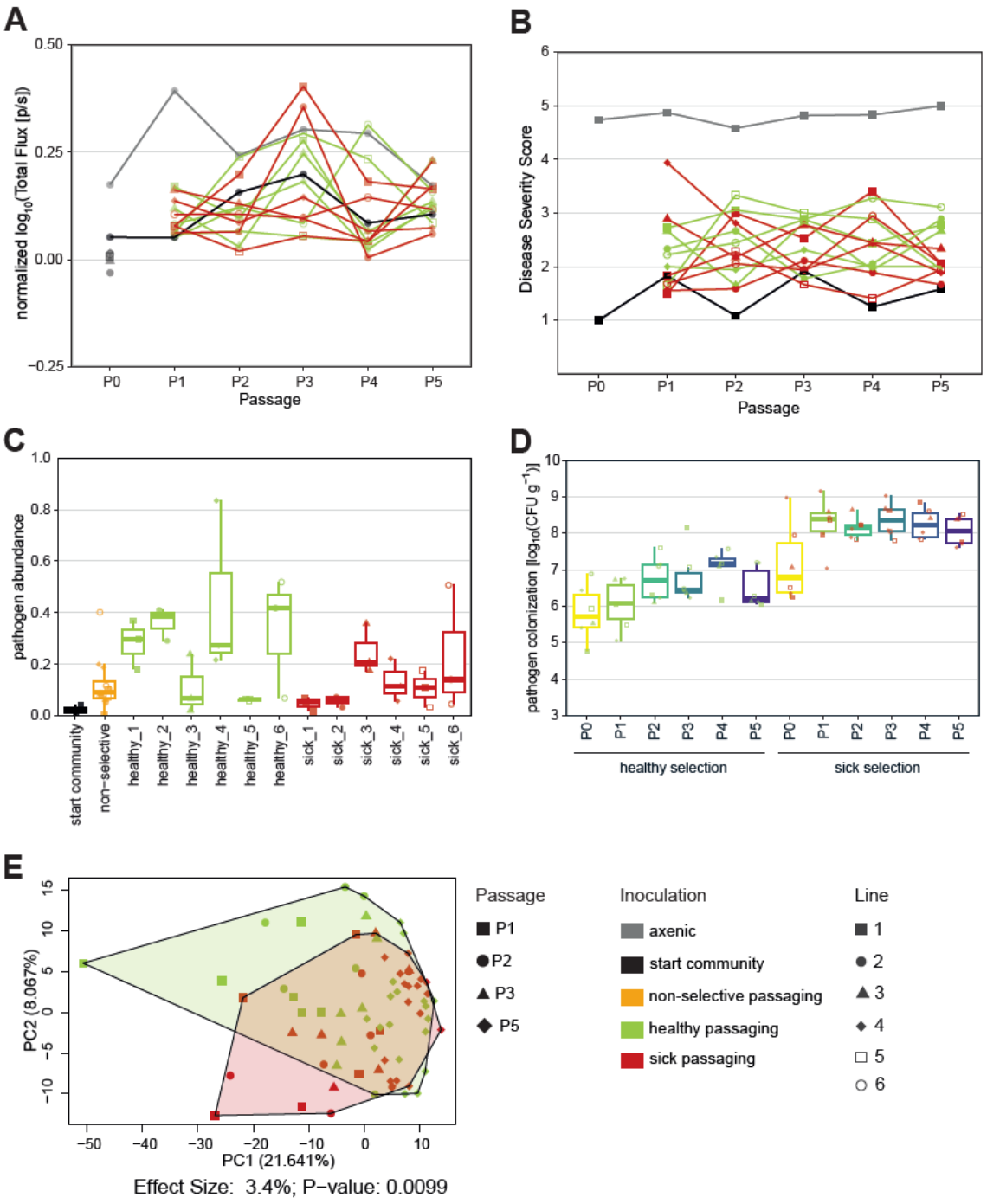


**Figure 5: Effect of selection type on microbiota composition and disease establishment through passages.** A. Mean of pathogen luminescence at 3 dpi of healthy (green) and sick (red) selection lines compared to axenic (grey) and start community controls (black). In P0, parental lines are depicted with grey symbols. Standard deviation was omitted for readability purposes. B. Mean of disease severity scores at 14 dpi of healthy (green) and sick (red) selection lines compared to axenic (grey) and start community controls (black). Standard deviation was omitted for readability purposes. C. Pathogen abundance of passage 5 in samples of healthy (green) and sick (red) selection lines compared to start community control (black) and non-selective passaging (orange). D. Pathogen colonization of pooled selected plants in each passage (boxplot colour) of healthy selection (green symbols) and sick selection (red symbols) E. Community composition comparison between healthy (green) and sick (red) selection of combined passages (P1-P5) with a PCA and PERMANOVA. Data of control conditions is repeated from Figure 2.

**Community composition of selective passaging**

We wanted to analyse whether the selection and passaging of microbiota associated with opposite disease phenotypes was reflected in the community composition. For this, we compared the community compositions of healthy versus sick selections of passage 1 through 5. We pooled the samples of the passages because we saw that the community was stable after the first passage event, as was seen for all passaging types (see above, Supplemental Figures 15,16). The difference of community composition between healthy and sick selection was 5.96 % (PERMANOVA, p-value 0.0099) when combining passage 1 through 5 (Figure 5E). The healthy and sick selections differed in each passage (Supplemental Figure 17), except for passage 3 (Supplemental Figure 17D). We saw a similar small effect size comparing the microbiota of healthy versus sick selected lines, when comparing healthy or sick selected passaged to the non-selective passaged microbiota (PERMANOVA, effect size 5.27 %, 5.04 %, respectively, p-value 0.0099 for both comparisons).

Since we could compare more representative plants in passage 5, we analysed the difference between samples associated with healthy and sick selection as well as samples associated with healthy and sick phenotype. The selection type had a small but significant effect on the community composition (PERMANOVA size effect 5.32 %, p-value 0.0297, Supplemental Figure 17E), while the disease phenotype did not (PERMANOVA size effect 3.07 %, p-value 0.802, Supplemental Figure 17F).

To understand what drove the difference in community composition between healthy and sick selection, we identified ASVs that changed in relative abundance between the selection types (Supplemental Table 22). We found ASVs that were more abundant (*Sanguibacter* Leaf3, *Sphingomonas* Leaf21, *Rhizobium* Leaf311, *Brevundimonas* Leaf280, *Microbacterium* Leaf159), as well as ASVs that were less abundant in the sick selection compared to healthy selection (*Microbacterium* Leaf179, *Sphingomonas* Leaf231, *Sphingomonas* Leaf16, *Methylobacterium* Leaf119, *Methylobacterium* Leaf456). Most notably, the ASV of *Sanguibacter* Leaf3 was significantly more abundant in sick selections in virtually all comparisons with a fold change of 49.6 when combining passages. In the same comparison, *Rhizobium* Leaf311 was also more abundant in sick selection (fold change 29.5), which was also more abundant in non-selective passaging compared to unchallenged passaging (Supplemental Table 14).

In conclusion, the selection and passaging of microbiota associated with opposite disease phenotype did drive community composition apart, though only with a small size effect. In each passage we found differences between protective and less protected plants that can attributed to the differential abundance of individual microbiota members.

**Validating selective passaging and adjustment of experimental design**

In a second passaging experiment, we validated the patterns seen for selectively passaging the microbiota based on the disease phenotype. To improve the variation of the disease phenotype in the parental passage, we selected only one plant as the "microbiota donor". This way, we picked the extreme ends of disease phenotype, but did not average the microbiota composition. We analysed the success of the passaging in the following passage. Additionally, we attempted to increase initial variation in disease progression by increasing infection titre (OD600 0.05, 0.1, 0.5), and by having two infection timepoints (early, late) resulting in selection at 14 dpi and 7 dpi, respectively. We tested different SynComs because we hypothesised that selective passaging in a lower complex community might be more successful in driving apart the disease phenotype. A previous passaging study also suggested that implementation of different levels of start community diversity could give insight into relevant functions, and the responsiveness to artificial selection, provided the initial composition is different enough [43]. To do so, we included four SynComs: SynCom-210 SynCom-48p, SynCom-15 and SynCom-15±5 (Supplemental Table 1, see methods for details).

Pathogen luminescence in the parental passage (P0) showed a smaller range for the early infection (Figure 6A) compared to late infection (Figure 6B), especially for SynCom-210. In general, the pathogen luminescence decreased from 3 dpi (Supplemental Figure 19A,B) to 6 dpi (Figure 6A,B), except for axenic infected samples. That meant that non-protected samples had a higher pathogen luminescence, as well as an increase in pathogen luminescence over time. We used this for selection of healthy and sick plants (see methods). Additionally, we decided to only passage plants infected with the highest infection titre common to all treatments ($OD_{600}$ 0.1). Thereby, we created 3 healthy selection lines and 2 sick selectin lines per infection time and SynCom. Pathogen colonization of these selected plates was generally lower in early infection (14 dpi) compared to late infection (7 dpi), suggesting that all synthetic communities reduce the pathogen colonization over time(Figure 6C). However, none of the comparisons of healthy versus sick selection was significantly different at P0 (Supplemental Table 24); however, this might be due to low sample number of each group (3 and 2, respectively).

Pathogen luminescence of early infection in the first passage (Supplemental Figure 19C,E)showed smaller variance compared to late infection (Supplemental Figure 19D,F), consistent with the earlier experiment. The luminescence of early infection was generally lower than that of the later infection, maybe because the plants are smaller at 21 days compared to 28 days giving the pathogen less surface to emit luminescence from. Comparing sick versus healthy selection lines within the inoculation treatments, the only significant comparison was found in SynCom-15 at 7 dpi (Supplemental Table 26), but not at 3 dpi (Supplemental Table 25). Specifically in SynCom-15, significant differences in pathogen luminescence at 7dpi were found between healthy line 2 and sick line 1 (p-value 0.00457) in the early infection, and for healthy line 1 versus sick lines 1 and 2 (p-value >0.00001, 0.00054) and healthy line 3 and sick line 1 (p-

value 0.00066) in the late infection (Supplemental Table 26). Interestingly, the sick lines of SynCom-15 of the late infection had a lower pathogen luminescence than the healthy selection (Supplemental Figure 19F).

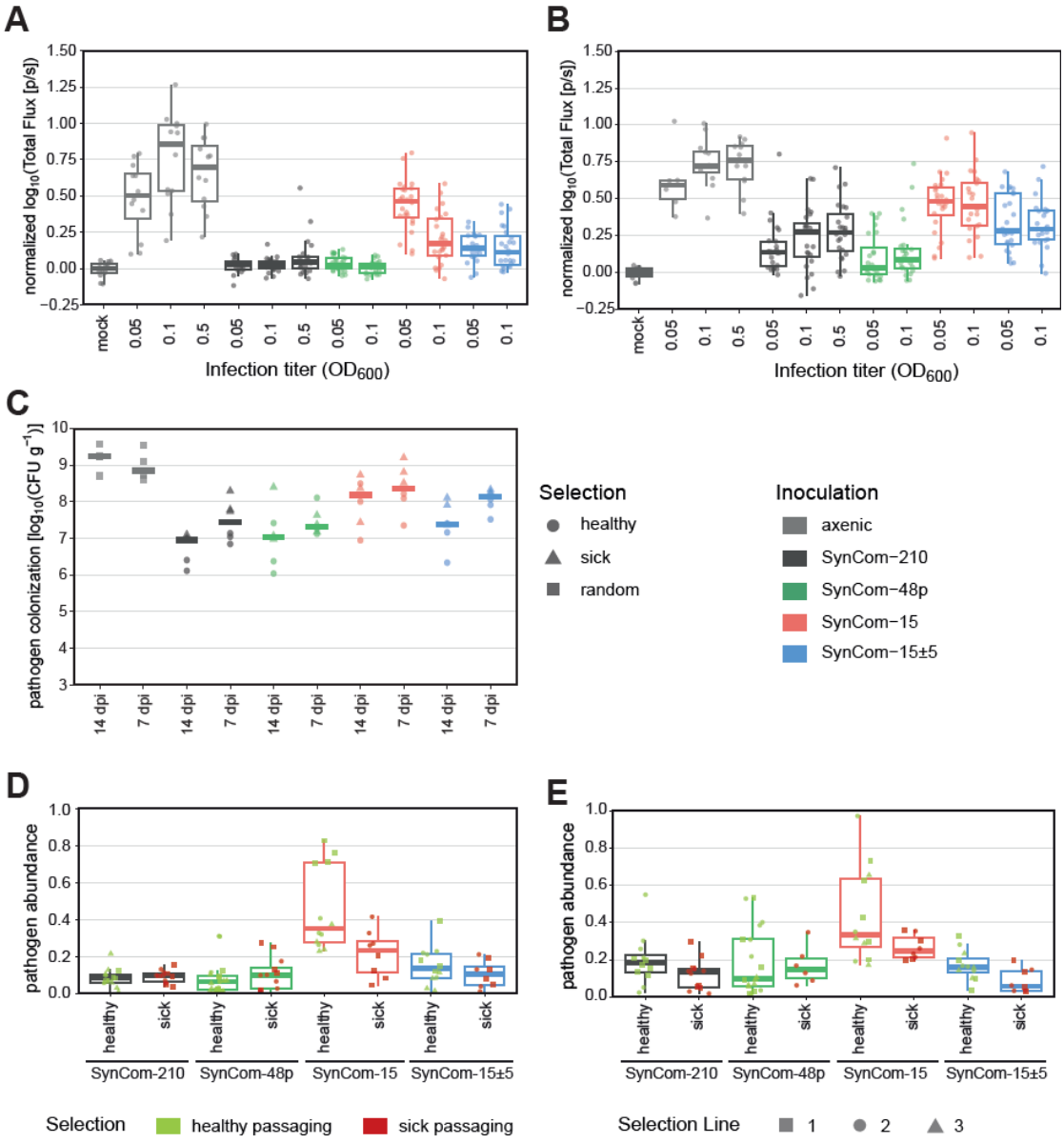


**Figure 6: Disease establishment in a second passaging experiment shows inconsistency in plant phenotype too.** A. Normalized pathogen luminescence of the parental passage at 6 dpi of different community composition (colours) with different infection titres in early infection (21 days old plants). B. Normalized pathogen luminescence of the parental passage at 6 dpi of different community composition (colours) with different infection titres in late infection (28 days old plants). C. Pathogen colonization of the parental passage of different community composition (colours) in 38 days old plants either at 14 dpi (early infection) or 7 dpi (late infection). Symbols represent selection type, either healthy (circles) or sick (triangles) selection. Axenic plants were selected at random (squares). D. Pathogen abundance of passage 1 in different community composition (box colour) and selection types (symbol colour) at 14 dpi (early infection). E. Pathogen abundance of passage 1 in different community composition (box colour) and selection types (symbol colour) at 7 dpi (late infection). Selection lines are indicated through symbol shape.

Next, we analysed whether pathogen abundance differed between healthy and sick selection after selective passaging (Figure 6D,E). Only SynCom-15 had significant differences in pathogen abundance between healthy and sick selection, as well as selection lines at 14 dpi (Supplemental Table 27). Interestingly, the sick selection had a lower pathogen abundance than healthy selection in SynCom-15 at 14 dpi. This trend could be seen for most treatments, both at 14 dpi and 7 dpi (early and late infection timepoint, respectively) (Figure 6D,E).

To investigate ASV changes between healthy and sick selections, we analysed the 16S rRNA amplicon sequencing of the first passage. As a first step, we looked at community diversity, evenness and richness scores of the different communities (Supplemental Figure 20, Supplemental Table 28). The species richness reflected the number of strains inoculated in the respective communities (Supplemental Figure 20C). The infection timepoint or selection did not affect diversity and richness of the communities (Supplemental Table 28). A higher evenness score was found in sick selections of SynCom-15 at 7 dpi and SynCom-15±5 14 dpi compared to their respective healthy lines.

In a second step, we investigated differences in the community composition between healthy and sick lines after passaging to analyse propagation of the disease phenotype. For SynCom-210, healthy and sick selections differed in community composition in both infection times points (Supplemental Figure 21A,B, PERMANOVA effect size 11.6 %, 13.6 %, respectively, p-value 0.0099 in both). The effect size was comparable to the first selective passaging, which was 12 % (Supplemental Figure 17B), suggesting we again observed differences in microbiota composition through selective passaging. Only one and two ASVs changed significantly in abundance at 14 and 7 dpi, respectively (Supplemental Table 29). Namely, the ASV of *Acidovorax* Leaf191 was reduced in healthy lines at 14 dpi. In the first selective passaging experiment, *Acidovorax* Leaf191 was more abundant in sick lines (Supplemental Table 29). At 7 dpi, *Aeromicrobium* Leaf272 and *Rhizobium* Leaf262 were more abundant in healthy lines. These strains were not found to be differently abundant in the previous experiment. SynCom-48p also showed significant differences between healthy and sick selection, both at 14 and 7 dpi (Supplemental Figure 21C,D, PERMANOVA effect size 13.1 %, 19.8 %, respectively, p-value 0.0099 for both). In each infection time, two ASV were significantly changed in abundance. At 14 dpi (early infection), *Arthrobacter* Leaf145 and *Sphingomonas* Leaf257 were lower in abundance in the sick selection. At 7 dpi (late infection), *Pseudomonas* Leaf15 and *Pseudomonas* Leaf98 were significantly lower in sick selection. Despite having significantly different pathogen abundance between sick and healthy selection, communities of healthy and sick selection of SynCom-15 did not differ in community composition (Supplemental Figure 21E,F). The same was true for the comparison of healthy and sick selection of SynCom-15±5 (Supplemental Figure 21G,H).

For passage 1, we did not only sample plants conforming with the selection type of the treatment, but in a representative manner spanning the plant phenotypes. In this way, we could analyse whether microbiota was differing based on the disease phenotype. Neither of the more complex communities showed significant

differences between healthy versus sick disease phenotypes (Supplemental Figure 22A-D), but did for the healthy versus sick selection comparison (Supplemental Figure 21A-D). This suggests that for the more complex communities, the selection based on phenotype introduced more pronounced changes, because there is more difference in community composition between the selection lines than between disease phenotypes in passage 1. As was seen for selection lines (Supplemental Figure 21E-H), the lower complex communities did not show community composition differences between disease phenotypes (Supplemental Figure 22E-H). The exception was the comparison of healthy versus sick disease phenotype in the late infection of SynCom-15±5 (Supplemental Figure 22H, PERMANOVA size effect 10.9 %, p-value 0.0495).

In conclusion, we found that selective passaging introduced microbiota changes, but the disease phenotype was more difficult to drive apart through passaging. We found that disease phenotype of the lower complex community SynCom-15 differed in pathogen abundance between healthy and sick selection, but not according to the selection phenotype (sick had lower pathogen abundance). However, the community compositions of SynCom-15 showed no microbiota changes between selection type (healthy versus sick). This suggests that the passaging of the disease phenotype relied on more than just ASV abundance changes.

**Summary of results**

We could show that a diverse community composed of the *At*-LSPHERE is stable, after a loss in low abundant ASVs in the first passaging event due to dilution of the washed-off communities prior to inoculation of the plants of the next passage. This was evidenced in a lower diversity and richness of the first passage compared to the parental passage (Figure 3C, Supplemental Table 10), and 25 % of ASVs decreasing in abundance from the parental passage to the first passage (Supplemental Table 11).

Introducing pathogen challenge seems to render the communities more stable (Supplemental Figure 11). Microbiota-conferred protection was reduced in the passaged communities (Figure 4D). Driving apart disease phenotype proved difficult, based on disease severity it was not successful (Supplemental Figure 1,2). The selected plants did show the expected differences in pathogen colonization (Figure 5D), and community composition was altered based on selection (Figure 5E). In a second selective passaging experiment, the microbiota composition was distinct for the selection types too, though pathogen abundance was not significantly different in the complex communities (SynCom-210, SynCom-48p) (Figure 6 DE, Supplemental Figure 22). The lower complex SynComs showed no microbiota composition differences, but SynCom-15 had differences in both pathogen luminescence and pathogen abundance between healthy and sick selection.

Taken together, we show that passaging the microbiota can result in changes in microbiota composition. The disease establishment and protection outcome seemed to have higher plant-to-plant variation compared to microbiota composition, and might be dependent on more than just ASV changes.

# Discussion

In our study, we showed that a synthetic community composed of 210 native bacterial strains of the *Arabidopsis thaliana* phyllosphere established in a stable manner after an initial loss of bacterial richness and diversity due to dilution of the inoculum (Figure 3). The loss of bacterial diversity was accompanied with increase in pathogen colonization and disease severity (Figure 4). These results outline, how loss of diversity in an ecosystem can diminish its function, which has been proposed to be an issue in a changing climate and human-influenced nature [2,3]. Loss of protection against a disease due to loss of microbiota diversity was shown in plant diseases [44-46]. This finding is not limited to the plant host as it was shown in human studies as well [11,12,33,46]. Previous passaging experiment also found a loss of diversity over passages [32,47], suggesting that the experimental procedure of passaging caused a loss of microbiota diversity, rather than the reduced diversity being a result of a functional or evolutionary process. However, no other study has presented an investigation of influence of experimental procedure of microbiota passaging, like we did when we sequenced and compared *in planta* versus washed-off communities (Supplemental Figure 7). Therefore, it is hard to say whether loss of diversity is due to selection of more adapted microbes, as was proposed before [32], or because microbes are lost during experimental procedures as we show. Ehau-Taumaunu and colleagues showed an increase in pathogen colonization over passages until passage 5, after which the pathogen decreased until returning to the level prior to passaging at passage 9 [33]. In contrast to that, we saw that pathogen colonization and disease severity was stable after passage 1, despite increasing pathogen pressure (Figure 4C,D, Supplemental Table 2). Our results therefor suggested that the passaged communities of similar diversities (P1-5) maintained their capacity to limit pathogen colonization.

Morella and colleagues also showed that their passaged communities are stable to invasion of non-passaged communities, suggesting that they selected for a better adapted microbiota that was more competitive [32]. It would be interesting to investigate not only how resistant to invasion of the non-passaged strains the passaged communities are, and also how the performance of non-passaged strains compares to their passaged counterparts in terms of plant protection in an equally sized community. It was proposed prior that adapted or well-colonizing strains might occupy the available niches better, leaving no niches for invaders [48]. Interestingly, a study analysing selective passaging for increased plant biomass showed that despite loss of bacterial diversity, the microbiota showed a higher connectivity in terms of co-occurrence analysis in selective passaging compared to random and non-selection passaging [25]. This suggests that loss of strain diversity might not be the only cause of loss of protection but is accompanied with loosing important microbe-microbe interactions that together would limit pathogen colonization and virulence.

Apart from passaging itself, we showed that the pathogen infection had an impact on community composition as well (Figure 4A, Supplemental Table 14). A shift in microbiota composition upon pathogen invasion has been reported before [46,49-52]. While in rhizosphere communities, the pathogen invasion induced

a reduction in diversity and abundance of non-pathogenic bacteria [46], diversity and evenness was not affected in phyllosphere communities [51]. Changes in community composition and structure could be a direct effect of pathogen invasion through resource competition, but also indirectly through the plant immunity and stress responses [52]. The reaction of the microbiota seems not only specific to the tissue, but also the invading pathogen species.

One of the aims of this study was to shift the microbiota through plant phenotype selection, and to investigate how well the leaf-associated microbiota trait will be transplanted to the next passage. Despite succeeding at shifting the microbiota between healthy and sick selection lines (Figure 5E, Supplemental Figures 17, 21), the plant phenotype associated with the selective passaging was unstable (Figure 5A-D, 6DE). Previous studies that tried to drive different microbiota-associated traits to extremes showed similar difficulties in doing so. For example, when passaging communities to improve degradation of a pollutant, no systematic increase in degradation was found within or across different selection types [27]. Selecting for low and high plant biomass production by successively passaging soil microbiota showed distinct biomass after passage 8, however the difference collapses twice in following passages, while higher diluted of inoculum of passages did not show any distinction [26]. Another study showed increased biomass in both selective and no-selective passaging compared to control but passaging of selected plants (random or highest biomass) showed similar effects on biomass [25]. Passaging the soil microbiota 10 times while selecting for induction of early or late flowering, a successful translation of flowering time to different plant species and genotypes was shown [28], which was also conserved when only the culturable strains of the microbiota were present [53]. However, they did not show the progression of phenotype over plant passages. Two other studies propagated rhizosphere and phyllosphere communities over 9 passages, though no improvement or distinction of plant phenotype was shown [33] [30]. Taken together, selecting for distinct traits that are conferred or associated with the microbiota proves to be difficult. Additionally, the trait on which selection is based needs careful evaluation, as was shown in a study for lower $CO_2$ emission of bacterial wastewater communities that despite being successfully selected for, resulted in reduced biomass production, which was an unfavourable outcome [54].

Passaging of a lower complex community in the second selective passaging experiment showed distinct pathogen abundance and pathogen luminescence of healthy versus sick selection in SynCom-15 (Figure 6D,E, Supplemental Table 25-27). However, the sick selection showed to have lower pathogen abundance, which was the opposite of what was selected for. We did not detect a shift in microbiota composition in SynCom-15 when comparing healthy and sick selected plants (Supplemental Figure 21E,F). At this time we cannot exclude that an initial higher pathogen abundance might determine the disease outcome, the latter might be impacted also by the initial colonization dynamics of commensal community members and by the recognition and response of the plant to the bacteria [55]. We could successfully show that different community compositions react differently to selective passaging, as was suggested prior [43].

We also associated ASVs that had different abundances in different selection types with pathogen infection or higher colonization (Supplemental Table 14,22). Some ASVs seemed to profit from the pathogen infection, one of which was *Xanthomonas* Leaf131, which was previously reported to be an opportunistic pathogen [42]. Interestingly, *Rhizobium* Leaf311 was more abundant upon pathogen challenge in random selection (unchallenged versus non-selective passaging), as well as being more abundant in sick selected passaging compared to healthy selected passaging. Other ASVs that were higher abundant in sick selection included *Sphingomonas* Leaf21, *Brevundimonas* Leaf280, *Microbacterium* Leaf159 and *Sanguibacter* Leaf3 which were more abundant in each passage analysed. ASVs that were more abundant in healthy selected passaging included *Microbacterium* Leaf179, *Sphingomonas* Leaf231, *Sphingomonas* Leaf16, *Methylobacterium* Leaf119 and *Methylobacterium* Leaf456. However, it is unclear whether these ASVs were differently abundant because of pathogen infection or prior to pathogen infection and to what extent they have a functional importance to protection against pathogen. The investigation of causal relationships needs further investigation.

We analysed the progression of host-associated phenotype of whole population of each selection type through pathogen luminescence and disease severity (Figure 4B,C, 5A,B, 6A,B). However, our dataset had no representative community composition of the whole population of each selection line. Therefore, we could not correlate a representative sampling of the population of community composition to disease progression. We propose future experiments to sample the selection lines representatively in addition to the selected plants to cover the phenotype variances.

Further investigation also needs to be done to analyse to what extend the genetic makeup of the passaged strains have changed in comparison to their non-passaged ancestors. Through re-isolation from passaged communities, we might also gain insight how fast leaf-associated strains can evolve. Once passaged strains are isolated, their plant protection potential could also be investigated in comparison to their ancestor to experimentally test whether a plant protection potential can be selected for.

In summary, we show that passaging of microbiota can give us insight in its stability over passages, as well as upon pathogen colonization. We showed that diversity of the phyllosphere microbiota is one important element for plant protection and reduction in pathogen since we lost diversity in the first passaging event and saw an increase in pathogen colonization. Driving plant protection apart proved difficult, but microbiota composition was different between healthy and sick selection of the more complex communities (SynCom-210, SynCom-48p). We highlight how important careful selection of the plant phenotype is and how robustness and potentially redundancy within microbiota might impact selection outcomes.

# Materials and Methods

### Plant growth conditions

In all experiments of this Chapter, *Arabidopsis thaliana* Col-0 were grown gnotobiotically in 6-well tissue culture plates (TechnoPlasticProducts), as previously described [56]. Briefly, 5 ml calcined clay (Diamond Pro Calcined Clay Drying Agent) was mixed with 2.5 ml 0.5× Murashige and Skoog (½ MS) medium including vitamins, pH 7 (M0222.0050, Duchefa). Surface sterilized seeds were stratified at 4 °C for 4 d and 1 seed was placed in the centre of each well. If a seed did not germinate, a new plant was transplanted at day 10 from surplus plates. Starting at 4 days, each well was watered twice a week with 200 µl ½ MS medium, except on the day of inoculation with bacteria. Plants were placed in growth chambers set to 22°C and 54 % relative humidity with a 11 h photoperiod. Combined light intensities were set to 200-210 µmol $m^{-2}$ $s^{-1}$ (400-700 nm, PAR) and 7 (4 in pre-inoculation chamber) µmol $m^{-2}$ $s^{-1}$ (280-400 nm, UV light). The plants were inoculated with bacterial suspensions at 10d. In the first passaging experiment presented, plants were infected on day 21, plants were selected 14 days post infection (dpi) on day 35, and harvested at 17 dpi, on day 38. In the second passaging experiment, plants were infected on two different timepoints, either at 21 or 28 days (early versus late infection), selected for on day 35 (7 or 14 dpi) and harvested on day 38 (9 or 17 dpi).

### Synthetic community mixing and initial plant inoculation of the first passaging experiment

The term "inoculation" is used to refer to treatment with commensal strains of the *At*-LSPHERE collection, whereas the term "infection" refers to spraying with the pathogen *P. syringae*. Bacterial strains were streaked out on R2A agar (Sigma-Aldrich) supplemented with 0.5 % (v/v) methanol (R2A+M) and incubated at 22°C for 6 days. Strains were resuspended individually in 1 ml 10 mM $MgCl_2$ buffer by transferring "one loop-full" of bacterial cell mass with a sterile 1 µl plastic loop vortexubg for 10 min. If aggregates were formed in bacterial suspensions, the suspension were left to settle, and the supernatant was transferred to a sterile Eppendorf tube.

The strains were mixed in a 1:1 ratio into their respective phyla (Supplemental Table 1). The $OD_{600}$ of each phylum was measured (Proteobacteria 1.37, Actinobacteria 1.63, Bacteroidetes 1.71, Firmicutes 3.53, *Deinococcus* 3.91). Based on this, the Firmicutes mix and *Deinococcus* were diluted 1:1 with 10 mM $MgCl_2$ buffer. In a 50 ml Falcon tube, the phyla were mixed based on their representation in the *At*-LSPHERE. Specifically, 25 ml Proteobacteria mix, 14.75 ml Actinobacteria mix, 7.5 ml Bacteroidetes mix, 2.5 ml Firmicutes mix and 0.25 ml *Deinococcus* were mixed. Three aliquots of 1.5 ml pre-inoculum were spun down in lysis matrix E tubes (FastDNA SPIN Kit for Soil, MP Biomedicals), and stored at −80 °C. Aliquots of pre-inoculum were mixed in a 1:1 ratio with 50 % (v/v) glycerol in R2A broth, acclimated for freezing for 30 min at 4 °C, and then slowly frozen to −80 °C. For start community control in the parental passage,

a frozen aliquot of pre-inoculum was thawed after 5 h of freezing, spun down at 6000 rcf for 10 min, and resuspended in 10 mM $MgCl_2$ buffer with supplemented phosphate buffer. The $OD_{600}$ was adjusted to 0.02, and the plants of the start community control were inoculated as described below.

The pre-inoculum was adjusted to $OD_{600}$ 0.02 in 50 ml 10 mM $MgCl_2$ buffer containing 500 µl Phosphate buffer with 0.2 % (v/v) Silwett L-77 (Leu+Gygax). 10 d old plants were inoculated with 200 µl of inoculum at $OD_{600}$ 0.02 or with buffer (axenic). The plates were labelled at random prior to inoculation with the intended inoculum and selection line (colour and number). To control the viability of strains in the inoculum, tenfold dilution series of all strains were prepared, and 4 µl of each dilution was spotted onto R2A+M agar square plates (Greiner) to determine colony-forming units (cfu).

**Plant infection with pathogen and monitoring**

Infection inoculum of *Pseudomonas syringae* pv. tomato DC3000 *luxCDABE* (*Ps*t) [57] was prepared as described in Innerebner et al. [58]. Briefly, a lawn of *Ps*t was grown on King's B agar [59] at 28°C overnight, resuspended in 10 ml 10 mM $MgCl_2$ buffer and $OD_{600}$ adjusted to the desired amount (see Supplemental Table 2). The plants were sprayed at day 21 or 28 with either buffer (non-infected controls, NI) or with *Ps*t suspension using a thin-layer chromatography reagent sprayer (Faust Laborbedarf AG). Each plate was at least 6 times, or until all plants appeared to be thoroughly wet. The amount sprayed was estimated by spraying into a Falcon tube ten times, and measuring its weight prior and after on an analytical balance (Mettler-Toledo) with an accuracy of 0.1 mg. In the second passaging experiment, several pathogen titres were used in the parental generation. They were sprayed starting with the highest dilution and cleaning the sprayer by spraying ten times into a separate Falcon tube to move the lower dilution out of the sprayer. The pathogen titre was controlled by cfu determination on King's B agar. An overview of pathogen titre in each passage and experiment can be found in Supplemental Table 2.

**Pathogen luminescence.** The pathogen used in this study carried a luminescence operon *luxCDABE* [57] enabling us to measure its luminescence as a proxy for pathogen colonization as described previously [18]. In the first passaging experiment, the luminescence was measured at 3 dpi, while in the second at both 3 and 6 (or 7) dpi. Briefly, plates were placed with a clean lid into the IVIS Spectrum Imaging System (Xenogen), and luminescence was acquired for 30 s at 500 nm wavelength. If the lids of the tissue plates showed condensation, they were dried in a laminar flow hood or exchanged with a new lid. In the Living Image Software v.4.2., circular region of interests (ROI) were set around each well and the total photon flux per ROI was exported.

**Disease phenotype.** A photograph of each plate was made at infection time, 7 dpi and 14 dpi. Disease severity was scored on the pictures of the plants on the same day. As described before [60], disease severity was scored from 1 no visible disease phenotypes, to 5 visibly diseased in the centre of the plant. Because disease symptoms on community inoculated plants are lessened, following rules are used: healthy plants

with no disease symptoms score a 1. If one lesion on one leave could be seen, the plant scored as a 2. A score 3 refers to several leaves with lesions. If additionally, the plant showed discoloration of leaves (usually darker green), it scored a 4. A score of 5 was given to plants with lesions, discoloration and a diseased meristem, which is the centre of the rosette, out of which the plant grows. If the meristem is diseased, the plant will likely not grow further (not assessed passed 14 dpi). For the second passaging experiment, disease phenotype was assessed, but not further analysed.

**Selection of plants in the first passaging experiment**

In the first passaging experiments, there were four selection types. Namely, the mock-infected microbiota chosen at random, called unchallenged passaging, the *Ps*t infected and chosen at random, called non-selective passaging, and *Ps*t infected and chosen based on disease severity, either healthy selection or sick selection passaging. To have enough washed off microbiota to create the inoculum for the next passage, analyse the microbiota by 16S rRNA amplicon sequcing, creating glycerolstocks and analysing total bacterial colonization and pathogen colonization by cfu enumeration, we selected four of the 18 plants in each selection line (Figure 7IV).

In the parental passage (P0), 6 selection lines per selection type with 3 replicate 6-well plates each were inoculated with the SynCom-210 (Figure 7I). 10 days after inoculation, the unchallenged passaging was mock-infected by spraying the plants with buffer, while the other selection types were infected by spraying the plants with a *Ps*t suspension. At 3 dpi, pathogen luminescence was assessed. At 7 and 14 dpi, the disease severity was scored for each plant in the morning. The three measurements were combined into one table and selected for at 15 dpi (Figure II).

For the unchallenged and non-selective passaging, plants were selected at random within each selection line using a random number generator to select four plants between 1 and 18 (www.randomizer.org) (Figure 7II). In case the selected plant did not grow, it was exchanged with another randomly chosen plant. The selection type was kept consistent throughout the passaging experiment. At no point were plants of different selections or selection lines mixed.

For the selective passaging, where plants were selected based on their phenotype, the parental passage included a "parental" line for each healthy and sick line (Figure 7, parental lines in grey). This meant that selection lines 1 through 6 of healthy and sick selection were initially selected from the healthiest and sickest four plants of the parental line 1 through 6. The healthiest and sickest plants were determined by ordering the plants within each selection line by the disease severity score of 14 dpi from lowest (1 = healthy) to highest (5 = comparable to axenic controls) (Figure 7II). The next level of ordering was based on disease severity score at 7 dpi, and the last level was the total flux [p/s] of pathogen luminescence at 3dpi. This way, plants were ordered from healthy to sick, and those that remained within the same disease score were then ordered by their increasing pathogen colonization proxy (luminescence). Within each selection line, the top

4 plants (aka the healthiest) were chosen for the healthy selection and the bottom 4 were chosen for the sick selection. The selection type (healthy or sick) was kept consistent throughout all of the passaging experiment (Figure 7 IV-VIII). At no point were plants of different selections or selection lines mixed.

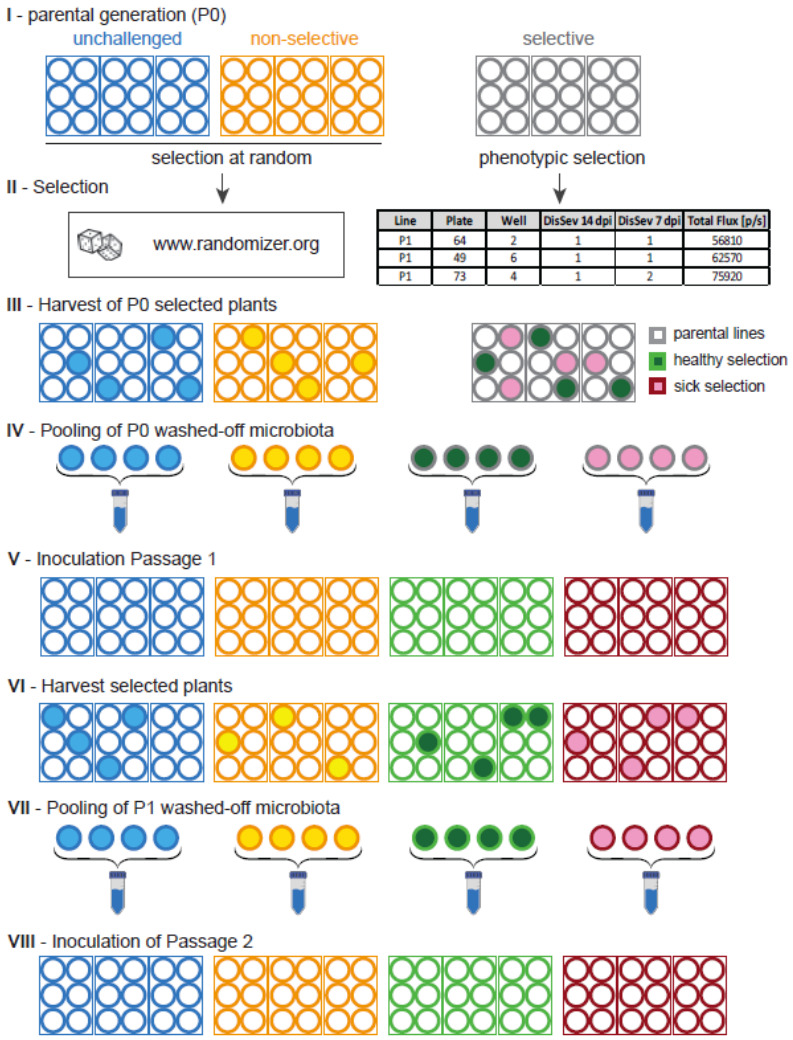


**Figure 7: Overview of selection process in the first passaging experiment.** The selection and harvest procedures are shown on a scheme for one explanatory selection line. The plants of the parental generation (I) were inoculated with the same community, then mock-infected (unchallenged, blue) or infected with pathogen (non-selective, selective). At 14 dpi, the plants were selected (II). The four plants of unchallenged and non-selective passaging were chosen at random with a webtool. For the selective passaging, the plants of each parental line (grey) were ordered by three levels: disease severity scores at 14 dpi, then by scores at 7 dpi and then pathogen luminescence at 3dpi (Total Flux). The top 4 plants were selected for healthy selection lines (green) and the bottom four for the sick selection lines (red). The selected plants were harvested (III), and half of each plant was washed off. The wash-off of the four plants was pooled for each selection line (IV) and diluted, forming the inoculation of the next passage (V). The new passage was cycled through infection, disease assessment and selection (II). The plants of each healthy (green) and sick (red) selection line were ordered as described for the parental passage, but now plants were selected according to their selection type and not divergently. In healthy lines, the healthiest plants (top four) were selected, in sick lines the bottom four were selected. The plants were harvested (VI), one half was used for community wash-off. The wash-off of the four plants was pooled for each selection line (VII) and used for inoculation of passage 2 (VIII). Then the steps VI trough VII were repeated for following passages.

**Microbiota wash-off, bacterial enumeration and inoculation of the following passage in the first passaging experiment**

At day 39, 17 days post infection, the four selected plants were harvested. Working through the selection lines at random, the plates of one selection line were moved into a laminar flow hood. The four selected plants were removed from the clay substrate with sterile tweezers, and cotyledons and roots were removed with a sterile scalpel. With a new set of sterile tweezers and scalpel, the plant was cut in half. One half of the plant was transferred into 2 ml Eppendorf tubes containing 1.3 ml 100 mM phosphate buffer (pH7) supplemented with 0.2 % (v/v) Silwet-L77 (Leu+Gygax). Plant fresh weight was measured on an analytical balance (Mettler-Toledo) with an accuracy of 0.1 mg. The tubes were subsequently washed off by shaking tubes for 15 min at 25 Hz with a TissueLyser II (Qiagen), followed by sonication (ultrasonic bath, Branson) for 5 min. The plants were spun down for 5 sec, and 1 ml of supernatant of the four selected plants per selection line was combined in a sterile 5 ml tube.

From the combined wash-off liquid, three 500 µl aliquots of pre-inoculum were mixed in a 1:1 ratio with 50 % (v/v)glycerol in R2A broth, acclimated for freezing for 30 min at 4 °C, and then slowly frozen to −80 °C. For 16S rRNA amplicon sequencing, 1 ml of wash-off was spun down in lysis matrix E tubes (FastDNA SPIN Kit for Soil, MP Biomedicals), supernatant was discarded and the tubes were stored at −80 °C. For bacterial enumeration, 100 µl of the combined wash-off was transferred to a 96-well plate and a tenfold dilution series in 100 mM phosphate buffer was performed. 4 µl of each dilution was spotted onto two square agar plates (Greiner), one with R2A+M and one with KB agar supplemented with 50 µg/ml kanamycin and 50 µg/ml rifampicin to select for *Ps*t. The plates were grown at room temperature until colonies could be counted, around 2 days for *Ps*t and 7 days for total commensal load.

For the next passage, a new set of axenic plants was seeded and grown for 10 days prior to harvest day of the previous passage. 100 µl of the combined wash-off of each selection line was diluted into 4.9 ml 10 mM $MgCl_2$ buffer to create the inoculum for the next passage. Dilution of the wash-off was made to ensure a non-toxic concentration of Silwett L-77. The selection lines were kept distinct from each other. After inoculation, plants of the new passage were put back into growth chambers and infection, selection and harvest cycle were repeated until passage 5.

**Plant harvest for DNA extraction in the first passaging experiment**

As described for the plant harvest for wash-off, the plants were harvested at day 39 or 17 days post infection. Working through the selection lines at random, the plates of one selection line were moved into a laminar flow hood. The four selected plants were removed from the clay substrate with sterile tweezers, and cotyledons and roots were removed with a sterile scalpel. With a new set of sterile tweezers and scalpel, the plant was cut in half. One half was used for wash-off, described above. The second halves of the plants of each selection line were combined in a lysis matrix E tube (FastDNA SPIN Kit for Soil, MP Biomedicals).

The plant fresh weight was measured on an analytical balance (Mettler-Toledo) with an accuracy of 0.1 mg. Then the samples were frozen in liquid nitrogen and stored at -80 °C until DNA extraction.

In passage 5, additional plants outside the four selected ones were harvested to analyse community composition to have a more representative sample. For this, the plants were harvested as described for selected ones, but not cut in half and directly placed into lysis matrix E tubes (FastDNA SPIN Kit for Soil, MP Biomedicals). The plant fresh weight was measured on an analytical balance (Mettler-Toledo) with an accuracy of 0.1 mg. Then the samples were frozen in liquid nitrogen and stored at -80 °C until DNA extraction.

**Synthetic community mixing and initial plant inoculation of the second passaging experiment**

For SynCom-210, a frozen aliquot was used in the same manner as the start community control of the above described first passaging experiment. For SynCom-48p, SynCom-15 and SynCom-15±5, bacterial strains were streaked out on R2A agar (Sigma-Aldrich) supplemented with 0.5 % (v/v) methanol (R2A+M) and incubated at 22°C for 6 days. Then, one loop full of bacterial biomass was transferred from the agar plates into 1 ml 10mM $MgCl_2$ buffer using a sterile 1 µl plastic loop. The strains were vortexed for 10 min. When necessary, strains that formed aggregates were left to settle and supernatant was transferred to a clean tube. Strains were mixed in a 1:1 ratio into the respective synthetic communities to form a pre-inoculum. 1.5 ml aliquots were spun down in lysis matrix E tubes (FastDNA SPIN Kit for Soil, MP Biomedicals), and stored at −80 °C. Aliquots of pre-inoculum were mixed in a 1:1 ratio with 50 % (v/v) glycerol in R2A broth, acclimated for freezing for 30 min at 4 °C, and then slowly frozen to −80 °C. $OD_{600}$ was adjusted to 0.02 in 25 ml 10 mM $MgCl_2$ buffer.

**Selection of plants in the second passaging experiment**

To ensure highest variation between opposite extremes of plant phenotype (healthy or sick), the plants of the highest applied common infection titre ($OD_{600}$ of 0.1) were used for passaging of the microbiota. The measured luminescence of 6 dpi was subtracted from the one of 3 dpi, and plants were ordered according to the difference. For the healthy lines, three plants with the biggest decrease in luminescence or lowest overall luminescence measurements were chosen. For the sick lines, two plants with the biggest increase in luminescence or highest overall luminescence measurements were chosen. The selection was done independently for each community and infection timepoint. The selected plants were chosen to be the "microbiota donor" or parent of the selection lines in the next passage. Plants of passage 1 were chosen in a similar manner, to cover both ends of phenotypic variation in each selection line. Since the microbiota was not passaged further, the community composition of each treatment was analysed with 16S rRNA amplicon sequencing.

**Plant harvest for microbiota passaging, bacterial enumeration and for 16S rRNA amplicon sequencing in the second passaging experiment**

For the parental passage, selected plants were harvested by removing cotyledons and roots with sterile tweezers and scalpels as described before [18]. The whole plant was transferred into a 2 ml Eppendorf tube filled with 1.3 ml phosphate buffer (pH7) complemented with 0.2 % (v/v) Silwett L-77 (Leu+Gygax) and then treated as described for the first passaging experiment. The wash-off suspension was divided into two 490 µl aliquots for glycerolstocks, 100 µl was transferred into inoculation tube (prefilled with 900 µl 10mM $MgCl_2$ buffer) and 100 µl was transferred into 96-well plate to assess bacterial enumeration (as described for first passaging experiment). The plants of passage 1 were pre-grown for 10 days, as described for the first passaging experiment, and inoculated with 200 µl of bacterial suspension.

For the harvest of the first passage, the selected plants were harvested by removing cotyledons and roots with sterile tweezers and scalpels and the remaining phyllosphere was directly placed into lysis matrix E tubes (FastDNA SPIN Kit for Soil, MP Biomedicals). Plant fresh weight was assessed as described before, and samples were frozen in liquid nitrogen and stored at -80 °C.

**DNA extraction and library preparation for 16S rRNA amplicon sequencing**

DNA extraction and 16S rRNA amplicon sequencing was done as described before [40-42,56]. Briefly, frozen plant and inoculum samples were lyophilised (Christ Alpha 2–4 LD Plus) overnight and subsequently homogenized with a TissueLyser II for 2 min at 25 Hz. The DNA was extracted with the FastDNA SPIN Kit for Soil (MP Biomedicals) according to the manufacturer's instructions. DNA was quantified (Promega QuantiFluor dsDNA, E2670) and normalized to a concentration of 2.5 ng/µl in 50 µl end volume. For samples with lower DNA concentration, the undiluted sample was taken. The 16S amplicon library was prepared as previously described [40-42,56]. The V5–V7 region of the 16S rRNA gene was amplified in triplicate with primers 799F [61] and 1193R [62] with the DFS Taq polymerase (Bioron). After pooling triplicate samples, amplification was verified by loading 5 µl of each sample on a 1.5 % (w/v) agarose gel. Primers were removed by enzymatic digestion with Antarctic phosphatase (NewEnglandBioLabs) and Exonuclease I (NewEnglandBioLabs). 10 cycles of barcoding-PCR were performed in triplicate with plate-specific forward and well-specific reverse. Triplicates were again pooled and the amplification was verified with a 1.5 % (w/v) agarose gel as described for the first PCR. Based on the intensity of the gel band, samples were pooled and to reduce the volume of the library, it was cleaned by bead clean-up (AMPure XP, Beckman Coulter) with a ratio of 0.8:1. Then the library was loaded on a 1.5 % (w/v) agarose gel and the band at approximately 500 bp was cleaned up with the QIAquick gel extraction kit (Qiagen) according to the manufacturer's protocol. The library was cleaned twice by bead clean-up (AMPure XP, Beckman Coulter) with a ratio of 0.8:1. Library sequencing was performed on the Illumina MiSeq platform using a v3 cycle kit (2 × 300 bp, paired-end) at the Genetic Diversity Center (ETH Zurich). The denatured library was diluted

to a final concentration of 10 or 20 pM with addition of 20-30 % PhiX. Sequencing was performed with custom sequencing primers as previously described [40]. The 16S rRNA amplicon sequencing samples presented in this study were split over three libraries. Validation of uniformity was done on the SynCom-210 inoculum sample of first passaging experiment, sequenced in triplicates in every library.

**Data analysis**

If not stated differently, data was analysed and visualized in the statistical software R v4.2.2 [63]. The packages used for data preparation, analysis and visualization included *tidyverse* v1.3.2 [64], *gridExtra* v2.3 [65], *ggpubr* v0.6.0 [66].

**Pathogen luminescence analysis.** Prior to data analysis, the total flux [p/s] measurements were $\log_{10}$-transformed and normalized to the median of axenic mock-infected controls (background luminescence) because we saw differences in background levels (Figure 2B). Differences in luminescence among different treatments were detected using pairwise Welch's t-tests with Bonferroni-correction on the p-value. In the analysis, measurements of the mock-infected axenic plants served as the background luminescence signal (no bacteria on top of plants).

**Disease severity score analysis.** Differences in disease severity scores among different treatments were detected through pairwise Welch's t-tests with Bonferroni-correction on the p-value. Disease severity scores were visualized with boxplots, or by calculating the mean and standard deviation.

**Bacterial colonization analysis.** Prior to data analysis, the calculated bacterial cfu per gram fresh weight were $\log_{10}$-transformed. Differences in colonization level among different treatments were detected using pairwise Welch's t-tests with Bonferroni-correction on the p-value. Comparisons of non-passaged controls, unchallenged and non-selective were separated into separate comparisons, while the data for selective passaging was compared to all other treatments. The data was visualized with the lower limit of the plot being the calculated detection limit.

**16S rRNA data processing.** The 16S rRNA amplicon sequencing data were analysed as described previously [41,42,56]. The raw reads of the paired-end sequencing were processed using USEARCH v.11.0.667-i86 linux64 [67]. The reads were merged with the *fastq_mergepairs* command with minimum identity of 90 % and minimum overlap of 16 bp. The merged reads were filtered using the command *fastq_filter* with a maximum expected error of 1 and a minimum length of 200 bp. A 16S rDNA reference database was composed based on the amplicon sequencing variants (ASV) of the V5-V7 region 16S rRNA gene sequences of the At-LPSHERE strains [56]. The command *otutab* was used to classify and count reads with 100 % identity to the 16S rDNA reference database and assign them to individual samples, to generate an ASV table. The sequences with a barcode corresponding to a sample but no match to the reference database were added up and included as an additional line for sequencing depth estimation, but not further investigated.

Control samples of axenic plants, and water controls of the extraction and processing controls were used to detect possible systematic contaminations but excluded for further analysis. The 16S rRNA amplicon sequencing samples presented in this study were split over three libraries. Validation of uniformity was done on the SynCom-210 inoculum sample of first passaging experiment, sequenced in triplicates in every library. ASV tables of all three libraries were combined and analysed together.

**Pathogen abundance.** For passage 5 of the first passaging experiment, and for passage 1 of the second passaging experiment, the pathogen abundances were calculated since the pathogen colonization was not determined. The number of sequences assigned to the pathogen *Ps*t in the ASV tables were divided by the total number of reads of the samples. Differences in pathogen abundance among different treatments were detected using pairwise Welch's t-tests with Bonferroni-correction on the p-value.

**Comparing and visualising community composition based on 16s rRNA amplicon sequencing.** Prior to data analysis, the pathogen abundance was omitted, and for low complex communities (SynCom-15s) ASV tables were reduced to ASVs present in the communities. The community composition comparisons and ASV changes between treatments were analysed as described before [41,42,56] with the R package *phylloR* version 1.0.1 available on GitHub (https://github.com/MicrobiologyETHZ/phylloR/). Briefly, after filtering for the comparisons of interest, the ASV table was log-normalized for sequence depth and variance-stabilized by DESeq2 v1.38.3 [68]. For visualization of the overall comparison of two treatments, the *plotPCA* function in the package *phylloR* was used. The function applies a principal component analysis (PCA) to the transformed OTU table using the *prcomp* command and calculated the effect size, which is the variance explained by the compared factor, and the p-value of the comparisons were calculated by PERMANOVA using the *adonis* function of the package *vegan* v2.6-4 [69] with Euclidean distance. In the *phylloR* package, PERMANOVA was modified to account for the batch effect between replicate experiments with the strata argument. Changes in ASV abundances between two groups was analysed through the function *plotCommunityChanges* in the *phylloR* package. The output of *DESeq2* provided $\log_2$-fold change values and p-values (Wald tests, Benjamini-Hochberg adjusted). The community composition was visualized through the function *plotCommunity*, where the relative abundance values were calculated by proportional normalization of each sample by its sequencing depth.

Shannon's diversity scores, Pielou's evenness and species richness were calculated using the package package *vegan* v2.6-4 [69]. In a first step, ASV tables were transposed, and samples of interest were filtered for. Next, the samples were rarefied based on the minimal sequencing depth (total number of reads per sample). For the first passaging experiment, the minimal size was 1798. For the second passaging experiment, samples with less than 1'000 reads were excluded and remaining were rarefied to the minimal size of 3309. The species number (richness) was calculated using the function *specnumber*. The diversity scores were calculated with the function *diversity* with index set to "shannon". Pielou's evenness was

calculated by dividing the diversity by the log-transformed species richness. Differences in diversity scores among different treatments were detected using pairwise Welch's t-tests with Bonferroni-correction on the p-value.

**Data and Code availability**

Data files and R script used for preparation of data, visualization and analysis are stored on a gitlab repository (https://gitlab.ethz.ch/thesisbe/june2022.git). Raw sequencing data are stored on TAPES (\\LTS22\biol_lts_cifs\biol-micro\gr_vorholt\OMICS\barbmuel).

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# Supplemental Tables

**Supplemental Table 1:** Representation of *At*-LSPHERE strains in synthetic communities described in chapter II.

| ASV representative | Strain | ASV size (n strains) | Phylum [1] | Class [1] | Genus [1] | SynCom-210 | SynCom-48p | SynCom-15 | SynCom-15±5 |
|---|---|---|---|---|---|---|---|---|---|
| Leaf2 | Leaf2 | 1 | Proteobacteria | Alphaproteobacteria | Novosphingobium | 1 | 0 | 0 | 0 |
| Leaf3 | Leaf3 | 1 | Actinobacteria | Actinobacteria | Sanguibacter | 1 | 0 | 0 | 0 |
| Leaf10 | Leaf10 | 1 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf11 | Leaf9 | 6 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf11 | Leaf11 | 6 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf11 | Leaf23 | 6 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf11 | Leaf25 | 6 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf11 | Leaf42 | 6 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf11 | Leaf407 | 6 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf13 | Leaf13 | 1 | Firmicutes | Bacilli | Bacillus | 1 | 0 | 0 | 0 |
| Leaf15 | Leaf15 | 2 | Proteobacteria | Gammaproteobacteria | Pseudomonas | 1 | 1 | 0 | 0 |
| Leaf15 | Leaf98 | 2 | Proteobacteria | Gammaproteobacteria | Pseudomonas | 1 | 1 | 0 | 0 |
| Leaf16 | Leaf16 | 3 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf16 | Leaf29 | 3 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf16 | Leaf32 | 3 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf17 | Leaf17 | 1 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf21 | Leaf21 | 1 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 1 | 1 | 0 |
| Leaf22 | Leaf22 | 2 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf22 | Leaf62 | 2 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf24 | Leaf5 | 2 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf24 | Leaf24 | 2 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf26 | Leaf26 | 1 | Proteobacteria | Alphaproteobacteria | Sphingobium | 1 | 0 | 0 | 0 |
| Leaf28 | Leaf28 | 1 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf30 | Leaf30 | 1 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf33 | Leaf33 | 1 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 1 |
| Leaf34 | Leaf34 | 2 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf34 | Leaf38 | 2 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf41 | Leaf41 | 1 | Bacteroidetes | Sphingobacteriia | Pedobacter | 1 | 0 | 1 | 1 |
| Leaf48 | Leaf48 | 1 | Proteobacteria | Gammaproteobacteria | Pseudomonas | 1 | 1 | 0 | 0 |
| Leaf49 | Leaf49 | 1 | Firmicutes | Bacilli | Bacillus | 1 | 1 | 0 | 0 |
| Leaf51 | Leaf51 | 1 | Proteobacteria | Gammaproteobacteria | Serratia | 1 | 1 | 0 | 0 |
| Leaf53 | Leaf53 | 1 | Proteobacteria | Gammaproteobacteria | Erwinia | 1 | 1 | 0 | 0 |
| Leaf58 | Leaf58 | 1 | Proteobacteria | Gammaproteobacteria | Pseudomonas | 1 | 1 | 0 | 0 |
| Leaf59 | Leaf59 | 1 | Proteobacteria | Gammaproteobacteria | Pseudomonas | 0 | 1 | 0 | 0 |
| Leaf61 | Leaf61 | 1 | Proteobacteria | Betaproteobacteria | Duganella | 1 | 1 | 0 | 1 |
| Leaf64 | Leaf64 | 1 | Proteobacteria | Alphaproteobacteria | Devosia | 1 | 0 | 0 | 0 |
| Leaf67 | Leaf67 | 1 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 1 | 1 | 1 |
| Leaf69 | Leaf69 | 1 | Actinobacteria | Actinobacteria | Arthrobacter | 1 | 0 | 0 | 0 |
| Leaf70 | Leaf70 | 1 | Proteobacteria | Gammaproteobacteria | Stenotrophomonas | 1 | 0 | 0 | 0 |
| Leaf72 | Leaf72 | 1 | Firmicutes | Bacilli | Paenibacillus | 1 | 0 | 0 | 0 |
| Leaf75 | Leaf75 | 1 | Firmicutes | Bacilli | Bacillus | 1 | 0 | 0 | 0 |
| Leaf78 | Leaf78 | 1 | Proteobacteria | Betaproteobacteria | Acidovorax | 1 | 0 | 0 | 0 |
| Leaf82 | Leaf82 | 1 | Bacteroidetes | Flavobacteriia | Flavobacterium | 1 | 0 | 1 | 0 |
| Leaf83 | Leaf83 | 1 | Proteobacteria | Gammaproteobacteria | Pseudomonas | 1 | 0 | 1 | 0 |
| Leaf85 | Leaf85 | 1 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf86 | Leaf86 | 1 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf88 | Leaf88 | 9 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 1 | 1 |
| Leaf88 | Leaf89 | 9 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf88 | Leaf94 | 9 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf88 | Leaf104 | 9 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf88 | Leaf111 | 9 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf88 | Leaf113 | 9 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf88 | Leaf117 | 9 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf88 | Leaf125 | 9 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf88 | Leaf465 | 9 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf91 | Leaf91 | 1 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf99 | Leaf99 | 1 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf100 | Leaf87 | 5 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf100 | Leaf100 | 5 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf100 | Leaf102 | 5 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf100 | Leaf112 | 5 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf100 | Leaf469 | 5 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf106 | Leaf93 | 2 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf106 | Leaf106 | 2 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf108 | Leaf108 | 3 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf108 | Leaf399 | 3 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf108 | Leaf466 | 3 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf119 | Leaf90 | 4 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf119 | Leaf119 | 4 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf119 | Leaf121 | 4 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf119 | Leaf123 | 4 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf122 | Leaf92 | 2 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf122 | Leaf122 | 2 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf126 | Leaf126 | 1 | Proteobacteria | Betaproteobacteria | Duganella | 1 | 2 | 1 | 0 |
| Leaf127 | Leaf127 | 1 | Proteobacteria | Gammaproteobacteria | Pseudomonas | 1 | 1 | 0 | 0 |
| Leaf129 | Leaf129 | 1 | Proteobacteria | Gammaproteobacteria | Pseudomonas | 1 | 1 | 0 | 1 |
| Leaf130 | Leaf130 | 1 | Proteobacteria | Gammaproteobacteria | Acinetobacter | 1 | 1 | 0 | 0 |
| Leaf131 | Leaf131 | 2 | Proteobacteria | Gammaproteobacteria | Xanthomonas | 1 | 1 | 0 | 0 |
| Leaf131 | Leaf148 | 2 | Proteobacteria | Gammaproteobacteria | Xanthomonas | 1 | 1 | 0 | 0 |
| Leaf137 | Leaf137 | 1 | Actinobacteria | Actinobacteria | Arthrobacter | 1 | 1 | 0 | 0 |
| Leaf139 | Leaf139 | 1 | Proteobacteria | Betaproteobacteria | Massilia | 1 | 0 | 0 | 0 |
| Leaf141 | Leaf141 | 1 | Actinobacteria | Actinobacteria | Arthrobacter | 1 | 1 | 0 | 0 |

Supplemental Table 1 continued

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Leaf145 | Leaf145 | 1 | Actinobacteria | Actinobacteria | Arthrobacter | 1 | 1 | 0 | 0 |
| Leaf151 | Leaf151 | 1 | Actinobacteria | Actinobacteria | Microbacterium | 1 | 1 | 0 | 0 |
| Leaf154 | Leaf154 | 2 | Actinobacteria | Actinobacteria | Curtobacterium | 1 | 1 | 0 | 0 |
| Leaf154 | Leaf183 | 2 | Actinobacteria | Actinobacteria | Curtobacterium | 1 | 1 | 0 | 0 |
| Leaf155 | Leaf202 | 4 | Proteobacteria | Alphaproteobacteria | Rhizobium | 1 | 0 | 0 | 0 |
| Leaf155 | Leaf68 | 4 | Proteobacteria | Alphaproteobacteria | Rhizobium | 1 | 1 | 1 | 0 |
| Leaf155 | Leaf155 | 4 | Proteobacteria | Alphaproteobacteria | Rhizobium | 1 | 1 | 0 | 0 |
| Leaf155 | Leaf167 | 4 | Proteobacteria | Alphaproteobacteria | Rhizobium | 0 | 1 | 0 | 0 |
| Leaf159 | Leaf159 | 3 | Actinobacteria | Actinobacteria | Microbacterium | 1 | 0 | 0 | 0 |
| Leaf159 | Leaf161 | 3 | Actinobacteria | Actinobacteria | Microbacterium | 1 | 0 | 0 | 0 |
| Leaf159 | Leaf320 | 3 | Actinobacteria | Actinobacteria | Microbacterium | 1 | 0 | 0 | 0 |
| Leaf160 | Leaf160 | 1 | Proteobacteria | Betaproteobacteria | Acidovorax | 1 | 1 | 0 | 0 |
| Leaf164 | Leaf164 | 1 | Actinobacteria | Actinobacteria | Rathayibacter | 1 | 0 | 0 | 0 |
| Leaf168 | Leaf168 | 1 | Proteobacteria | Alphaproteobacteria | Brevundimonas | 1 | 0 | 0 | 0 |
| Leaf171 | Leaf1 | 3 | Actinobacteria | Actinobacteria | Plantibacter | 1 | 0 | 0 | 0 |
| Leaf171 | Leaf171 | 3 | Actinobacteria | Actinobacteria | Plantibacter | 1 | 0 | 0 | 0 |
| Leaf171 | Leaf314 | 3 | Actinobacteria | Actinobacteria | Plantibacter | 1 | 0 | 0 | 0 |
| Leaf172 | Leaf172 | 2 | Actinobacteria | Actinobacteria | Clavibacter | 1 | 0 | 0 | 0 |
| Leaf172 | Leaf263 | 2 | Actinobacteria | Actinobacteria | Clavibacter | 1 | 0 | 0 | 0 |
| Leaf176 | Leaf176 | 1 | Bacteroidetes | Sphingobacteriia | Pedobacter | 1 | 0 | 0 | 0 |
| Leaf177 | Leaf177 | 1 | Proteobacteria | Betaproteobacteria | Burkholderia | 1 | 1 | 0 | 0 |
| Leaf179 | Leaf179 | 3 | Actinobacteria | Actinobacteria | Microbacterium | 1 | 1 | 0 | 0 |
| Leaf179 | Leaf203 | 3 | Actinobacteria | Actinobacteria | Microbacterium | 1 | 1 | 0 | 0 |
| Leaf179 | Leaf436 | 3 | Actinobacteria | Actinobacteria | Microbacterium | 1 | 1 | 0 | 0 |
| Leaf180 | Leaf180 | 1 | Bacteroidetes | Flavobacteriia | Chryseobacterium | 1 | 0 | 0 | 0 |
| Leaf182 | Leaf182 | 1 | Firmicutes | Bacilli | Brevibacillus | 1 | 0 | 0 | 0 |
| Leaf185 | Leaf185 | 2 | Actinobacteria | Actinobacteria | Rathayibacter | 1 | 0 | 0 | 0 |
| Leaf185 | Leaf294 | 2 | Actinobacteria | Actinobacteria | Rathayibacter | 1 | 0 | 0 | 0 |
| Leaf186 | Leaf44 | 5 | Actinobacteria | Actinobacteria | Frigoribacterium | 1 | 1 | 0 | 0 |
| Leaf186 | Leaf254 | 5 | Actinobacteria | Actinobacteria | Frigoribacterium | 1 | 1 | 0 | 0 |
| Leaf186 | Leaf8 | 5 | Actinobacteria | Actinobacteria | Frigoribacterium | 1 | 0 | 0 | 0 |
| Leaf186 | Leaf186 | 5 | Actinobacteria | Actinobacteria | Frigoribacterium | 1 | 0 | 0 | 0 |
| Leaf186 | Leaf415 | 5 | Actinobacteria | Actinobacteria | Frigoribacterium | 1 | 0 | 0 | 0 |
| Leaf187 | Leaf187 | 2 | Firmicutes | Bacilli | Exiguobacterium | 1 | 0 | 1 | 1 |
| Leaf187 | Leaf196 | 2 | Firmicutes | Bacilli | Exiguobacterium | 1 | 0 | 0 | 0 |
| Leaf189 | Leaf189 | 1 | Bacteroidetes | Cytophagia | Dyadobacter | 1 | 0 | 0 | 0 |
| Leaf191 | Leaf76 | 3 | Proteobacteria | Betaproteobacteria | Acidovorax | 1 | 0 | 0 | 0 |
| Leaf191 | Leaf84 | 3 | Proteobacteria | Betaproteobacteria | Acidovorax | 1 | 0 | 0 | 0 |
| Leaf191 | Leaf191 | 3 | Proteobacteria | Betaproteobacteria | Acidovorax | 1 | 0 | 0 | 0 |
| Leaf194 | Leaf194 | 1 | Bacteroidetes | Sphingobacteriia | Pedobacter | 1 | 0 | 0 | 0 |
| Leaf198 | Leaf198 | 5 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 1 | 0 | 0 |
| Leaf198 | Leaf230 | 5 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 1 | 0 | 0 |
| Leaf198 | Leaf242 | 5 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 1 | 0 | 0 |
| Leaf198 | Leaf20 | 5 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf198 | Leaf205 | 5 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf201 | Leaf201 | 1 | Bacteroidetes | Flavobacteriia | Chryseobacterium | 1 | 0 | 0 | 0 |
| Leaf208 | Leaf208 | 1 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf210 | Leaf335 | 4 | Actinobacteria | Actinobacteria | Agreia | 1 | 1 | 0 | 0 |
| Leaf210 | Leaf210 | 4 | Actinobacteria | Actinobacteria | Agreia | 1 | 0 | 0 | 0 |
| Leaf210 | Leaf244 | 4 | Actinobacteria | Actinobacteria | Agreia | 1 | 0 | 0 | 0 |
| Leaf210 | Leaf283 | 4 | Actinobacteria | Actinobacteria | Agreia | 1 | 0 | 0 | 0 |
| Leaf216 | Leaf216 | 1 | Bacteroidetes | Sphingobacteriia | Pedobacter | 1 | 0 | 0 | 0 |
| Leaf220 | Leaf220 | 1 | Proteobacteria | Betaproteobacteria | Variovorax | 1 | 0 | 1 | 1 |
| Leaf222 | Leaf222 | 1 | Actinobacteria | Actinobacteria | Agromyces | 1 | 0 | 0 | 0 |
| Leaf226 | Leaf226 | 1 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 1 | 0 | 0 |
| Leaf231 | Leaf231 | 1 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf233 | Leaf233 | 2 | Actinobacteria | Actinobacteria | Rhodococcus | 1 | 1 | 0 | 0 |
| Leaf233 | Leaf278 | 2 | Actinobacteria | Actinobacteria | Rhodococcus | 1 | 0 | 0 | 0 |
| Leaf234 | Leaf234 | 1 | Actinobacteria | Actinobacteria | Arthrobacter | 1 | 0 | 0 | 0 |
| Leaf245 | Leaf245 | 1 | Actinobacteria | Actinobacteria | Aeromicrobium | 1 | 0 | 0 | 0 |
| Leaf247 | Leaf7 | 4 | Actinobacteria | Actinobacteria | Rhodococcus | 1 | 1 | 0 | 0 |
| Leaf247 | Leaf225 | 4 | Actinobacteria | Actinobacteria | Rhodococcus | 1 | 0 | 0 | 0 |
| Leaf247 | Leaf247 | 4 | Actinobacteria | Actinobacteria | Rhodococcus | 1 | 0 | 0 | 0 |
| Leaf247 | Leaf258 | 4 | Actinobacteria | Actinobacteria | Rhodococcus | 1 | 0 | 0 | 0 |
| Leaf257 | Leaf257 | 1 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 1 | 0 | 0 |
| Leaf261 | Leaf261 | 1 | Actinobacteria | Actinobacteria | Curtobacterium | 1 | 0 | 0 | 0 |
| Leaf262 | Leaf262 | 1 | Proteobacteria | Alphaproteobacteria | Rhizobium | 1 | 1 | 0 | 0 |
| Leaf264 | Leaf264 | 2 | Actinobacteria | Actinobacteria | Leifsonia | 1 | 0 | 0 | 0 |
| Leaf264 | Leaf325 | 2 | Actinobacteria | Actinobacteria | Leifsonia | 1 | 0 | 0 | 0 |
| Leaf265 | Leaf265 | 1 | Proteobacteria | Betaproteobacteria | Pseudorhodoferax | 1 | 0 | 0 | 0 |
| Leaf267 | Leaf267 | 1 | Proteobacteria | Betaproteobacteria | Variovorax | 1 | 0 | 0 | 0 |
| Leaf272 | Leaf272 | 1 | Actinobacteria | Actinobacteria | Aeromicrobium | 1 | 0 | 1 | 1 |
| Leaf274 | Leaf274 | 1 | Proteobacteria | Betaproteobacteria | Pseudorhodoferax | 1 | 0 | 0 | 0 |
| Leaf280 | Leaf280 | 1 | Proteobacteria | Alphaproteobacteria | Brevundimonas | 1 | 0 | 0 | 0 |
| Leaf285 | Leaf285 | 2 | Actinobacteria | Actinobacteria | Nocardioides | 1 | 0 | 0 | 0 |
| Leaf285 | Leaf307 | 2 | Actinobacteria | Actinobacteria | Nocardioides | 1 | 0 | 0 | 0 |
| Leaf288 | Leaf288 | 1 | Actinobacteria | Actinobacteria | Microbacterium | 1 | 0 | 0 | 0 |
| Leaf289 | Leaf289 | 2 | Actinobacteria | Actinobacteria | Aeromicrobium | 1 | 0 | 0 | 0 |
| Leaf289 | Leaf291 | 2 | Actinobacteria | Actinobacteria | Aeromicrobium | 1 | 0 | 0 | 0 |
| Leaf299 | Leaf299 | 2 | Actinobacteria | Actinobacteria | Rathayibacter | 1 | 0 | 1 | 1 |
| Leaf299 | Leaf296 | 2 | Actinobacteria | Actinobacteria | Rathayibacter | 1 | 0 | 0 | 0 |
| Leaf304 | Leaf304 | 1 | Actinobacteria | Actinobacteria | Frondihabitans | 1 | 0 | 0 | 0 |
| Leaf306 | Leaf306 | 2 | Proteobacteria | Alphaproteobacteria | Rhizobium | 1 | 0 | 0 | 0 |
| Leaf306 | Leaf321 | 2 | Proteobacteria | Alphaproteobacteria | Rhizobium | 1 | 0 | 0 | 0 |
| Leaf311 | Leaf311 | 1 | Proteobacteria | Alphaproteobacteria | Rhizobium | 1 | 1 | 0 | 0 |
| Leaf324 | Leaf324 | 1 | Proteobacteria | Alphaproteobacteria | Aureimonas | 1 | 0 | 0 | 0 |
| Leaf326 | Leaf326 | 1 | Deinococcus-Thermus | Deinococci | Deinococcus | 1 | 0 | 0 | 0 |

Supplemental Table 1 continued

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Leaf334 | Leaf334 | 2 | Actinobacteria | Actinobacteria | Cellulomonas | 1 | 0 | 0 | 0 |
| Leaf334 | Leaf395 | 2 | Actinobacteria | Actinobacteria | Cellulomonas | 1 | 0 | 0 | 0 |
| Leaf336 | Leaf336 | 1 | Actinobacteria | Actinobacteria | Leifsonia | 1 | 0 | 0 | 0 |
| Leaf337 | Leaf337 | 1 | Actinobacteria | Actinobacteria | Arthrobacter | 1 | 1 | 0 | 0 |
| Leaf339 | Leaf339 | 1 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf343 | Leaf343 | 1 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf344 | Leaf344 | 1 | Proteobacteria | Alphaproteobacteria | Bosea | 1 | 0 | 0 | 0 |
| Leaf347 | Leaf347 | 2 | Actinobacteria | Actinobacteria | Microbacterium | 1 | 1 | 0 | 0 |
| Leaf347 | Leaf351 | 2 | Actinobacteria | Actinobacteria | Microbacterium | 1 | 1 | 0 | 0 |
| Leaf354 | Leaf354 | 1 | Actinobacteria | Actinobacteria | Williamsia | 1 | 0 | 0 | 0 |
| Leaf357 | Leaf357 | 1 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf359 | Leaf359 | 1 | Bacteroidetes | Flavobacteriia | Flavobacterium | 1 | 0 | 0 | 1 |
| Leaf361 | Leaf361 | 1 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf363 | Leaf363 | 1 | Proteobacteria | Alphaproteobacteria | Brevundimonas | 1 | 0 | 0 | 0 |
| Leaf369 | Leaf369 | 1 | Actinobacteria | Actinobacteria | Geodermatophilus | 1 | 0 | 0 | 0 |
| Leaf371 | Leaf371 | 4 | Proteobacteria | Alphaproteobacteria | Rhizobium | 1 | 0 | 0 | 1 |
| Leaf371 | Leaf341 | 4 | Proteobacteria | Alphaproteobacteria | Rhizobium | 1 | 0 | 0 | 0 |
| Leaf371 | Leaf383 | 4 | Proteobacteria | Alphaproteobacteria | Rhizobium | 1 | 0 | 0 | 0 |
| Leaf371 | Leaf384 | 4 | Proteobacteria | Alphaproteobacteria | Rhizobium | 1 | 0 | 0 | 0 |
| Leaf374 | Leaf374 | 1 | Actinobacteria | Actinobacteria | Nocardioides | 0 | 0 | 1 | 1 |
| Leaf380 | Leaf380 | 1 | Actinobacteria | Actinobacteria | Blastococcus | 1 | 0 | 0 | 0 |
| Leaf386 | Leaf453 | 3 | Proteobacteria | Alphaproteobacteria | Rhizobium | 1 | 1 | 0 | 0 |
| Leaf386 | Leaf386 | 3 | Proteobacteria | Alphaproteobacteria | Rhizobium | 1 | 0 | 0 | 0 |
| Leaf386 | Leaf391 | 3 | Proteobacteria | Alphaproteobacteria | Rhizobium | 1 | 0 | 0 | 0 |
| Leaf394 | Leaf394 | 1 | Bacteroidetes | Flavobacteriia | Chryseobacterium | 1 | 0 | 0 | 0 |
| Leaf396 | Leaf396 | 1 | Proteobacteria | Alphaproteobacteria | Bradyrhizobium | 1 | 0 | 0 | 0 |
| Leaf400 | Leaf400 | 1 | Proteobacteria | Betaproteobacteria | Acidovorax | 1 | 0 | 0 | 0 |
| Leaf404 | Leaf404 | 1 | Bacteroidetes | Flavobacteriia | Chryseobacterium | 1 | 0 | 0 | 0 |
| Leaf405 | Leaf405 | 1 | Bacteroidetes | Flavobacteriia | Chryseobacterium | 1 | 0 | 1 | 1 |
| Leaf406 | Leaf406 | 1 | Firmicutes | Bacilli | Bacillus | 1 | 0 | 0 | 0 |
| Leaf412 | Leaf412 | 1 | Proteobacteria | Alphaproteobacteria | Sphingomonas | 1 | 0 | 0 | 0 |
| Leaf414 | Leaf408 | 3 | Proteobacteria | Betaproteobacteria | Methylophilus | 1 | 0 | 0 | 0 |
| Leaf414 | Leaf414 | 3 | Proteobacteria | Betaproteobacteria | Methylophilus | 1 | 0 | 0 | 0 |
| Leaf414 | Leaf416 | 3 | Proteobacteria | Betaproteobacteria | Methylophilus | 1 | 0 | 0 | 0 |
| Leaf420 | Leaf420 | 1 | Proteobacteria | Alphaproteobacteria | Devosia | 1 | 0 | 0 | 0 |
| Leaf427 | Leaf427 | 2 | Proteobacteria | Alphaproteobacteria | Aurantimonas | 1 | 0 | 1 | 1 |
| Leaf427 | Leaf460 | 2 | Proteobacteria | Alphaproteobacteria | Aurantimonas | 1 | 0 | 0 | 0 |
| Leaf434 | Leaf434 | 1 | Proteobacteria | Gammaproteobacteria | Pseudomonas | 1 | 1 | 0 | 0 |
| Leaf443 | Leaf443 | 1 | Proteobacteria | Alphaproteobacteria | Aurantimonas | 1 | 0 | 0 | 0 |
| Leaf446 | Leaf446 | 1 | Actinobacteria | Actinobacteria | Marmoricola | 1 | 0 | 0 | 0 |
| Leaf454 | Leaf454 | 1 | Proteobacteria | Alphaproteobacteria | Aurantimonas | 1 | 0 | 0 | 0 |
| Leaf456 | Leaf456 | 1 | Proteobacteria | Alphaproteobacteria | Methylobacterium | 1 | 0 | 0 | 0 |
| Leaf459 | Leaf459 | 1 | Proteobacteria | Betaproteobacteria | Methylophilus | 1 | 0 | 0 | 0 |

[1] Y. Bai et al., Functional overlap of the Arabidopsis leaf and root microbiota. Nature 528, 364-369 (2015).

**Supplemental Table 2:** Pathogen titre used in each passage of the first and second passaging experiment presented in chapter II.

| Experiment | Passage | Sprayed | $OD_{600}$ measured | amount per spray [ml] | pathogen [CFU/ml] | pathogen CFUs sprayed | Comment |
|---|---|---|---|---|---|---|---|
| first | P0 | buffer | | 39.54674595 | | | |
| first | P0 | pathogen suspension | 0.011 | 28.42391023 | 1.05E+07 | 3.69E+05 | |
| first | P1 | buffer | | 32.74667145 | | | |
| first | P1 | pathogen suspension | 0.025 | 67.05796181 | 2.50E+07 | 3.73E+05 | |
| first | P2 | buffer | | 29.18616046 | | | |
| first | P2 | pathogen suspension | 0.046 | 56.57702102 | 4.38E+07 | 7.73E+05 | |
| first | P3 | buffer | | 36.39744891 | | | |
| first | P3 | pathogen suspension | 0.064 | 64.44023401 | 5.13E+07 | 7.95E+05 | |
| first | P4 | buffer | | 52.92624356 | | | |
| first | P4 | pathogen suspension | 0.091 | 55.46373449 | 5.50E+07 | 9.92E+05 | |
| first | P5 | buffer | | 83.13542409 | | | |
| first | P5 | pathogen suspension | 0.12 | 37.52076505 | 1.75E+08 | 4.66E+06 | |
| second selective | 2_P0 | buffer | | 33.65936581 | | | early infection (21d) |
| second selective | 2_P0 | pathogen suspension | 0.049 | 56.98823497 | | | early infection (21d) |
| second selective | 2_P0 | pathogen suspension | 0.101 | 50.12798282 | | | early infection (21d) |
| second selective | 2_P0 | pathogen suspension | 0.504 | 39.51665712 | | | early infection (21d) |
| second selective | 2_P0 | buffer | | | | | late infection (28d) |
| second selective | 2_P0 | pathogen suspension | 0.054 | 56.09559982 | | | late infection (28d) |
| second selective | 2_P0 | pathogen suspension | 0.109 | 49.11499237 | | | late infection (28d) |
| second selective | 2_P0 | pathogen suspension | 0.524 | 49.89730182 | | | late infection (28d) |
| second selective | 2_P1 | buffer | | 61.9639237 | | | early infection (21d) |
| second selective | 2_P1 | pathogen suspension | 0.249 | 58.95403822 | | | early infection (21d) |
| second selective | 2_P1 | buffer | | 36.66824834 | | | late infection (28d) |
| second selective | 2_P1 | pathogen suspension | 0.1975 | 50.93035149 | | | late infection (28d) |

**Supplemental Table 3:** Median pathogen luminescence (total flux) and bacterial colonization of treatments in each passage of the first passaging experiment.

| Passage | Selection/Treatment | Pst luminescence [$log_{10}$(p/s)] | Pst colonization [$log_{10}$(CFU $g^{-1}$] | commensal [$log_{10}$(CFU $g^{-1}$] |
|---|---|---|---|---|
| P0 | axenic mock-infected | 4.823 | *NA* | *NA* |
| P1 | axenic mock-infected | 4.699 | *NA* | *NA* |
| P2 | axenic mock-infected | 4.829 | *NA* | *NA* |
| P3 | axenic mock-infected | 4.84 | *NA* | *NA* |
| P4 | axenic mock-infected | 4.844 | *NA* | *NA* |
| P5 | axenic mock-infected | 4.837 | *NA* | *NA* |
| P0 | axenic infected | 4.982 | 9.274 | *NA* |
| P1 | axenic infected | 5.092 | 9.502 | *NA* |
| P2 | axenic infected | 5.071 | 9.056 | *NA* |
| P3 | axenic infected | 5.31 | 9.131 | *NA* |
| P4 | axenic infected | 5.138 | 9.325 | *NA* |
| P5 | axenic infected | 5.009 | 9.108 | *NA* |
| P0 | start community mock-infected | 4.82 | *NA* | 8.267 |
| P1 | start community mock-infected | 4.74 | *NA* | 8.125 |
| P2 | start community mock-infected | 4.804 | *NA* | 7.737 |
| P3 | start community mock-infected | 4.845 | *NA* | 8.224 |
| P4 | start community mock-infected | 4.827 | *NA* | 8.728 |
| P5 | start community mock-infected | 4.825 | *NA* | *NA* |
| P0 | start community infected | 4.862 | 6.136 | 7.751 |
| P1 | start community infected | 4.75 | 6.541 | 8.185 |
| P2 | start community infected | 4.986 | 6.067 | 8.149 |
| P3 | start community infected | 5.038 | 6.428 | 8.262 |
| P4 | start community infected | 4.929 | 6.55 | 8.312 |
| P5 | start community infected | 4.943 | 6.263 | *NA* |
| P0 | unchallenged passaging | *NA* | *NA* | 7.215 |
| P1 | unchallenged passaging | *NA* | *NA* | 8.168 |
| P2 | unchallenged passaging | *NA* | *NA* | 8.185 |
| P3 | unchallenged passaging | *NA* | *NA* | 8.102 |
| P4 | unchallenged passaging | *NA* | *NA* | 8.129 |
| P5 | unchallenged passaging | *NA* | *NA* | *NA* |
| P0 | non-selective passaging | 4.829 | 6.132 | 8.242 |
| P1 | non-selective passaging | 4.828 | 7.819 | 8.213 |
| P2 | non-selective passaging | 4.769 | 7.94 | 8.344 |
| P3 | non-selective passaging | 4.986 | 7.304 | 8.349 |
| P4 | non-selective passaging | 5.015 | 7.747 | 8.328 |
| P5 | non-selective passaging | 4.917 | 7.251 | *NA* |
| P0 | healthy selected passaging | 4.829 | 5.73 | 8.142 |
| P1 | healthy selected passaging | 4.919 | 6.073 | 8.158 |
| P2 | healthy selected passaging | 4.942 | 6.706 | 8.169 |
| P3 | healthy selected passaging | 5.017 | 6.449 | 8.111 |
| P4 | healthy selected passaging | 4.925 | 7.176 | 8.149 |
| P5 | healthy selected passaging | 4.966 | 6.201 | *NA* |
| P0 | sick selected passaging | 4.829 | 6.788 | 8.749 |
| P1 | sick selected passaging | 4.792 | 8.389 | 8.979 |
| P2 | sick selected passaging | 4.933 | 8.168 | 8.507 |
| P3 | sick selected passaging | 4.944 | 8.352 | 8.592 |
| P4 | sick selected passaging | 4.92 | 8.215 | 8.511 |
| P5 | sick selected passaging | 4.941 | 8.067 | *NA* |

**Supplemental Table 4:** Bonferroni-corrected p-values of pairwise Welch's t-tests of bacterial colonization of control conditions in first passaging experiment. Note that commensal colonization data of passage 5 is missing.

| colonization by | Treatment/Passage | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|---|
| Commensal | P0 | start community *Ps* t infected | start community mock-infected | 1 | |
| Commensal | P1 | start community *Ps* t infected | start community mock-infected | 1 | |
| Commensal | P2 | start community *Ps* t infected | start community mock-infected | 1 | |
| Commensal | P3 | start community *Ps* t infected | start community mock-infected | 1 | |
| Commensal | P4 | start community *Ps* t infected | start community mock-infected | 1 | |
| Commensal | start community mock-infected | P0 | P1 | 1 | |
| Commensal | start community mock-infected | P0 | P2 | 1 | |
| Commensal | start community mock-infected | P0 | P3 | 1 | |
| Commensal | start community mock-infected | P0 | P4 | 1 | |
| Commensal | start community mock-infected | P1 | P2 | 1 | |
| Commensal | start community mock-infected | P1 | P3 | 1 | |
| Commensal | start community mock-infected | P1 | P4 | 0.12497 | |
| Commensal | start community mock-infected | P2 | P3 | 1 | |
| Commensal | start community mock-infected | P2 | P4 | 0.01913 | * |
| Commensal | start community mock-infected | P3 | P4 | 1 | |
| Commensal | start community infected | P0 | P1 | 1 | |
| Commensal | start community infected | P0 | P2 | 1 | |
| Commensal | start community infected | P0 | P3 | 1 | |
| Commensal | start community infected | P0 | P4 | 1 | |
| Commensal | start community infected | P1 | P2 | 1 | |
| Commensal | start community infected | P1 | P3 | 1 | |
| Commensal | start community infected | P1 | P4 | 1 | |
| Commensal | start community infected | P2 | P3 | 1 | |
| Commensal | start community infected | P2 | P4 | 1 | |
| Commensal | start community infected | P3 | P4 | 1 | |
| pathogen (*Ps* t) | P0 | start community *Ps* t infected | axenic infected | 0 | **** |
| pathogen (*Ps* t) | P1 | start community *Ps* t infected | axenic infected | 0 | **** |
| pathogen (*Ps* t) | P2 | start community *Ps* t infected | axenic infected | 0 | **** |
| pathogen (*Ps* t) | P3 | start community *Ps* t infected | axenic infected | 0 | **** |
| pathogen (*Ps* t) | P4 | start community *Ps* t infected | axenic infected | 0 | **** |
| pathogen (*Ps* t) | P5 | start community *Ps* t infected | axenic infected | 0 | **** |
| pathogen (*Ps* t) | start community infected | P0 | P1 | 1 | |
| pathogen (*Ps* t) | start community infected | P0 | P2 | 1 | |
| pathogen (*Ps* t) | start community infected | P0 | P3 | 1 | |
| pathogen (*Ps* t) | start community infected | P0 | P4 | 1 | |
| pathogen (*Ps* t) | start community infected | P0 | P5 | 1 | |
| pathogen (*Ps* t) | start community infected | P1 | P2 | 1 | |
| pathogen (*Ps* t) | start community infected | P1 | P3 | 1 | |
| pathogen (*Ps* t) | start community infected | P1 | P4 | 1 | |
| pathogen (*Ps* t) | start community infected | P1 | P5 | 1 | |
| pathogen (*Ps* t) | start community infected | P2 | P3 | 1 | |
| pathogen (*Ps* t) | start community infected | P2 | P4 | 1 | |
| pathogen (*Ps* t) | start community infected | P2 | P5 | 1 | |
| pathogen (*Ps* t) | start community infected | P3 | P4 | 1 | |
| pathogen (*Ps* t) | start community infected | P3 | P5 | 1 | |
| pathogen (*Ps* t) | start community infected | P4 | P5 | 1 | |
| pathogen (*Ps* t) | axenic infected | P0 | P1 | 1 | |
| pathogen (*Ps* t) | axenic infected | P0 | P2 | 1 | |
| pathogen (*Ps* t) | axenic infected | P0 | P3 | 1 | |
| pathogen (*Ps* t) | axenic infected | P0 | P4 | 1 | |
| pathogen (*Ps* t) | axenic infected | P0 | P5 | 1 | |
| pathogen (*Ps* t) | axenic infected | P1 | P2 | 1 | |
| pathogen (*Ps* t) | axenic infected | P1 | P3 | 1 | |
| pathogen (*Ps* t) | axenic infected | P1 | P4 | 1 | |
| pathogen (*Ps* t) | axenic infected | P1 | P5 | 1 | |
| pathogen (*Ps* t) | axenic infected | P2 | P3 | 1 | |
| pathogen (*Ps* t) | axenic infected | P2 | P4 | 1 | |
| pathogen (*Ps* t) | axenic infected | P2 | P5 | 1 | |
| pathogen (*Ps* t) | axenic infected | P3 | P4 | 1 | |
| pathogen (*Ps* t) | axenic infected | P3 | P5 | 1 | |
| pathogen (*Ps* t) | axenic infected | P4 | P5 | 1 | |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 5:** Bonferroni-corrected p-values of pairwise Welch's t-tests of pathogen luminescence of control conditions in first passaging experiment.

| Comment | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|
| P0 | axenic mock-infected | axenic infected | 0.00124 | ** |
| P0 | axenic mock-infected | start community infected | 1 | |
| P0 | axenic infected | start community infected | 1 | |
| P1 | axenic mock-infected | axenic infected | 0 | **** |
| P1 | axenic mock-infected | start community infected | 1 | |
| P1 | axenic infected | start community infected | 0.05365 | |
| P2 | axenic mock-infected | axenic infected | 0.04792 | * |
| P2 | axenic mock-infected | start community infected | 1 | |
| P2 | axenic infected | start community infected | 1 | |
| P3 | axenic mock-infected | axenic infected | 0.00001 | **** |
| P3 | axenic mock-infected | start community infected | 0.21413 | |
| P3 | axenic infected | start community infected | 1 | |
| P4 | axenic mock-infected | axenic infected | 0.00001 | **** |
| P4 | axenic mock-infected | start community infected | 1 | |
| P4 | axenic infected | start community infected | 1 | |
| P5 | axenic mock-infected | axenic infected | 0.49667 | |
| P5 | axenic mock-infected | start community infected | 0.00083 | *** |
| P5 | axenic infected | start community infected | 1 | |
| axenic mock-infected | P0 | P1 | 1 | |
| axenic mock-infected | P0 | P2 | 1 | |
| axenic mock-infected | P0 | P3 | 1 | |
| axenic mock-infected | P0 | P4 | 1 | |
| axenic mock-infected | P0 | P5 | 1 | |
| axenic mock-infected | P1 | P2 | 1 | |
| axenic mock-infected | P1 | P3 | 1 | |
| axenic mock-infected | P1 | P4 | 1 | |
| axenic mock-infected | P1 | P5 | 1 | |
| axenic mock-infected | P2 | P3 | 1 | |
| axenic mock-infected | P2 | P4 | 1 | |
| axenic mock-infected | P2 | P5 | 1 | |
| axenic mock-infected | P3 | P4 | 1 | |
| axenic mock-infected | P3 | P5 | 1 | |
| axenic mock-infected | P4 | P5 | 1 | |
| axenic infected | P0 | P1 | 0.49692 | |
| axenic infected | P0 | P2 | 1 | |
| axenic infected | P0 | P3 | 1 | |
| axenic infected | P0 | P4 | 1 | |
| axenic infected | P0 | P5 | 1 | |
| axenic infected | P1 | P2 | 1 | |
| axenic infected | P1 | P3 | 1 | |
| axenic infected | P1 | P4 | 1 | |
| axenic infected | P1 | P5 | 1 | |
| axenic infected | P2 | P3 | 1 | |
| axenic infected | P2 | P4 | 1 | |
| axenic infected | P2 | P5 | 1 | |
| axenic infected | P3 | P4 | 1 | |
| axenic infected | P3 | P5 | 1 | |
| axenic infected | P4 | P5 | 1 | |
| start community infected | P0 | P1 | 1 | |
| start community infected | P0 | P2 | 1 | |
| start community infected | P0 | P3 | 1 | |
| start community infected | P0 | P4 | 1 | |
| start community infected | P0 | P5 | 0.04894 | * |
| start community infected | P1 | P2 | 1 | |
| start community infected | P1 | P3 | 1 | |
| start community infected | P1 | P4 | 1 | |
| start community infected | P1 | P5 | 1 | |
| start community infected | P2 | P3 | 1 | |
| start community infected | P2 | P4 | 1 | |
| start community infected | P2 | P5 | 1 | |
| start community infected | P3 | P4 | 1 | |
| start community infected | P3 | P5 | 1 | |
| start community infected | P4 | P5 | 1 | |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 6:** Bonferroni-corrected p-values of pairwise Welch's t-tests of disease severity scores at 7 dpi of control conditions in first passaging experiment. Note that data of passage 4 is missing.

| Comment | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|
| P0 | axenic mock-infected | axenic infected | 0 | **** |
| P0 | axenic mock-infected | start community, non-infected | 1 | |
| P0 | axenic mock-infected | start community, infected | 1 | |
| P0 | axenic infected | start community, infected | 0 | **** |
| P1 | axenic mock-infected | axenic infected | 0 | **** |
| P1 | axenic mock-infected | start community, non-infected | 1 | |
| P1 | axenic mock-infected | start community, infected | 1 | |
| P1 | axenic infected | start community, infected | 0.0518 | |
| P2 | axenic mock-infected | axenic infected | 0.00072 | *** |
| P2 | axenic mock-infected | start community, non-infected | 1 | |
| P2 | axenic mock-infected | start community, infected | 1 | |
| P2 | axenic infected | start community, infected | 0.11033 | |
| P3 | axenic mock-infected | axenic infected | 0 | **** |
| P3 | axenic mock-infected | start community, non-infected | 1 | |
| P3 | axenic mock-infected | start community, infected | 1 | |
| P3 | axenic infected | start community, infected | 1 | |
| P5 | axenic mock-infected | axenic infected | 0 | **** |
| P5 | axenic mock-infected | start community, non-infected | 1 | |
| P5 | axenic mock-infected | start community, infected | 1 | |
| P5 | axenic infected | start community, infected | 0.00429 | ** |
| axenic mock-infected | P0 | P1 | 1 | |
| axenic mock-infected | P0 | P2 | 1 | |
| axenic mock-infected | P0 | P3 | 1 | |
| axenic mock-infected | P0 | P5 | 1 | |
| axenic mock-infected | P1 | P2 | 1 | |
| axenic mock-infected | P1 | P3 | 1 | |
| axenic mock-infected | P1 | P5 | 1 | |
| axenic mock-infected | P2 | P3 | 1 | |
| axenic mock-infected | P2 | P5 | 1 | |
| axenic mock-infected | P3 | P5 | 1 | |
| axenic infected | P0 | P1 | 1 | |
| axenic infected | P0 | P2 | 1 | |
| axenic infected | P0 | P3 | 1 | |
| axenic infected | P0 | P5 | 1 | |
| axenic infected | P1 | P2 | 1 | |
| axenic infected | P1 | P3 | 1 | |
| axenic infected | P1 | P5 | 1 | |
| axenic infected | P2 | P3 | 1 | |
| axenic infected | P2 | P5 | 1 | |
| axenic infected | P3 | P5 | 1 | |
| start community, infected | P0 | P1 | 1 | |
| start community, infected | P0 | P2 | 1 | |
| start community, infected | P0 | P3 | 1 | |
| start community, infected | P0 | P5 | 1 | |
| start community, infected | P1 | P2 | 1 | |
| start community, infected | P1 | P3 | 1 | |
| start community, infected | P1 | P5 | 1 | |
| start community, infected | P2 | P3 | 1 | |
| start community, infected | P2 | P5 | 1 | |
| start community, infected | P3 | P5 | 1 | |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 7:** Bonferroni-corrected p-values of pairwise Welch's t-tests of disease severity scores at14 dpi of control conditions in first passaging experiment.

| Comment | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|
| P0 | axenic mock-infected | axenic infected | 0 | **** |
| P0 | axenic mock-infected | start community, non-infected | 1 | |
| P0 | axenic mock-infected | start community, infected | 1 | |
| P0 | axenic infected | start community, infected | 0 | **** |
| P1 | axenic mock-infected | axenic infected | 0 | **** |
| P1 | axenic mock-infected | start community, non-infected | 1 | |
| P1 | axenic mock-infected | start community, infected | 1 | |
| P1 | axenic infected | start community, infected | 0 | **** |
| P2 | axenic mock-infected | axenic infected | 0 | **** |
| P2 | axenic mock-infected | start community, non-infected | 1 | |
| P2 | axenic mock-infected | start community, infected | 1 | |
| P2 | axenic infected | start community, infected | 0 | **** |
| P3 | axenic mock-infected | axenic infected | 0 | **** |
| P3 | axenic mock-infected | start community, non-infected | 1 | |
| P3 | axenic mock-infected | start community, infected | 1 | |
| P3 | axenic infected | start community, infected | 0.00001 | **** |
| P4 | axenic mock-infected | axenic infected | 0 | **** |
| P4 | axenic mock-infected | start community, non-infected | 1 | |
| P4 | axenic mock-infected | start community, infected | 1 | |
| P4 | axenic infected | start community, infected | 0 | **** |
| P5 | axenic mock-infected | axenic infected | 0 | **** |
| P5 | axenic mock-infected | start community, non-infected | 1 | |
| P5 | axenic mock-infected | start community, infected | 1 | |
| P5 | axenic infected | start community, infected | 0 | **** |
| axenic mock-infected | P0 | P1 | 1 | |
| axenic mock-infected | P0 | P2 | 1 | |
| axenic mock-infected | P0 | P3 | 1 | |
| axenic mock-infected | P0 | P4 | 1 | |
| axenic mock-infected | P0 | P5 | 1 | |
| axenic mock-infected | P1 | P2 | 1 | |
| axenic mock-infected | P1 | P3 | 1 | |
| axenic mock-infected | P1 | P4 | 1 | |
| axenic mock-infected | P1 | P5 | 1 | |
| axenic mock-infected | P2 | P3 | 1 | |
| axenic mock-infected | P2 | P4 | 1 | |
| axenic mock-infected | P2 | P5 | 1 | |
| axenic mock-infected | P3 | P4 | 1 | |
| axenic mock-infected | P3 | P5 | 1 | |
| axenic mock-infected | P4 | P5 | 1 | |
| axenic infected | P0 | P1 | 1 | |
| axenic infected | P0 | P2 | 1 | |
| axenic infected | P0 | P3 | 1 | |
| axenic infected | P0 | P4 | 1 | |
| axenic infected | P0 | P5 | 1 | |
| axenic infected | P1 | P2 | 1 | |
| axenic infected | P1 | P3 | 1 | |
| axenic infected | P1 | P4 | 1 | |
| axenic infected | P1 | P5 | 1 | |
| axenic infected | P2 | P3 | 1 | |
| axenic infected | P2 | P4 | 1 | |
| axenic infected | P2 | P5 | 1 | |
| axenic infected | P3 | P4 | 1 | |
| axenic infected | P3 | P5 | 1 | |
| axenic infected | P4 | P5 | 1 | |
| start community, infected | P0 | P1 | 1 | |
| start community, infected | P0 | P2 | 1 | |
| start community, infected | P0 | P3 | 1 | |
| start community, infected | P0 | P4 | 1 | |
| start community, infected | P0 | P5 | 1 | |
| start community, infected | P1 | P2 | 1 | |
| start community, infected | P1 | P3 | 1 | |
| start community, infected | P1 | P4 | 1 | |
| start community, infected | P1 | P5 | 1 | |
| start community, infected | P2 | P3 | 1 | |
| start community, infected | P2 | P4 | 1 | |
| start community, infected | P2 | P5 | 1 | |
| start community, infected | P3 | P4 | 1 | |
| start community, infected | P3 | P5 | 1 | |
| start community, infected | P4 | P5 | 1 | |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 8:** Bonferroni-corrected p-values of pairwise Welch's t-tests of community diversity scores of start community control conditions in first passaging experiment.

| Diversity score | Passage Group 1 | Passage Group 2 | p-value | significance [1] |
|---|---|---|---|---|
| Shannon Diversity | P0 | P1 | 1 | |
| Shannon Diversity | P0 | P2 | 1 | |
| Shannon Diversity | P0 | P3 | 0.3747 | |
| Shannon Diversity | P0 | P4 | 1 | |
| Shannon Diversity | P0 | P5 | 1 | |
| Shannon Diversity | P1 | P2 | 1 | |
| Shannon Diversity | P1 | P3 | 0.04952 | * |
| Shannon Diversity | P1 | P4 | 1 | |
| Shannon Diversity | P1 | P5 | 1 | |
| Shannon Diversity | P2 | P3 | 0.67051 | |
| Shannon Diversity | P2 | P4 | 1 | |
| Shannon Diversity | P2 | P5 | 1 | |
| Shannon Diversity | P3 | P4 | 1 | |
| Shannon Diversity | P3 | P5 | 0.06619 | |
| Shannon Diversity | P4 | P5 | 1 | |
| Pielou's Evenness | P0 | P1 | 1 | |
| Pielou's Evenness | P0 | P2 | 1 | |
| Pielou's Evenness | P0 | P3 | 0.06886 | |
| Pielou's Evenness | P0 | P4 | 1 | |
| Pielou's Evenness | P0 | P5 | 1 | |
| Pielou's Evenness | P1 | P2 | 1 | |
| Pielou's Evenness | P1 | P3 | 0.09806 | |
| Pielou's Evenness | P1 | P4 | 1 | |
| Pielou's Evenness | P1 | P5 | 1 | |
| Pielou's Evenness | P2 | P3 | 0.90334 | |
| Pielou's Evenness | P2 | P4 | 1 | |
| Pielou's Evenness | P2 | P5 | 1 | |
| Pielou's Evenness | P3 | P4 | 1 | |
| Pielou's Evenness | P3 | P5 | 0.17763 | |
| Pielou's Evenness | P4 | P5 | 1 | |
| Richness | P0 | P1 | 0.49891 | |
| Richness | P0 | P2 | 0.79282 | |
| Richness | P0 | P3 | 1 | |
| Richness | P0 | P4 | 1 | |
| Richness | P0 | P5 | 0.205 | |
| Richness | P1 | P2 | 1 | |
| Richness | P1 | P3 | 1 | |
| Richness | P1 | P4 | 1 | |
| Richness | P1 | P5 | 1 | |
| Richness | P2 | P3 | 1 | |
| Richness | P2 | P4 | 1 | |
| Richness | P2 | P5 | 1 | |
| Richness | P3 | P4 | 1 | |
| Richness | P3 | P5 | 1 | |
| Richness | P4 | P5 | 1 | |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 9:** Bonferroni-corrected p-values of pairwise Welch's t-tests of bacterial colonization of non-selective passaged communities in first passaging experiment. Note that the commensal colonization data of passage 5 is missing.

| colonization by | Comment | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|---|
| Commensals | P0 | start community | unchallenged | 1 | |
| Commensals | P1 | start community | unchallenged | 1 | |
| Commensals | P2 | start community | unchallenged | 1 | |
| Commensals | P3 | start community | unchallenged | 1 | |
| Commensals | P4 | start community | unchallenged | 1 | |
| Commensals | unchallenged | P0 | P1 | 1 | |
| Commensals | unchallenged | P1 | P2 | 1 | |
| Commensals | unchallenged | P2 | P3 | 1 | |
| Commensals | unchallenged | P3 | P4 | 1 | |
| Commensals | P0 | start community | non-selective | 1 | |
| Commensals | P0 | unchallenged | non-selective | 1 | |
| Commensals | P1 | start community | non-selective | 1 | |
| Commensals | P1 | unchallenged | non-selective | 1 | |
| Commensals | P2 | start community | non-selective | 1 | |
| Commensals | P2 | unchallenged | non-selective | 1 | |
| Commensals | P3 | start community | non-selective | 1 | |
| Commensals | P3 | unchallenged | non-selective | 1 | |
| Commensals | P4 | start community | non-selective | 1 | |
| Commensals | P4 | unchallenged | non-selective | 1 | |
| Commensals | non-selective | P0 | P1 | 1 | |
| Commensals | non-selective | P1 | P2 | 1 | |
| Commensals | non-selective | P2 | P3 | 1 | |
| Commensals | non-selective | P3 | P4 | 1 | |
| *Ps* t | P0 | axenic | non-selective | 0 | **** |
| *Ps* t | P0 | start community | non-selective | 1 | |
| *Ps* t | P1 | axenic | non-selective | 0 | **** |
| *Ps* t | P1 | start community | non-selective | 0.50401 | |
| *Ps* t | P2 | axenic | non-selective | 0.02005 | * |
| *Ps* t | P2 | start community | non-selective | 0 | **** |
| *Ps* t | P3 | axenic | non-selective | 0 | **** |
| *Ps* t | P3 | start community | non-selective | 0.0325 | * |
| *Ps* t | P4 | axenic | non-selective | 0.00017 | *** |
| *Ps* t | P4 | start community | non-selective | 0.00061 | *** |
| *Ps* t | P5 | axenic | non-selective | 0.00018 | *** |
| *Ps* t | P5 | start community | non-selective | 0.20859 | |
| *Ps* t abundance | P5 | start community | non-selective | 1 | |
| *Ps* t | non-selective | P0 | P1 | 0.03646 | * |
| *Ps* t | non-selective | P1 | P2 | 1 | |
| *Ps* t | non-selective | P2 | P3 | 1 | |
| *Ps* t | non-selective | P3 | P4 | 1 | |
| *Ps* t | non-selective | P4 | P5 | 1 | |
| *Ps* t | non-selective | P0 | P5 | 0.09792 | |
| *Ps* t | non-selective | P1 | P5 | 1 | |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 10:** Bonferroni-corrected p-values of pairwise Welch's t-tests of community diversity scores of unchallenged passaged communities in first passaging experiment.

| Score | Comment | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|---|
| Shannon's Diversity | P0 | start community | unchallenged | 1 | |
| Shannon's Diversity | P1 | start community | unchallenged | 0 | **** |
| Shannon's Diversity | P2 | start community | unchallenged | 0 | **** |
| Shannon's Diversity | P3 | start community | unchallenged | 0 | **** |
| Shannon's Diversity | P4 | start community | unchallenged | 0 | **** |
| Shannon's Diversity | P5 | start community | unchallenged | 0 | **** |
| Shannon's Diversity | unchallenged | P0 | P1 | 0 | **** |
| Shannon's Diversity | unchallenged | P1 | P2 | 1 | |
| Shannon's Diversity | unchallenged | P2 | P3 | 1 | |
| Shannon's Diversity | unchallenged | P3 | P4 | 1 | |
| Shannon's Diversity | unchallenged | P4 | P5 | 0.26934 | |
| Pielou's Evenness | P0 | start community | unchallenged | 1 | |
| Pielou's Evenness | P1 | start community | unchallenged | 1 | |
| Pielou's Evenness | P2 | start community | unchallenged | 1 | |
| Pielou's Evenness | P3 | start community | unchallenged | 1 | |
| Pielou's Evenness | P4 | start community | unchallenged | 1 | |
| Pielou's Evenness | P5 | start community | unchallenged | 1 | |
| Pielou's Evenness | unchallenged | P0 | P1 | 1 | |
| Pielou's Evenness | unchallenged | P1 | P2 | 1 | |
| Pielou's Evenness | unchallenged | P2 | P3 | 1 | |
| Pielou's Evenness | unchallenged | P3 | P4 | 1 | |
| Pielou's Evenness | unchallenged | P4 | P5 | 0.00395 | ** |
| Richness | P0 | start community | unchallenged | 1 | |
| Richness | P1 | start community | unchallenged | 0 | **** |
| Richness | P2 | start community | unchallenged | 0 | **** |
| Richness | P3 | start community | unchallenged | 0 | **** |
| Richness | P4 | start community | unchallenged | 0 | **** |
| Richness | P5 | start community | unchallenged | 0 | **** |
| Richness | unchallenged | P0 | P1 | 0 | **** |
| Richness | unchallenged | P1 | P2 | 0.80748 | |
| Richness | unchallenged | P2 | P3 | 1 | |
| Richness | unchallenged | P3 | P4 | 1 | |
| Richness | unchallenged | P4 | P5 | 1 | |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 11:** Table to summarize relative abundance changes of ASVs over passages in unchallenged passaging.

| Treatment | Group 1 | Group 2 | ASV representative StrainID | fold change (factor) | p-value | significance [1] | Not Detected in Group 2 |
|---|---|---|---|---|---|---|---|
| P0 | start community | unchallenged | Leaf58 | 0.09 | 0.00187 | ** | 2 of 12 |
| P0 | start community | unchallenged | Leaf233 | 0.471 | 0.00586 | ** | |
| P0 | start community | unchallenged | Leaf21 | 3.26 | 0.00187 | ** | |
| P0 | start community | unchallenged | Leaf220 | 0.0446 | 0.00586 | ** | 9 of 12 |
| P0 | start community | unchallenged | Leaf262 | 0.269 | 0.000747 | *** | |
| unchallenged | P0 | P1 | Leaf10 | 0.00372 | 0.0145 | * | 6 of 6 |
| unchallenged | P0 | P1 | Leaf106 | 0.0136 | 0.000000125 | **** | 4 of 6 |
| unchallenged | P0 | P1 | Leaf108 | 0.00511 | 0.000000125 | **** | 4 of 6 |
| unchallenged | P0 | P1 | Leaf127 | 0.013 | 0.0187 | * | 6 of 6 |
| unchallenged | P0 | P1 | Leaf131 | 0.0216 | 0.00000951 | **** | 2 of 6 |
| unchallenged | P0 | P1 | Leaf137 | 0.00522 | 0.000000154 | **** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf151 | 0.000602 | 2.14E-16 | **** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf154 | 0.0438 | 0.00416 | ** | 4 of 6 |
| unchallenged | P0 | P1 | Leaf159 | 0.0324 | 0.0000187 | **** | 4 of 6 |
| unchallenged | P0 | P1 | Leaf16 | 0.00172 | 6.67E-15 | **** | 5 of 6 |
| unchallenged | P0 | P1 | Leaf160 | 0.00334 | 0.000131 | *** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf168 | 0.00247 | 4.78E-10 | **** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf171 | 0.00183 | 4.73E-14 | **** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf177 | 2.31 | 0.0185 | * | |
| unchallenged | P0 | P1 | Leaf186 | 0.0144 | 0.00416 | ** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf191 | 0.0695 | 0.0443 | * | 4 of 6 |
| unchallenged | P0 | P1 | Leaf198 | 0.00776 | 0.00000685 | **** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf201 | 0.0000955 | 1.16E-34 | **** | 5 of 6 |
| unchallenged | P0 | P1 | Leaf208 | 0.0185 | 0.018 | * | 5 of 6 |
| unchallenged | P0 | P1 | Leaf210 | 0.00905 | 7.78E-09 | **** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf233 | 0.0108 | 5.19E-12 | **** | 1 of 6 |
| unchallenged | P0 | P1 | Leaf247 | 0.00116 | 0.0000663 | **** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf257 | 0.0121 | 0.000000245 | **** | 5 of 6 |
| unchallenged | P0 | P1 | Leaf26 | 0.00179 | 1.57E-14 | **** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf264 | 0.0504 | 0.0438 | * | 6 of 6 |
| unchallenged | P0 | P1 | Leaf265 | 0.0149 | 0.000412 | *** | 5 of 6 |
| unchallenged | P0 | P1 | Leaf272 | 0.000822 | 1.68E-16 | **** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf285 | 0.005 | 1.88E-09 | **** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf288 | 0.0121 | 0.00272 | ** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf289 | 0.0029 | 1.85E-10 | **** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf299 | 0.00766 | 0.000000881 | **** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf306 | 0.00582 | 0.000000245 | **** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf324 | 0.00187 | 2.67E-12 | **** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf363 | 0.0204 | 0.000929 | *** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf371 | 0.000788 | 8.41E-22 | **** | 5 of 6 |
| unchallenged | P0 | P1 | Leaf386 | 0.0231 | 0.00000685 | **** | 2 of 6 |
| unchallenged | P0 | P1 | Leaf396 | 0.0316 | 0.0067 | ** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf404 | 0.000975 | 8.64E-13 | **** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf405 | 0.000366 | 1.57E-19 | **** | 4 of 6 |
| unchallenged | P0 | P1 | Leaf414 | 0.000155 | 1.27E-58 | **** | 3 of 6 |
| unchallenged | P0 | P1 | Leaf420 | 0.000131 | 5.75E-35 | **** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf427 | 0.00854 | 5.12E-08 | **** | 5 of 6 |
| unchallenged | P0 | P1 | Leaf443 | 0.00933 | 5.82E-08 | **** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf454 | 0.0382 | 0.00754 | ** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf456 | 0.00655 | 0.00000431 | **** | 5 of 6 |
| unchallenged | P0 | P1 | Leaf48 | 0.00408 | 6.81E-16 | **** | 4 of 6 |
| unchallenged | P0 | P1 | Leaf51 | 0.0264 | 0.000079 | **** | 4 of 6 |
| unchallenged | P0 | P1 | Leaf53 | 0.0462 | 0.00754 | ** | 4 of 6 |
| unchallenged | P0 | P1 | Leaf58 | 0.0113 | 0.00133 | ** | 6 of 6 |
| unchallenged | P0 | P1 | Leaf64 | 0.000814 | 3.41E-10 | **** | 5 of 6 |
| unchallenged | P0 | P1 | Leaf78 | 0.0323 | 0.0078 | ** | 4 of 6 |
| unchallenged | P0 | P1 | Leaf82 | 0.0508 | 0.00352 | ** | 2 of 6 |
| unchallenged | P0 | P1 | Leaf85 | 0.324 | 0.0161 | * | 6 of 6 |
| unchallenged | P0 | P1 | Leaf91 | 0.0143 | 2.68E-08 | **** | 2 of 6 |
| unchallenged | P1 | P5 | Leaf122 | 0.00285 | 0.0209 | * | 6 of 6 |
| unchallenged | P1 | P5 | Leaf145 | 0.0186 | 0.00218 | ** | |
| unchallenged | P1 | P5 | Leaf231 | 0.00425 | 0.0000595 | **** | 3 of 6 |
| unchallenged | P1 | P5 | Leaf88 | 0.00612 | 0.0468 | * | 5 of 6 |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 12:** Table to summarize relative abundance changes of ASVs between passages of non-selective passaging.

| Group 1 | Group 2 | ASV representative StrainID | fold change | p-value | significance [1] | Not detected in Group 2 |
|---|---|---|---|---|---|---|
| P0 | P1 | Leaf108 | 0.0024 | 2.02E-10 | **** | 6 |
| P0 | P1 | Leaf11 | 0.0251 | 0.00316 | ** | 5 |
| P0 | P1 | Leaf127 | 0.0115 | 0.0036 | ** | 6 |
| P0 | P1 | Leaf137 | 0.0222 | 0.00115 | ** | 6 |
| P0 | P1 | Leaf141 | 0.483 | 0.0229 | * | 6 |
| P0 | P1 | Leaf151 | 0.0145 | 0.000126 | *** | 4 |
| P0 | P1 | Leaf154 | 0.035 | 0.0019 | ** | 5 |
| P0 | P1 | Leaf16 | 0.000278 | 1.28E-17 | **** | 6 |
| P0 | P1 | Leaf168 | 0.00137 | 9.62E-13 | **** | 5 |
| P0 | P1 | Leaf171 | 0.00349 | 4.02E-09 | **** | 6 |
| P0 | P1 | Leaf198 | 0.0208 | 0.00302 | ** | 6 |
| P0 | P1 | Leaf2 | 0.0245 | 0.00739 | ** | 6 |
| P0 | P1 | Leaf201 | 0.0279 | 0.0071 | ** | 3 |
| P0 | P1 | Leaf216 | 0.0502 | 0.0303 | * | 6 |
| P0 | P1 | Leaf233 | 0.0295 | 0.0000204 | **** | 1 |
| P0 | P1 | Leaf24 | 0.0138 | 0.0174 | * | 6 |
| P0 | P1 | Leaf247 | 0.0166 | 0.000176 | *** | 6 |
| P0 | P1 | Leaf257 | 0.00771 | 0.00000108 | **** | 6 |
| P0 | P1 | Leaf264 | 0.0418 | 0.0117 | * | 6 |
| P0 | P1 | Leaf272 | 0.000729 | 4.56E-17 | **** | 5 |
| P0 | P1 | Leaf285 | 0.00473 | 3.83E-07 | **** | 6 |
| P0 | P1 | Leaf288 | 0.0247 | 0.00302 | ** | 6 |
| P0 | P1 | Leaf289 | 0.00523 | 0.00000415 | **** | 6 |
| P0 | P1 | Leaf299 | 0.00874 | 0.00008 | **** | 6 |
| P0 | P1 | Leaf306 | 0.00498 | 9.83E-08 | **** | 6 |
| P0 | P1 | Leaf324 | 0.00851 | 0.00000516 | **** | 5 |
| P0 | P1 | Leaf344 | 0.00317 | 0.0000989 | **** | 6 |
| P0 | P1 | Leaf361 | 0.0105 | 9.17E-08 | **** | 5 |
| P0 | P1 | Leaf371 | 0.00125 | 1.51E-12 | **** | 6 |
| P0 | P1 | Leaf386 | 0.0438 | 0.0386 | * | 6 |
| P0 | P1 | Leaf414 | 0.000199 | 1.09E-40 | **** | 2 |
| P0 | P1 | Leaf420 | 0.00785 | 0.00008 | **** | 4 |
| P0 | P1 | Leaf427 | 0.00964 | 6.48E-07 | **** | 6 |
| P0 | P1 | Leaf443 | 0.00897 | 0.0019 | ** | 6 |
| P0 | P1 | Leaf51 | 0.0381 | 0.00302 | ** | 5 |
| P0 | P1 | Leaf53 | 0.0024 | 8.69E-12 | **** | 6 |
| P0 | P1 | Leaf64 | 0.00206 | 2.08E-08 | **** | 6 |
| P0 | P1 | Leaf85 | 0.0538 | 0.00385 | ** | 5 |
| P0 | P1 | Leaf86 | 0.016 | 0.000129 | *** | 5 |
| P1 | P5 | Leaf280 | 0.00172 | 0.00326 | ** | 4 |
| P1 | P5 | Leaf78 | 0.00269 | 0.0437 | * | 6 |
| P1 | P5 | Leaf82 | 0.000319 | 0.00183 | ** | 4 |
| P1 | P5 | Leaf91 | 0.000000209 | 6.07E-12 | **** | 6 |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 13:** Bonferroni-corrected p-values of pairwise Welch's t-tests of community diversity scores of non-selective passaged communities in first passaging experiment.

| Score | Comment | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|---|
| Shannon's Diversity | P0 | start community | non-selective | 1 | |
| Shannon's Diversity | P0 | unchallenged | non-selective | 1 | |
| Shannon's Diversity | P1 | start community | non-selective | 0 | **** |
| Shannon's Diversity | P1 | unchallenged | non-selective | 1 | |
| Shannon's Diversity | P2 | start community | non-selective | 0 | **** |
| Shannon's Diversity | P2 | unchallenged | non-selective | 1 | |
| Shannon's Diversity | P3 | start community | non-selective | 0 | **** |
| Shannon's Diversity | P3 | unchallenged | non-selective | 1 | |
| Shannon's Diversity | P4 | start community | non-selective | 0 | **** |
| Shannon's Diversity | P4 | unchallenged | non-selective | 1 | |
| Shannon's Diversity | P5 | start community | non-selective | 0 | **** |
| Shannon's Diversity | P5 | unchallenged | non-selective | 1 | |
| Shannon's Diversity | non-selective | P0 | P1 | 0 | **** |
| Shannon's Diversity | non-selective | P1 | P2 | 1 | |
| Shannon's Diversity | non-selective | P2 | P3 | 1 | |
| Shannon's Diversity | non-selective | P3 | P4 | 1 | |
| Shannon's Diversity | non-selective | P4 | P5 | 1 | |
| Shannon's Diversity | non-selective | P1 | P5 | 1 | |
| Pielou's Evenness | P0 | start community | non-selective | 1 | |
| Pielou's Evenness | P0 | unchallenged | non-selective | 1 | |
| Pielou's Evenness | P1 | start community | non-selective | 1 | |
| Pielou's Evenness | P1 | unchallenged | non-selective | 1 | |
| Pielou's Evenness | P2 | start community | non-selective | 1 | |
| Pielou's Evenness | P2 | unchallenged | non-selective | 1 | |
| Pielou's Evenness | P3 | start community | non-selective | 1 | |
| Pielou's Evenness | P3 | unchallenged | non-selective | 1 | |
| Pielou's Evenness | P4 | start community | non-selective | 1 | |
| Pielou's Evenness | P4 | unchallenged | non-selective | 1 | |
| Pielou's Evenness | P5 | start community | non-selective | 1 | |
| Pielou's Evenness | P5 | unchallenged | non-selective | 1 | |
| Pielou's Evenness | non-selective | P0 | P1 | 1 | |
| Pielou's Evenness | non-selective | P1 | P2 | 1 | |
| Pielou's Evenness | non-selective | P2 | P3 | 1 | |
| Pielou's Evenness | non-selective | P3 | P4 | 1 | |
| Pielou's Evenness | non-selective | P4 | P5 | 1 | |
| Pielou's Evenness | non-selective | P1 | P5 | 1 | |
| Richness | P0 | start community | non-selective | 1 | |
| Richness | P0 | unchallenged | non-selective | 1 | |
| Richness | P1 | start community | non-selective | 0 | **** |
| Richness | P1 | unchallenged | non-selective | 1 | |
| Richness | P2 | start community | non-selective | 0 | **** |
| Richness | P2 | unchallenged | non-selective | 1 | |
| Richness | P3 | start community | non-selective | 0 | **** |
| Richness | P3 | unchallenged | non-selective | 1 | |
| Richness | P4 | start community | non-selective | 0 | **** |
| Richness | P4 | unchallenged | non-selective | 1 | |
| Richness | P5 | start community | non-selective | 0 | **** |
| Richness | P5 | unchallenged | non-selective | 1 | |
| Richness | non-selective | P0 | P1 | 0 | **** |
| Richness | non-selective | P1 | P2 | 1 | |
| Richness | non-selective | P2 | P3 | 1 | |
| Richness | non-selective | P3 | P4 | 1 | |
| Richness | non-selective | P4 | P5 | 1 | |
| Richness | non-selective | P1 | P5 | 0.15768 | |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 14:** Table to summarize relative abundance changes of ASVs comparing unchallenged and non-selective passaging to investigate influence of pathogen infection on microbiota.

| Passage | Group 1 | Group 2 | ASV Representative | fold change | p-value | significance [1] | Comment |
|---|---|---|---|---|---|---|---|
| P2 | unchallenged | non-selective | Leaf131 | 73.2 | 0.0203 | * | |
| P2 | unchallenged | non-selective | Leaf106 | 9880000 | 1.14E-13 | **** | not detected in without pathogen lines |
| P2 | unchallenged | non-selective | Leaf280 | 7680000 | 2.23E-16 | **** | not detected in without pathogen lines |
| P2 | unchallenged | non-selective | Leaf262 | 597 | 0.00536 | ** | not detected in 5/6 without pathogen lines |
| P2 | unchallenged | non-selective | Leaf361 | 1.17E-08 | 5.47E-17 | **** | not detected in with pathogen lines |
| P3 | unchallenged | non-selective | Leaf131 | 403 | 0.00132 | ** | |
| P3 | unchallenged | non-selective | Leaf21 | 16300000 | 6.94E-14 | **** | not detected in without pathogen lines |
| P3 | unchallenged | non-selective | Leaf280 | 195 | 0.0159 | * | |
| P3 | unchallenged | non-selective | Leaf262 | 1400 | 0.00576 | ** | not detected in 5/6 without pathogen lines |
| P3 | unchallenged | non-selective | Leaf361 | 0.00142 | 0.00228 | ** | |
| P4 | unchallenged | non-selective | Leaf131 | 403 | 0.00132 | ** | |
| P4 | unchallenged | non-selective | Leaf21 | 16300000 | 6.94E-14 | **** | not detected in without pathogen lines |
| P4 | unchallenged | non-selective | Leaf280 | 195 | 0.0159 | * | |
| P4 | unchallenged | non-selective | Leaf262 | 1400 | 0.00576 | ** | not detected in 5/6 without pathogen lines |
| P4 | unchallenged | non-selective | Leaf361 | 0.00142 | 0.00228 | ** | |
| P5 | unchallenged | non-selective | Leaf131 | 194 | 0.00794 | ** | not-detected in most samples of both groups |
| combined P1 to P5 | unchallenged | non-selective | Leaf86 | 0.00496 | 0.0000643 | **** | |
| combined P1 to P5 | unchallenged | non-selective | Leaf131 | 126 | 3.68E-11 | **** | |
| combined P1 to P5 | unchallenged | non-selective | Leaf106 | 298 | 0.0000165 | **** | |
| combined P1 to P5 | unchallenged | non-selective | Leaf78 | 104 | 0.00145 | ** | |
| combined P1 to P5 | unchallenged | non-selective | Leaf311 | 14.4 | 0.00371 | ** | |
| combined P1 to P5 | unchallenged | non-selective | Leaf361 | 0.00338 | 0.000994 | *** | |
| combined P1 to P5 | unchallenged | non-selective | Leaf82 | 0.00132 | 4.95E-12 | **** | |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 15:** Bonferroni-corrected p-values of pairwise Welch's t-tests of pathogen luminescence of non-selective passaged communities in first passaging experiment.

| Comment | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|
| non-selective line 1 | P0 | P1 | 1 | |
| non-selective line 1 | P1 | P2 | 1 | |
| non-selective line 1 | P2 | P3 | 1 | |
| non-selective line 1 | P3 | P4 | 1 | |
| non-selective line 1 | P4 | P5 | 1 | |
| non-selective line 1 | P1 | P5 | 1 | |
| non-selective line 2 | P0 | P1 | 1 | |
| non-selective line 2 | P1 | P2 | 1 | |
| non-selective line 2 | P2 | P3 | 1 | |
| non-selective line 2 | P3 | P4 | 1 | |
| non-selective line 2 | P4 | P5 | 1 | |
| non-selective line 2 | P1 | P5 | 1 | |
| non-selective line 3 | P0 | P1 | 1 | |
| non-selective line 3 | P1 | P2 | 1 | |
| non-selective line 3 | P2 | P3 | 1 | |
| non-selective line 3 | P3 | P4 | 1 | |
| non-selective line 3 | P4 | P5 | 1 | |
| non-selective line 3 | P1 | P5 | 1 | |
| non-selective line 4 | P0 | P1 | 1 | |
| non-selective line 4 | P1 | P2 | 1 | |
| non-selective line 4 | P2 | P3 | 1 | |
| non-selective line 4 | P3 | P4 | 1 | |
| non-selective line 4 | P4 | P5 | 1 | |
| non-selective line 4 | P1 | P5 | 1 | |
| non-selective line 5 | P0 | P1 | 1 | |
| non-selective line 5 | P1 | P2 | 1 | |
| non-selective line 5 | P2 | P3 | 1 | |
| non-selective line 5 | P3 | P4 | 1 | |
| non-selective line 5 | P4 | P5 | 1 | |
| non-selective line 5 | P1 | P5 | 1 | |
| non-selective line 6 | P0 | P1 | 1 | |
| non-selective line 6 | P1 | P2 | 1 | |
| non-selective line 6 | P2 | P3 | 1 | |
| non-selective line 6 | P3 | P4 | 1 | |
| non-selective line 6 | P4 | P5 | 1 | |
| non-selective line 6 | P1 | P5 | 1 | |
| P0 | non-selective line 1 | non-selective line 2 | 1 | |
| P0 | non-selective line 1 | non-selective line 3 | 1 | |
| P0 | non-selective line 1 | non-selective line 4 | 1 | |
| P0 | non-selective line 1 | non-selective line 5 | 1 | |
| P0 | non-selective line 1 | non-selective line 6 | 1 | |
| P0 | non-selective line 2 | non-selective line 3 | 1 | |
| P0 | non-selective line 2 | non-selective line 4 | 1 | |
| P0 | non-selective line 2 | non-selective line 5 | 1 | |
| P0 | non-selective line 2 | non-selective line 6 | 1 | |
| P0 | non-selective line 3 | non-selective line 4 | 1 | |
| P0 | non-selective line 3 | non-selective line 5 | 1 | |
| P0 | non-selective line 3 | non-selective line 6 | 1 | |
| P0 | non-selective line 4 | non-selective line 5 | 1 | |
| P0 | non-selective line 4 | non-selective line 6 | 1 | |
| P0 | non-selective line 5 | non-selective line 6 | 1 | |
| P1 | non-selective line 1 | non-selective line 2 | 1 | |
| P1 | non-selective line 1 | non-selective line 3 | 1 | |
| P1 | non-selective line 1 | non-selective line 4 | 1 | |
| P1 | non-selective line 1 | non-selective line 5 | 1 | |
| P1 | non-selective line 1 | non-selective line 6 | 1 | |

Supplemental Table 15 continued

| | | | |
|---|---|---|---|
| P1 | non-selective line 2 | non-selective line 3 | 1 |
| P1 | non-selective line 2 | non-selective line 4 | 1 |
| P1 | non-selective line 2 | non-selective line 5 | 1 |
| P1 | non-selective line 2 | non-selective line 6 | 1 |
| P1 | non-selective line 3 | non-selective line 4 | 1 |
| P1 | non-selective line 3 | non-selective line 5 | 1 |
| P1 | non-selective line 3 | non-selective line 6 | 1 |
| P1 | non-selective line 4 | non-selective line 5 | 1 |
| P1 | non-selective line 4 | non-selective line 6 | 1 |
| P1 | non-selective line 5 | non-selective line 6 | 1 |
| P2 | non-selective line 1 | non-selective line 2 | 1 |
| P2 | non-selective line 1 | non-selective line 3 | 1 |
| P2 | non-selective line 1 | non-selective line 4 | 1 |
| P2 | non-selective line 1 | non-selective line 5 | 1 |
| P2 | non-selective line 1 | non-selective line 6 | 1 |
| P2 | non-selective line 2 | non-selective line 3 | 1 |
| P2 | non-selective line 2 | non-selective line 4 | 1 |
| P2 | non-selective line 2 | non-selective line 5 | 1 |
| P2 | non-selective line 2 | non-selective line 6 | 1 |
| P2 | non-selective line 3 | non-selective line 4 | 1 |
| P2 | non-selective line 3 | non-selective line 5 | 1 |
| P2 | non-selective line 3 | non-selective line 6 | 1 |
| P2 | non-selective line 4 | non-selective line 5 | 1 |
| P2 | non-selective line 4 | non-selective line 6 | 1 |
| P2 | non-selective line 5 | non-selective line 6 | 1 |
| P3 | non-selective line 1 | non-selective line 2 | 1 |
| P3 | non-selective line 1 | non-selective line 3 | 1 |
| P3 | non-selective line 1 | non-selective line 4 | 1 |
| P3 | non-selective line 1 | non-selective line 5 | 1 |
| P3 | non-selective line 1 | non-selective line 6 | 1 |
| P3 | non-selective line 2 | non-selective line 3 | 1 |
| P3 | non-selective line 2 | non-selective line 4 | 1 |
| P3 | non-selective line 2 | non-selective line 5 | 1 |
| P3 | non-selective line 2 | non-selective line 6 | 1 |
| P3 | non-selective line 3 | non-selective line 4 | 1 |
| P3 | non-selective line 3 | non-selective line 5 | 1 |
| P3 | non-selective line 3 | non-selective line 6 | 1 |
| P3 | non-selective line 4 | non-selective line 5 | 1 |
| P3 | non-selective line 4 | non-selective line 6 | 1 |
| P3 | non-selective line 5 | non-selective line 6 | 1 |
| P4 | non-selective line 1 | non-selective line 2 | 1 |
| P4 | non-selective line 1 | non-selective line 3 | 1 |
| P4 | non-selective line 1 | non-selective line 4 | 1 |
| P4 | non-selective line 1 | non-selective line 5 | 1 |
| P4 | non-selective line 1 | non-selective line 6 | 1 |
| P4 | non-selective line 2 | non-selective line 3 | 1 |
| P4 | non-selective line 2 | non-selective line 4 | 1 |
| P4 | non-selective line 2 | non-selective line 5 | 1 |
| P4 | non-selective line 2 | non-selective line 6 | 1 |
| P4 | non-selective line 3 | non-selective line 4 | 1 |
| P4 | non-selective line 3 | non-selective line 5 | 1 |
| P4 | non-selective line 3 | non-selective line 6 | 1 |
| P4 | non-selective line 4 | non-selective line 5 | 1 |
| P4 | non-selective line 4 | non-selective line 6 | 1 |
| P4 | non-selective line 5 | non-selective line 6 | 1 |
| P5 | non-selective line 1 | non-selective line 2 | 1 |
| P5 | non-selective line 1 | non-selective line 3 | 1 |
| P5 | non-selective line 1 | non-selective line 4 | 1 |

Supplemental Table 15 continued

| | | | |
|---|---|---|---|
| P5 | non-selective line 1 | non-selective line 5 | 1 |
| P5 | non-selective line 1 | non-selective line 6 | 1 |
| P5 | non-selective line 2 | non-selective line 3 | 1 |
| P5 | non-selective line 2 | non-selective line 4 | 1 |
| P5 | non-selective line 2 | non-selective line 5 | 1 |
| P5 | non-selective line 2 | non-selective line 6 | 1 |
| P5 | non-selective line 3 | non-selective line 4 | 1 |
| P5 | non-selective line 3 | non-selective line 5 | 1 |
| P5 | non-selective line 3 | non-selective line 6 | 1 |
| P5 | non-selective line 4 | non-selective line 5 | 1 |
| P5 | non-selective line 4 | non-selective line 6 | 1 |
| P5 | non-selective line 5 | non-selective line 6 | 1 |
| P0 | start community infected | non-selective line 1 | 1 |
| P0 | start community infected | non-selective line 2 | 1 |
| P0 | start community infected | non-selective line 3 | 1 |
| P0 | start community infected | non-selective line 4 | 1 |
| P0 | start community infected | non-selective line 5 | 1 |
| P0 | start community infected | non-selective line 6 | 1 |
| P1 | start community infected | non-selective line 1 | 1 |
| P1 | start community infected | non-selective line 2 | 1 |
| P1 | start community infected | non-selective line 3 | 1 |
| P1 | start community infected | non-selective line 4 | 1 |
| P1 | start community infected | non-selective line 5 | 1 |
| P1 | start community infected | non-selective line 6 | 1 |
| P2 | start community infected | non-selective line 1 | 1 |
| P2 | start community infected | non-selective line 2 | 1 |
| P2 | start community infected | non-selective line 3 | 1 |
| P2 | start community infected | non-selective line 4 | 1 |
| P2 | start community infected | non-selective line 5 | 1 |
| P2 | start community infected | non-selective line 6 | 1 |
| P3 | start community infected | non-selective line 1 | 1 |
| P3 | start community infected | non-selective line 2 | 1 |
| P3 | start community infected | non-selective line 3 | 1 |
| P3 | start community infected | non-selective line 4 | 1 |
| P3 | start community infected | non-selective line 5 | 1 |
| P3 | start community infected | non-selective line 6 | 1 |
| P4 | start community infected | non-selective line 1 | 1 |
| P4 | start community infected | non-selective line 2 | 1 |
| P4 | start community infected | non-selective line 3 | 1 |
| P4 | start community infected | non-selective line 4 | 1 |
| P4 | start community infected | non-selective line 5 | 1 |
| P4 | start community infected | non-selective line 6 | 1 |
| P5 | start community infected | non-selective line 1 | 1 |
| P5 | start community infected | non-selective line 2 | 1 |
| P5 | start community infected | non-selective line 3 | 1 |
| P5 | start community infected | non-selective line 4 | 1 |
| P5 | start community infected | non-selective line 5 | 1 |
| P5 | start community infected | non-selective line 6 | 1 |
| P0 | axenic mock-infected | non-selective line 1 | 1 |
| P0 | axenic mock-infected | non-selective line 2 | 1 |
| P0 | axenic mock-infected | non-selective line 3 | 1 |
| P0 | axenic mock-infected | non-selective line 4 | 1 |
| P0 | axenic mock-infected | non-selective line 5 | 1 |
| P0 | axenic mock-infected | non-selective line 6 | 1 |
| P1 | axenic mock-infected | non-selective line 1 | 1 |
| P1 | axenic mock-infected | non-selective line 2 | 1 |
| P1 | axenic mock-infected | non-selective line 3 | 1 |
| P1 | axenic mock-infected | non-selective line 4 | 1 |

Supplemental Table 15 continued

| | | | | |
|---|---|---|---|---|
| P1 | axenic mock-infected | non-selective line 5 | 1 | |
| P1 | axenic mock-infected | non-selective line 6 | 1 | |
| P2 | axenic mock-infected | non-selective line 1 | 1 | |
| P2 | axenic mock-infected | non-selective line 2 | 1 | |
| P2 | axenic mock-infected | non-selective line 3 | 1 | |
| P2 | axenic mock-infected | non-selective line 4 | 1 | |
| P2 | axenic mock-infected | non-selective line 5 | 0.43426 | |
| P2 | axenic mock-infected | non-selective line 6 | 1 | |
| P3 | axenic mock-infected | non-selective line 1 | 1 | |
| P3 | axenic mock-infected | non-selective line 2 | 1 | |
| P3 | axenic mock-infected | non-selective line 3 | 1 | |
| P3 | axenic mock-infected | non-selective line 4 | 1 | |
| P3 | axenic mock-infected | non-selective line 5 | 1 | |
| P3 | axenic mock-infected | non-selective line 6 | 1 | |
| P4 | axenic mock-infected | non-selective line 1 | 1 | |
| P4 | axenic mock-infected | non-selective line 2 | 1 | |
| P4 | axenic mock-infected | non-selective line 3 | 1 | |
| P4 | axenic mock-infected | non-selective line 4 | 1 | |
| P4 | axenic mock-infected | non-selective line 5 | 1 | |
| P4 | axenic mock-infected | non-selective line 6 | 1 | |
| P5 | axenic mock-infected | non-selective line 1 | 1 | |
| P5 | axenic mock-infected | non-selective line 2 | 1 | |
| P5 | axenic mock-infected | non-selective line 3 | 1 | |
| P5 | axenic mock-infected | non-selective line 4 | 1 | |
| P5 | axenic mock-infected | non-selective line 5 | 1 | |
| P5 | axenic mock-infected | non-selective line 6 | 1 | |
| P0 | axenic infected | non-selective line 1 | 1 | |
| P0 | axenic infected | non-selective line 2 | 1 | |
| P0 | axenic infected | non-selective line 3 | 1 | |
| P0 | axenic infected | non-selective line 4 | 1 | |
| P0 | axenic infected | non-selective line 5 | 1 | |
| P0 | axenic infected | non-selective line 6 | 1 | |
| P1 | axenic infected | non-selective line 1 | 0.72003 | |
| P1 | axenic infected | non-selective line 2 | 0.54698 | |
| P1 | axenic infected | non-selective line 3 | 0.03651 | * |
| P1 | axenic infected | non-selective line 4 | 0.29568 | |
| P1 | axenic infected | non-selective line 5 | 0.00672 | ** |
| P1 | axenic infected | non-selective line 6 | 0.17397 | |
| P2 | axenic infected | non-selective line 1 | 1 | |
| P2 | axenic infected | non-selective line 2 | 1 | |
| P2 | axenic infected | non-selective line 3 | 1 | |
| P2 | axenic infected | non-selective line 4 | 1 | |
| P2 | axenic infected | non-selective line 5 | 1 | |
| P2 | axenic infected | non-selective line 6 | 1 | |
| P3 | axenic infected | non-selective line 1 | 1 | |
| P3 | axenic infected | non-selective line 2 | 1 | |
| P3 | axenic infected | non-selective line 3 | 1 | |
| P3 | axenic infected | non-selective line 4 | 1 | |
| P3 | axenic infected | non-selective line 5 | 1 | |
| P3 | axenic infected | non-selective line 6 | 1 | |
| P4 | axenic infected | non-selective line 1 | 1 | |
| P4 | axenic infected | non-selective line 2 | 1 | |
| P4 | axenic infected | non-selective line 3 | 1 | |
| P4 | axenic infected | non-selective line 4 | 1 | |
| P4 | axenic infected | non-selective line 5 | 1 | |
| P4 | axenic infected | non-selective line 6 | 1 | |
| P5 | axenic infected | non-selective line 1 | 1 | |
| P5 | axenic infected | non-selective line 2 | 1 | |

Supplemental Table 15 continued

| | | | |
|---|---|---|---|
| P5 | axenic infected | non-selective line 3 | 1 |
| P5 | axenic infected | non-selective line 4 | 1 |
| P5 | axenic infected | non-selective line 5 | 1 |
| P5 | axenic infected | non-selective line 6 | 1 |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 16:** Bonferroni-corrected p-values of pairwise Welch's t-tests of disease severity scores at 7 dpi of non-selective passaged communities in first passaging experiment. Note that data of passage 4 is missing.

| Comment | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|
| non-selective line 1 | P0 | P1 | 1 | |
| non-selective line 1 | P1 | P2 | 1 | |
| non-selective line 1 | P2 | P3 | 1 | |
| non-selective line 1 | P3 | P5 | 1 | |
| non-selective line 1 | P1 | P5 | 1 | |
| non-selective line 2 | P0 | P1 | 1 | |
| non-selective line 2 | P1 | P2 | 1 | |
| non-selective line 2 | P2 | P3 | 1 | |
| non-selective line 2 | P3 | P5 | 1 | |
| non-selective line 2 | P1 | P5 | 1 | |
| non-selective line 3 | P0 | P1 | 1 | |
| non-selective line 3 | P1 | P2 | 1 | |
| non-selective line 3 | P2 | P3 | 1 | |
| non-selective line 3 | P3 | P5 | 1 | |
| non-selective line 3 | P1 | P5 | 1 | |
| non-selective line 3 | P0 | P1 | 1 | |
| non-selective line 4 | P1 | P2 | 1 | |
| non-selective line 4 | P2 | P3 | 1 | |
| non-selective line 4 | P3 | P5 | 1 | |
| non-selective line 4 | P1 | P5 | 1 | |
| non-selective line 5 | P0 | P1 | 1 | |
| non-selective line 5 | P1 | P2 | 0.00305 | ** |
| non-selective line 5 | P2 | P3 | 1 | |
| non-selective line 5 | P3 | P5 | 1 | |
| non-selective line 5 | P1 | P5 | 1 | |
| non-selective line 6 | P0 | P1 | 1 | |
| non-selective line 6 | P1 | P2 | 1 | |
| non-selective line 6 | P2 | P3 | 1 | |
| non-selective line 6 | P3 | P5 | 1 | |
| non-selective line 6 | P1 | P5 | 1 | |
| P0 | non-selective line 1 | non-selective line 2 | 1 | |
| P0 | non-selective line 1 | non-selective line 3 | 1 | |
| P0 | non-selective line 1 | non-selective line 4 | 1 | |
| P0 | non-selective line 1 | non-selective line 5 | 1 | |
| P0 | non-selective line 1 | non-selective line 6 | 1 | |
| P0 | non-selective line 2 | non-selective line 3 | 1 | |
| P0 | non-selective line 2 | non-selective line 4 | 1 | |
| P0 | non-selective line 2 | non-selective line 5 | 1 | |
| P0 | non-selective line 2 | non-selective line 6 | 1 | |
| P0 | non-selective line 3 | non-selective line 4 | 1 | |
| P0 | non-selective line 3 | non-selective line 5 | 1 | |
| P0 | non-selective line 3 | non-selective line 6 | 1 | |
| P0 | non-selective line 4 | non-selective line 5 | 1 | |
| P0 | non-selective line 4 | non-selective line 6 | 1 | |
| P0 | non-selective line 5 | non-selective line 6 | 1 | |
| P1 | non-selective line 1 | non-selective line 2 | 1 | |
| P1 | non-selective line 1 | non-selective line 3 | 1 | |
| P1 | non-selective line 1 | non-selective line 4 | 1 | |
| P1 | non-selective line 1 | non-selective line 5 | 1 | |
| P1 | non-selective line 1 | non-selective line 6 | 1 | |
| P1 | non-selective line 2 | non-selective line 3 | 1 | |
| P1 | non-selective line 2 | non-selective line 4 | 1 | |
| P1 | non-selective line 2 | non-selective line 5 | 1 | |
| P1 | non-selective line 2 | non-selective line 6 | 1 | |

Supplemental Table 16 continued

| | | | | |
|---|---|---|---|---|
| P1 | non-selective line 3 | non-selective line 4 | 1 | |
| P1 | non-selective line 3 | non-selective line 5 | 1 | |
| P1 | non-selective line 3 | non-selective line 6 | 1 | |
| P1 | non-selective line 4 | non-selective line 5 | 1 | |
| P1 | non-selective line 4 | non-selective line 6 | 1 | |
| P1 | non-selective line 5 | non-selective line 6 | 1 | |
| P2 | non-selective line 1 | non-selective line 2 | 1 | |
| P2 | non-selective line 1 | non-selective line 3 | 1 | |
| P2 | non-selective line 1 | non-selective line 4 | 1 | |
| P2 | non-selective line 1 | non-selective line 5 | 1 | |
| P2 | non-selective line 1 | non-selective line 6 | 1 | |
| P2 | non-selective line 2 | non-selective line 3 | 1 | |
| P2 | non-selective line 2 | non-selective line 4 | 1 | |
| P2 | non-selective line 2 | non-selective line 5 | 0.10311 | |
| P2 | non-selective line 2 | non-selective line 6 | 1 | |
| P2 | non-selective line 3 | non-selective line 4 | 1 | |
| P2 | non-selective line 3 | non-selective line 5 | 1 | |
| P2 | non-selective line 3 | non-selective line 6 | 1 | |
| P2 | non-selective line 4 | non-selective line 5 | 1 | |
| P2 | non-selective line 4 | non-selective line 6 | 1 | |
| P2 | non-selective line 5 | non-selective line 6 | 1 | |
| P3 | non-selective line 1 | non-selective line 2 | 1 | |
| P3 | non-selective line 1 | non-selective line 3 | 1 | |
| P3 | non-selective line 1 | non-selective line 4 | 1 | |
| P3 | non-selective line 1 | non-selective line 5 | 1 | |
| P3 | non-selective line 1 | non-selective line 6 | 1 | |
| P3 | non-selective line 2 | non-selective line 3 | 1 | |
| P3 | non-selective line 2 | non-selective line 4 | 1 | |
| P3 | non-selective line 2 | non-selective line 5 | 1 | |
| P3 | non-selective line 2 | non-selective line 6 | 1 | |
| P3 | non-selective line 3 | non-selective line 4 | 1 | |
| P3 | non-selective line 3 | non-selective line 5 | 1 | |
| P3 | non-selective line 3 | non-selective line 6 | 1 | |
| P3 | non-selective line 4 | non-selective line 5 | 1 | |
| P3 | non-selective line 4 | non-selective line 6 | 1 | |
| P3 | non-selective line 5 | non-selective line 6 | 1 | |
| P5 | non-selective line 1 | non-selective line 2 | 1 | |
| P5 | non-selective line 1 | non-selective line 3 | 1 | |
| P5 | non-selective line 1 | non-selective line 4 | 1 | |
| P5 | non-selective line 1 | non-selective line 5 | 1 | |
| P5 | non-selective line 1 | non-selective line 6 | 0.04615 | * |
| P5 | non-selective line 2 | non-selective line 3 | 1 | |
| P5 | non-selective line 2 | non-selective line 4 | 1 | |
| P5 | non-selective line 2 | non-selective line 5 | 1 | |
| P5 | non-selective line 2 | non-selective line 6 | 1 | |
| P5 | non-selective line 3 | non-selective line 4 | 1 | |
| P5 | non-selective line 3 | non-selective line 5 | 1 | |
| P5 | non-selective line 3 | non-selective line 6 | 0.1473 | |
| P5 | non-selective line 4 | non-selective line 5 | 1 | |
| P5 | non-selective line 4 | non-selective line 6 | 1 | |
| P5 | non-selective line 5 | non-selective line 6 | 1 | |
| P0 | start community infected | non-selective line 1 | 1 | |
| P0 | start community infected | non-selective line 2 | 1 | |
| P0 | start community infected | non-selective line 3 | 1 | |
| P0 | start community infected | non-selective line 4 | 1 | |
| P0 | start community infected | non-selective line 5 | 1 | |
| P0 | start community infected | non-selective line 6 | 1 | |
| P1 | start community infected | non-selective line 1 | 1 | |

Supplemental Table 16 continued

| | | | | |
|---|---|---|---|---|
| P1 | start community infected | non-selective line 2 | 1 | |
| P1 | start community infected | non-selective line 3 | 1 | |
| P1 | start community infected | non-selective line 4 | 1 | |
| P1 | start community infected | non-selective line 5 | 1 | |
| P1 | start community infected | non-selective line 6 | 1 | |
| P2 | start community infected | non-selective line 1 | 1 | |
| P2 | start community infected | non-selective line 2 | 1 | |
| P2 | start community infected | non-selective line 3 | 1 | |
| P2 | start community infected | non-selective line 4 | 1 | |
| P2 | start community infected | non-selective line 5 | 0.05409 | |
| P2 | start community infected | non-selective line 6 | 1 | |
| P3 | start community infected | non-selective line 1 | 1 | |
| P3 | start community infected | non-selective line 2 | 1 | |
| P3 | start community infected | non-selective line 3 | 1 | |
| P3 | start community infected | non-selective line 4 | 1 | |
| P3 | start community infected | non-selective line 5 | 1 | |
| P3 | start community infected | non-selective line 6 | 1 | |
| P5 | start community infected | non-selective line 1 | 1 | |
| P5 | start community infected | non-selective line 2 | 1 | |
| P5 | start community infected | non-selective line 3 | 1 | |
| P5 | start community infected | non-selective line 4 | 1 | |
| P5 | start community infected | non-selective line 5 | 1 | |
| P5 | start community infected | non-selective line 6 | 1 | |
| P0 | axenic mock-infected | non-selective line 1 | 1 | |
| P0 | axenic mock-infected | non-selective line 2 | 1 | |
| P0 | axenic mock-infected | non-selective line 3 | 1 | |
| P0 | axenic mock-infected | non-selective line 4 | 1 | |
| P0 | axenic mock-infected | non-selective line 5 | 1 | |
| P0 | axenic mock-infected | non-selective line 6 | 1 | |
| P1 | axenic mock-infected | non-selective line 1 | 1 | |
| P1 | axenic mock-infected | non-selective line 2 | 0.0869 | |
| P1 | axenic mock-infected | non-selective line 3 | 1 | |
| P1 | axenic mock-infected | non-selective line 4 | 1 | |
| P1 | axenic mock-infected | non-selective line 5 | 1 | |
| P1 | axenic mock-infected | non-selective line 6 | 1 | |
| P2 | axenic mock-infected | non-selective line 1 | 1 | |
| P2 | axenic mock-infected | non-selective line 2 | 1 | |
| P2 | axenic mock-infected | non-selective line 3 | 1 | |
| P2 | axenic mock-infected | non-selective line 4 | 0.00018 | *** |
| P2 | axenic mock-infected | non-selective line 5 | 0.01676 | * |
| P2 | axenic mock-infected | non-selective line 6 | 1 | |
| P3 | axenic mock-infected | non-selective line 1 | 1 | |
| P3 | axenic mock-infected | non-selective line 2 | 0.47419 | |
| P3 | axenic mock-infected | non-selective line 3 | 0.00072 | *** |
| P3 | axenic mock-infected | non-selective line 4 | 1 | |
| P3 | axenic mock-infected | non-selective line 5 | 1 | |
| P3 | axenic mock-infected | non-selective line 6 | 1 | |
| P5 | axenic mock-infected | non-selective line 1 | 1 | |
| P5 | axenic mock-infected | non-selective line 2 | 1 | |
| P5 | axenic mock-infected | non-selective line 3 | 1 | |
| P5 | axenic mock-infected | non-selective line 4 | 1 | |
| P5 | axenic mock-infected | non-selective line 5 | 1 | |
| P5 | axenic mock-infected | non-selective line 6 | 0.01239 | * |
| P0 | axenic infected | non-selective line 1 | 0.00001 | **** |
| P0 | axenic infected | non-selective line 2 | 0 | **** |
| P0 | axenic infected | non-selective line 3 | 0 | **** |
| P0 | axenic infected | non-selective line 4 | 0 | **** |
| P0 | axenic infected | non-selective line 5 | 0 | **** |

Supplemental Table 16 continued

| | | | | |
|---|---|---|---|---|
| P0 | axenic infected | non-selective line 6 | 0 | **** |
| P1 | axenic infected | non-selective line 1 | 0.00005 | **** |
| P1 | axenic infected | non-selective line 2 | 0.01278 | * |
| P1 | axenic infected | non-selective line 3 | 0.00009 | **** |
| P1 | axenic infected | non-selective line 4 | 0.00003 | **** |
| P1 | axenic infected | non-selective line 5 | 0 | **** |
| P1 | axenic infected | non-selective line 6 | 1 | |
| P2 | axenic infected | non-selective line 1 | 0.24107 | |
| P2 | axenic infected | non-selective line 2 | 1 | |
| P2 | axenic infected | non-selective line 3 | 1 | |
| P2 | axenic infected | non-selective line 4 | 1 | |
| P2 | axenic infected | non-selective line 5 | 1 | |
| P2 | axenic infected | non-selective line 6 | 0.80573 | |
| P3 | axenic infected | non-selective line 1 | 1 | |
| P3 | axenic infected | non-selective line 2 | 1 | |
| P3 | axenic infected | non-selective line 3 | 1 | |
| P3 | axenic infected | non-selective line 4 | 1 | |
| P3 | axenic infected | non-selective line 5 | 1 | |
| P3 | axenic infected | non-selective line 6 | 1 | |
| P5 | axenic infected | non-selective line 1 | 0 | **** |
| P5 | axenic infected | non-selective line 2 | 0.07186 | |
| P5 | axenic infected | non-selective line 3 | 0.00001 | **** |
| P5 | axenic infected | non-selective line 4 | 1 | |
| P5 | axenic infected | non-selective line 5 | 0.36607 | |
| P5 | axenic infected | non-selective line 6 | 1 | |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 17:** Bonferroni-corrected p-values of pairwise Welch's t-tests of disease severity scores at 14 dpi of non-selective passaged communities in first passaging experiment.

| Comment | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|
| non-selective line 1 | P0 | P1 | 1 | |
| non-selective line 1 | P1 | P2 | 1 | |
| non-selective line 1 | P2 | P3 | 1 | |
| non-selective line 1 | P3 | P5 | 1 | |
| non-selective line 1 | P1 | P5 | 1 | |
| non-selective line 2 | P0 | P1 | 1 | |
| non-selective line 2 | P1 | P2 | 1 | |
| non-selective line 2 | P2 | P3 | 1 | |
| non-selective line 2 | P3 | P5 | 1 | |
| non-selective line 2 | P1 | P5 | 1 | |
| non-selective line 3 | P0 | P1 | 1 | |
| non-selective line 3 | P1 | P2 | 1 | |
| non-selective line 3 | P2 | P3 | 1 | |
| non-selective line 3 | P3 | P5 | 1 | |
| non-selective line 3 | P1 | P5 | 1 | |
| non-selective line 3 | P0 | P1 | 1 | |
| non-selective line 4 | P1 | P2 | 1 | |
| non-selective line 4 | P2 | P3 | 1 | |
| non-selective line 4 | P3 | P5 | 1 | |
| non-selective line 4 | P1 | P5 | 1 | |
| non-selective line 5 | P0 | P1 | 1 | |
| non-selective line 5 | P1 | P2 | 1 | |
| non-selective line 5 | P2 | P3 | 1 | |
| non-selective line 5 | P3 | P5 | 1 | |
| non-selective line 5 | P1 | P5 | 1 | |
| non-selective line 6 | P0 | P1 | 1 | |
| non-selective line 6 | P1 | P2 | 1 | |
| non-selective line 6 | P2 | P3 | 1 | |
| non-selective line 6 | P3 | P5 | 1 | |
| non-selective line 6 | P1 | P5 | 1 | |
| P0 | non-selective line 1 | non-selective line 2 | 1 | |
| P0 | non-selective line 1 | non-selective line 3 | 1 | |
| P0 | non-selective line 1 | non-selective line 4 | 1 | |
| P0 | non-selective line 1 | non-selective line 5 | 1 | |
| P0 | non-selective line 1 | non-selective line 6 | 1 | |
| P0 | non-selective line 2 | non-selective line 3 | 1 | |
| P0 | non-selective line 2 | non-selective line 4 | 1 | |
| P0 | non-selective line 2 | non-selective line 5 | 1 | |
| P0 | non-selective line 2 | non-selective line 6 | 1 | |
| P0 | non-selective line 3 | non-selective line 4 | 1 | |
| P0 | non-selective line 3 | non-selective line 5 | 1 | |
| P0 | non-selective line 3 | non-selective line 6 | 1 | |
| P0 | non-selective line 4 | non-selective line 5 | 1 | |
| P0 | non-selective line 4 | non-selective line 6 | 1 | |
| P0 | non-selective line 5 | non-selective line 6 | 1 | |
| P1 | non-selective line 1 | non-selective line 2 | 1 | |
| P1 | non-selective line 1 | non-selective line 3 | 1 | |
| P1 | non-selective line 1 | non-selective line 4 | 1 | |
| P1 | non-selective line 1 | non-selective line 5 | 1 | |
| P1 | non-selective line 1 | non-selective line 6 | 1 | |
| P1 | non-selective line 2 | non-selective line 3 | 1 | |
| P1 | non-selective line 2 | non-selective line 4 | 1 | |
| P1 | non-selective line 2 | non-selective line 5 | 1 | |
| P1 | non-selective line 2 | non-selective line 6 | 1 | |
| P1 | non-selective line 3 | non-selective line 4 | 1 | |

Supplemental Table 17 continued

| | | | | |
|---|---|---|---|---|
| P1 | non-selective line 3 | non-selective line 5 | 1 | |
| P1 | non-selective line 3 | non-selective line 6 | 1 | |
| P1 | non-selective line 4 | non-selective line 5 | 1 | |
| P1 | non-selective line 4 | non-selective line 6 | 1 | |
| P1 | non-selective line 5 | non-selective line 6 | 1 | |
| P2 | non-selective line 1 | non-selective line 2 | 1 | |
| P2 | non-selective line 1 | non-selective line 3 | 1 | |
| P2 | non-selective line 1 | non-selective line 4 | 1 | |
| P2 | non-selective line 1 | non-selective line 5 | 1 | |
| P2 | non-selective line 1 | non-selective line 6 | 1 | |
| P2 | non-selective line 2 | non-selective line 3 | 1 | |
| P2 | non-selective line 2 | non-selective line 4 | 1 | |
| P2 | non-selective line 2 | non-selective line 5 | 1 | |
| P2 | non-selective line 2 | non-selective line 6 | 1 | |
| P2 | non-selective line 3 | non-selective line 4 | 1 | |
| P2 | non-selective line 3 | non-selective line 5 | 1 | |
| P2 | non-selective line 3 | non-selective line 6 | 1 | |
| P2 | non-selective line 4 | non-selective line 5 | 1 | |
| P2 | non-selective line 4 | non-selective line 6 | 1 | |
| P2 | non-selective line 5 | non-selective line 6 | 1 | |
| P3 | non-selective line 1 | non-selective line 2 | 1 | |
| P3 | non-selective line 1 | non-selective line 3 | 1 | |
| P3 | non-selective line 1 | non-selective line 4 | 1 | |
| P3 | non-selective line 1 | non-selective line 5 | 1 | |
| P3 | non-selective line 1 | non-selective line 6 | 1 | |
| P3 | non-selective line 2 | non-selective line 3 | 1 | |
| P3 | non-selective line 2 | non-selective line 4 | 1 | |
| P3 | non-selective line 2 | non-selective line 5 | 1 | |
| P3 | non-selective line 2 | non-selective line 6 | 1 | |
| P3 | non-selective line 3 | non-selective line 4 | 1 | |
| P3 | non-selective line 3 | non-selective line 5 | 1 | |
| P3 | non-selective line 3 | non-selective line 6 | 1 | |
| P3 | non-selective line 4 | non-selective line 5 | 1 | |
| P3 | non-selective line 4 | non-selective line 6 | 1 | |
| P3 | non-selective line 5 | non-selective line 6 | 1 | |
| P4 | non-selective line 1 | non-selective line 2 | 1 | |
| P4 | non-selective line 1 | non-selective line 3 | 1 | |
| P4 | non-selective line 1 | non-selective line 4 | 1 | |
| P4 | non-selective line 1 | non-selective line 5 | 1 | |
| P4 | non-selective line 1 | non-selective line 6 | 1 | |
| P4 | non-selective line 2 | non-selective line 3 | 1 | |
| P4 | non-selective line 2 | non-selective line 4 | 1 | |
| P4 | non-selective line 2 | non-selective line 5 | 1 | |
| P4 | non-selective line 2 | non-selective line 6 | 1 | |
| P4 | non-selective line 3 | non-selective line 4 | 1 | |
| P4 | non-selective line 3 | non-selective line 5 | 1 | |
| P4 | non-selective line 3 | non-selective line 6 | 1 | |
| P4 | non-selective line 4 | non-selective line 5 | 1 | |
| P4 | non-selective line 4 | non-selective line 6 | 1 | |
| P4 | non-selective line 5 | non-selective line 6 | 1 | |
| P5 | non-selective line 1 | non-selective line 2 | 1 | |
| P5 | non-selective line 1 | non-selective line 3 | 1 | |
| P5 | non-selective line 1 | non-selective line 4 | 1 | |
| P5 | non-selective line 1 | non-selective line 5 | 0.00148 | ** |
| P5 | non-selective line 1 | non-selective line 6 | 1 | |
| P5 | non-selective line 2 | non-selective line 3 | 0.00113 | ** |
| P5 | non-selective line 2 | non-selective line 4 | 1 | |
| P5 | non-selective line 2 | non-selective line 5 | 1 | |

Supplemental Table 17 continued

| | | | |
|---|---|---|---|
| P5 | non-selective line 2 | non-selective line 6 | 1 |
| P5 | non-selective line 3 | non-selective line 4 | 0.38751 |
| P5 | non-selective line 3 | non-selective line 5 | 1 |
| P5 | non-selective line 3 | non-selective line 6 | 0.36622 |
| P5 | non-selective line 4 | non-selective line 5 | 1 |
| P5 | non-selective line 4 | non-selective line 6 | 1 |
| P5 | non-selective line 5 | non-selective line 6 | 1 |
| P0 | start community infected | non-selective line 1 | 1 |
| P0 | start community infected | non-selective line 2 | 1 |
| P0 | start community infected | non-selective line 3 | 1 |
| P0 | start community infected | non-selective line 4 | 1 |
| P0 | start community infected | non-selective line 5 | 1 |
| P0 | start community infected | non-selective line 6 | 1 |
| P1 | start community infected | non-selective line 1 | 1 |
| P1 | start community infected | non-selective line 2 | 1 |
| P1 | start community infected | non-selective line 3 | 1 |
| P1 | start community infected | non-selective line 4 | 1 |
| P1 | start community infected | non-selective line 5 | 1 |
| P1 | start community infected | non-selective line 6 | 1 |
| P2 | start community infected | non-selective line 1 | 1 |
| P2 | start community infected | non-selective line 2 | 1 |
| P2 | start community infected | non-selective line 3 | 1 |
| P2 | start community infected | non-selective line 4 | 1 |
| P2 | start community infected | non-selective line 5 | 1 |
| P2 | start community infected | non-selective line 6 | 0.51482 |
| P3 | start community infected | non-selective line 1 | 1 |
| P3 | start community infected | non-selective line 2 | 1 |
| P3 | start community infected | non-selective line 3 | 1 |
| P3 | start community infected | non-selective line 4 | 1 |
| P3 | start community infected | non-selective line 5 | 1 |
| P3 | start community infected | non-selective line 6 | 1 |
| P4 | start community infected | non-selective line 1 | 1 |
| P4 | start community infected | non-selective line 2 | 1 |
| P4 | start community infected | non-selective line 3 | 1 |
| P4 | start community infected | non-selective line 4 | 1 |
| P4 | start community infected | non-selective line 5 | 1 |
| P4 | start community infected | non-selective line 6 | 0.16597 |
| P5 | start community infected | non-selective line 1 | 1 |
| P5 | start community infected | non-selective line 2 | 1 |
| P5 | start community infected | non-selective line 3 | 1 |
| P5 | start community infected | non-selective line 4 | 0.29923 |
| P5 | start community infected | non-selective line 5 | 1 |
| P5 | start community infected | non-selective line 6 | 0.29466 |
| P0 | axenic mock-infected | non-selective line 1 | 1 |
| P0 | axenic mock-infected | non-selective line 2 | 1 |
| P0 | axenic mock-infected | non-selective line 3 | 1 |
| P0 | axenic mock-infected | non-selective line 4 | 1 |
| P0 | axenic mock-infected | non-selective line 5 | 1 |
| P0 | axenic mock-infected | non-selective line 6 | 1 |
| P1 | axenic mock-infected | non-selective line 1 | 1 |
| P1 | axenic mock-infected | non-selective line 2 | 1 |
| P1 | axenic mock-infected | non-selective line 3 | 1 |
| P1 | axenic mock-infected | non-selective line 4 | 1 |
| P1 | axenic mock-infected | non-selective line 5 | 1 |
| P1 | axenic mock-infected | non-selective line 6 | 1 |
| P2 | axenic mock-infected | non-selective line 1 | 1 |
| P2 | axenic mock-infected | non-selective line 2 | 1 |
| P2 | axenic mock-infected | non-selective line 3 | 0.55872 |

Supplemental Table 17 continued

| | | | | |
|---|---|---|---|---|
| P2 | axenic mock-infected | non-selective line 4 | 1 | |
| P2 | axenic mock-infected | non-selective line 5 | 1 | |
| P2 | axenic mock-infected | non-selective line 6 | 0.22159 | |
| P3 | axenic mock-infected | non-selective line 1 | 1 | |
| P3 | axenic mock-infected | non-selective line 2 | 1 | |
| P3 | axenic mock-infected | non-selective line 3 | 1 | |
| P3 | axenic mock-infected | non-selective line 4 | 0.00829 | ** |
| P3 | axenic mock-infected | non-selective line 5 | 1 | |
| P3 | axenic mock-infected | non-selective line 6 | 1 | |
| P4 | axenic mock-infected | non-selective line 1 | 1 | |
| P4 | axenic mock-infected | non-selective line 2 | 1 | |
| P4 | axenic mock-infected | non-selective line 3 | 0.39026 | |
| P4 | axenic mock-infected | non-selective line 4 | 0.67642 | |
| P4 | axenic mock-infected | non-selective line 5 | 1 | |
| P4 | axenic mock-infected | non-selective line 6 | 0.01029 | * |
| P5 | axenic mock-infected | non-selective line 1 | 1 | |
| P5 | axenic mock-infected | non-selective line 2 | 1 | |
| P5 | axenic mock-infected | non-selective line 3 | 1 | |
| P5 | axenic mock-infected | non-selective line 4 | 0.00039 | *** |
| P5 | axenic mock-infected | non-selective line 5 | 1 | |
| P5 | axenic mock-infected | non-selective line 6 | 0.00032 | *** |
| P0 | axenic infected | non-selective line 1 | 0 | **** |
| P0 | axenic infected | non-selective line 2 | 0 | **** |
| P0 | axenic infected | non-selective line 3 | 0 | **** |
| P0 | axenic infected | non-selective line 4 | 0 | **** |
| P0 | axenic infected | non-selective line 5 | 0 | **** |
| P0 | axenic infected | non-selective line 6 | 0 | **** |
| P1 | axenic infected | non-selective line 1 | 0 | **** |
| P1 | axenic infected | non-selective line 2 | 0 | **** |
| P1 | axenic infected | non-selective line 3 | 0 | **** |
| P1 | axenic infected | non-selective line 4 | 0 | **** |
| P1 | axenic infected | non-selective line 5 | 0 | **** |
| P1 | axenic infected | non-selective line 6 | 0 | **** |
| P2 | axenic infected | non-selective line 1 | 0.01424 | * |
| P2 | axenic infected | non-selective line 2 | 0 | **** |
| P2 | axenic infected | non-selective line 3 | 0.09747 | |
| P2 | axenic infected | non-selective line 4 | 0.0008 | *** |
| P2 | axenic infected | non-selective line 5 | 0.00095 | *** |
| P2 | axenic infected | non-selective line 6 | 0.16597 | |
| P3 | axenic infected | non-selective line 1 | 0.0001 | *** |
| P3 | axenic infected | non-selective line 2 | 0.00002 | **** |
| P3 | axenic infected | non-selective line 3 | 0.00086 | *** |
| P3 | axenic infected | non-selective line 4 | 1 | |
| P3 | axenic infected | non-selective line 5 | 0.00007 | **** |
| P3 | axenic infected | non-selective line 6 | 0.00171 | ** |
| P4 | axenic infected | non-selective line 1 | 0 | **** |
| P4 | axenic infected | non-selective line 2 | 0 | **** |
| P4 | axenic infected | non-selective line 3 | 0.00531 | ** |
| P4 | axenic infected | non-selective line 4 | 0.00269 | ** |
| P4 | axenic infected | non-selective line 5 | 0.00014 | *** |
| P4 | axenic infected | non-selective line 6 | 0.22159 | |
| P5 | axenic infected | non-selective line 1 | 0 | **** |
| P5 | axenic infected | non-selective line 2 | 0 | **** |
| P5 | axenic infected | non-selective line 3 | 0 | **** |
| P5 | axenic infected | non-selective line 4 | 1 | |
| P5 | axenic infected | non-selective line 5 | 0 | **** |
| P5 | axenic infected | non-selective line 6 | 0.67642 | |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 18:** Bonferroni-corrected p-values of pairwise Welch's t-tests of pathogen luminescence of selectively passaged communities in first passaging experiment.

| Comment | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|
| parental to healthy selection | parental line 1, P0 | healthy line 1, P1 | 1 | |
| parental to sick selection | parental line 1, P0 | sick line 1, P1 | 1 | |
| healthy vs sick selection | healthy line 1, P1 | sick line 1, P1 | 1 | |
| healthy vs sick selection | healthy line 1, P2 | sick line 1, P2 | 1 | |
| healthy vs sick selection | healthy line 1, P3 | sick line 1, P3 | 1 | |
| healthy vs sick selection | healthy line 1, P4 | sick line 1, P4 | 1 | |
| healthy vs sick selection | healthy line 1, P5 | sick line 1, P5 | 1 | |
| parental to healthy selection | parental line 2, P0 | healthy line 2, P1 | 1 | |
| parental to sick selection | parental line 2, P0 | sick line 2, P1 | 1 | |
| healthy vs sick selection | healthy line 2, P1 | sick line 2, P1 | 1 | |
| healthy vs sick selection | healthy line 2, P2 | sick line 2, P2 | 1 | |
| healthy vs sick selection | healthy line 2, P3 | sick line 2, P3 | 1 | |
| healthy vs sick selection | healthy line 2, P4 | sick line 2, P4 | 1 | |
| healthy vs sick selection | healthy line 2, P5 | sick line 2, P5 | 1 | |
| parental to healthy selection | parental line 3, P0 | healthy line 3, P1 | 1 | |
| parental to sick selection | parental line 3, P0 | sick line 3, P1 | 1 | |
| healthy vs sick selection | healthy line 3, P1 | sick line 3, P1 | 1 | |
| healthy vs sick selection | healthy line 3, P2 | sick line 3, P2 | 1 | |
| healthy vs sick selection | healthy line 3, P3 | sick line 3, P3 | 1 | |
| healthy vs sick selection | healthy line 3, P4 | sick line 3, P4 | 1 | |
| healthy vs sick selection | healthy line 3, P5 | sick line 3, P5 | 1 | |
| parental to healthy selection | parental line 4, P0 | healthy line 4, P1 | 1 | |
| parental to sick selection | parental line 4, P0 | sick line 4, P1 | 1 | |
| healthy vs sick selection | healthy line 4, P1 | sick line 4, P1 | 1 | |
| healthy vs sick selection | healthy line 4, P2 | sick line 4, P2 | 1 | |
| healthy vs sick selection | healthy line 4, P3 | sick line 4, P3 | 1 | |
| healthy vs sick selection | healthy line 4, P4 | sick line 4, P4 | 1 | |
| healthy vs sick selection | healthy line 4, P5 | sick line 4, P5 | 1 | |
| parental to healthy selection | parental line 5, P0 | healthy line 5, P1 | 1 | |
| parental to sick selection | parental line 5, P0 | sick line 5, P1 | 1 | |
| healthy vs sick selection | healthy line 5, P1 | sick line 5, P1 | 1 | |
| healthy vs sick selection | healthy line 5, P2 | sick line 5, P2 | 1 | |
| healthy vs sick selection | healthy line 5, P3 | sick line 5, P3 | 1 | |
| healthy vs sick selection | healthy line 5, P4 | sick line 5, P4 | 1 | |
| healthy vs sick selection | healthy line 5, P5 | sick line 5, P5 | 1 | |
| parental to healthy selection | parental line 6, P0 | healthy line 6, P1 | 1 | |
| parental to sick selection | parental line 6, P0 | sick line 6, P1 | 1 | |
| healthy vs sick selection | healthy line 6, P1 | sick line 6, P1 | 1 | |
| healthy vs sick selection | healthy line 6, P2 | sick line 6, P2 | 1 | |
| healthy vs sick selection | healthy line 6, P3 | sick line 6, P3 | 1 | |
| healthy vs sick selection | healthy line 6, P4 | sick line 6, P4 | 1 | |
| healthy vs sick selection | healthy line 6, P5 | sick line 6, P5 | 1 | |
| healthy line 1 | P1 | P2 | 1 | |
| healthy line 1 | P2 | P3 | 1 | |
| healthy line 1 | P3 | P4 | 1 | |
| healthy line 1 | P4 | P5 | 1 | |
| healthy line 1 | P0 | P5 | 1 | |
| healthy line 2 | P1 | P2 | 1 | |
| healthy line 2 | P2 | P3 | 1 | |
| healthy line 2 | P3 | P4 | 1 | |
| healthy line 2 | P4 | P5 | 1 | |
| healthy line 2 | P0 | P5 | 0.12352 | |
| healthy line 3 | P1 | P2 | 1 | |
| healthy line 3 | P2 | P3 | 1 | |
| healthy line 3 | P3 | P4 | 1 | |

Supplemental Table 18 continued

| | | | | |
|---|---|---|---|---|
| healthy line 3 | P4 | P5 | 1 | |
| healthy line 3 | P0 | P5 | 0.25143 | |
| healthy line 4 | P1 | P2 | 1 | |
| healthy line 4 | P2 | P3 | 1 | |
| healthy line 4 | P3 | P4 | 1 | |
| healthy line 4 | P4 | P5 | 1 | |
| healthy line 4 | P0 | P5 | 1 | |
| healthy line 5 | P1 | P2 | 1 | |
| healthy line 5 | P2 | P3 | 1 | |
| healthy line 5 | P3 | P4 | 1 | |
| healthy line 5 | P4 | P5 | 1 | |
| healthy line 5 | P0 | P5 | 1 | |
| healthy line 6 | P1 | P2 | 1 | |
| healthy line 6 | P2 | P3 | 1 | |
| healthy line 6 | P3 | P4 | 1 | |
| healthy line 6 | P4 | P5 | 1 | |
| healthy line 6 | P0 | P5 | 1 | |
| sick line 1 | P1 | P2 | 1 | |
| sick line 1 | P2 | P3 | 1 | |
| sick line 1 | P3 | P4 | 1 | |
| sick line 1 | P4 | P5 | 1 | |
| sick line 1 | P0 | P5 | 0.04079 | * |
| sick line 2 | P1 | P2 | 1 | |
| sick line 2 | P2 | P3 | 1 | |
| sick line 2 | P3 | P4 | 1 | |
| sick line 2 | P4 | P5 | 1 | |
| sick line 2 | P0 | P5 | 1 | |
| sick line 3 | P1 | P2 | 1 | |
| sick line 3 | P2 | P3 | 1 | |
| sick line 3 | P3 | P4 | 1 | |
| sick line 3 | P4 | P5 | 1 | |
| sick line 3 | P0 | P5 | 0.01962 | * |
| sick line 4 | P1 | P2 | 1 | |
| sick line 4 | P2 | P3 | 1 | |
| sick line 4 | P3 | P4 | 1 | |
| sick line 4 | P4 | P5 | 1 | |
| sick line 4 | P0 | P5 | 1 | |
| sick line 5 | P1 | P2 | 1 | |
| sick line 5 | P2 | P3 | 1 | |
| sick line 5 | P3 | P4 | 1 | |
| sick line 5 | P4 | P5 | 1 | |
| sick line 5 | P0 | P5 | 1 | |
| sick line 6 | P1 | P2 | 1 | |
| sick line 6 | P2 | P3 | 1 | |
| sick line 6 | P3 | P4 | 1 | |
| sick line 6 | P4 | P5 | 1 | |
| sick line 6 | P0 | P5 | 1 | |
| P0 | start community infected | parental line 1 | 1 | |
| P0 | start community infected | parental line 2 | 1 | |
| P0 | start community infected | parental line 3 | 1 | |
| P0 | start community infected | parental line 4 | 1 | |
| P0 | start community infected | parental line 5 | 1 | |
| P0 | start community infected | parental line 6 | 1 | |
| P1 | start community infected | healthy line 1 | 1 | |
| P1 | start community infected | healthy line 2 | 1 | |
| P1 | start community infected | healthy line 3 | 1 | |
| P1 | start community infected | healthy line 4 | 1 | |

Supplemental Table 18 continued

| | | | |
|---|---|---|---|
| P1 | start community infected | healthy line 5 | 1 |
| P1 | start community infected | healthy line 6 | 1 |
| P1 | start community infected | sick line 1 | 1 |
| P1 | start community infected | sick line 2 | 1 |
| P1 | start community infected | sick line 3 | 1 |
| P1 | start community infected | sick line 4 | 1 |
| P1 | start community infected | sick line 5 | 1 |
| P1 | start community infected | sick line 6 | 1 |
| P2 | start community infected | healthy line 1 | 1 |
| P2 | start community infected | healthy line 2 | 1 |
| P2 | start community infected | healthy line 3 | 1 |
| P2 | start community infected | healthy line 4 | 1 |
| P2 | start community infected | healthy line 5 | 1 |
| P2 | start community infected | healthy line 6 | 1 |
| P2 | start community infected | sick line 1 | 1 |
| P2 | start community infected | sick line 2 | 1 |
| P2 | start community infected | sick line 3 | 1 |
| P2 | start community infected | sick line 4 | 1 |
| P2 | start community infected | sick line 5 | 1 |
| P2 | start community infected | sick line 6 | 1 |
| P3 | start community infected | healthy line 1 | 1 |
| P3 | start community infected | healthy line 2 | 1 |
| P3 | start community infected | healthy line 3 | 1 |
| P3 | start community infected | healthy line 4 | 1 |
| P3 | start community infected | healthy line 5 | 1 |
| P3 | start community infected | healthy line 6 | 1 |
| P3 | start community infected | sick line 1 | 1 |
| P3 | start community infected | sick line 2 | 1 |
| P3 | start community infected | sick line 3 | 1 |
| P3 | start community infected | sick line 4 | 1 |
| P3 | start community infected | sick line 5 | 1 |
| P3 | start community infected | sick line 6 | 1 |
| P4 | start community infected | healthy line 1 | 1 |
| P4 | start community infected | healthy line 2 | 1 |
| P4 | start community infected | healthy line 3 | 1 |
| P4 | start community infected | healthy line 4 | 1 |
| P4 | start community infected | healthy line 5 | 1 |
| P4 | start community infected | healthy line 6 | 1 |
| P4 | start community infected | sick line 1 | 1 |
| P4 | start community infected | sick line 2 | 1 |
| P4 | start community infected | sick line 3 | 1 |
| P4 | start community infected | sick line 4 | 1 |
| P4 | start community infected | sick line 5 | 1 |
| P4 | start community infected | sick line 6 | 1 |
| P5 | start community infected | healthy line 1 | 1 |
| P5 | start community infected | healthy line 2 | 1 |
| P5 | start community infected | healthy line 3 | 1 |
| P5 | start community infected | healthy line 4 | 1 |
| P5 | start community infected | healthy line 5 | 1 |
| P5 | start community infected | healthy line 6 | 1 |
| P5 | start community infected | sick line 1 | 1 |
| P5 | start community infected | sick line 2 | 1 |
| P5 | start community infected | sick line 3 | 1 |
| P5 | start community infected | sick line 4 | 1 |
| P5 | start community infected | sick line 5 | 1 |
| P5 | start community infected | sick line 6 | 1 |
| P0 | axenic mock-infected | parental line 1 | 1 |

Supplemental Table 18 continued

| | | | | |
|---|---|---|---|---|
| P0 | axenic mock-infected | parental line 2 | 1 | |
| P0 | axenic mock-infected | parental line 3 | 1 | |
| P0 | axenic mock-infected | parental line 4 | 1 | |
| P0 | axenic mock-infected | parental line 5 | 1 | |
| P0 | axenic mock-infected | parental line 6 | 1 | |
| P1 | axenic mock-infected | healthy line 1 | 1 | |
| P1 | axenic mock-infected | healthy line 2 | 1 | |
| P1 | axenic mock-infected | healthy line 3 | 1 | |
| P1 | axenic mock-infected | healthy line 4 | 1 | |
| P1 | axenic mock-infected | healthy line 5 | 1 | |
| P1 | axenic mock-infected | healthy line 6 | 1 | |
| P1 | axenic mock-infected | sick line 1 | 1 | |
| P1 | axenic mock-infected | sick line 2 | 1 | |
| P1 | axenic mock-infected | sick line 3 | 0.53745 | |
| P1 | axenic mock-infected | sick line 4 | 1 | |
| P1 | axenic mock-infected | sick line 5 | 1 | |
| P1 | axenic mock-infected | sick line 6 | 1 | |
| P2 | axenic mock-infected | healthy line 1 | 1 | |
| P2 | axenic mock-infected | healthy line 2 | 1 | |
| P2 | axenic mock-infected | healthy line 3 | 1 | |
| P2 | axenic mock-infected | healthy line 4 | 1 | |
| P2 | axenic mock-infected | healthy line 5 | 0.04646 | * |
| P2 | axenic mock-infected | healthy line 6 | 1 | |
| P2 | axenic mock-infected | sick line 1 | 1 | |
| P2 | axenic mock-infected | sick line 2 | 1 | |
| P2 | axenic mock-infected | sick line 3 | 1 | |
| P2 | axenic mock-infected | sick line 4 | 1 | |
| P2 | axenic mock-infected | sick line 5 | 1 | |
| P2 | axenic mock-infected | sick line 6 | 1 | |
| P3 | axenic mock-infected | healthy line 1 | 0.43588 | |
| P3 | axenic mock-infected | healthy line 2 | 1 | |
| P3 | axenic mock-infected | healthy line 3 | 1 | |
| P3 | axenic mock-infected | healthy line 4 | 1 | |
| P3 | axenic mock-infected | healthy line 5 | 1 | |
| P3 | axenic mock-infected | healthy line 6 | 1 | |
| P3 | axenic mock-infected | sick line 1 | 1 | |
| P3 | axenic mock-infected | sick line 2 | 1 | |
| P3 | axenic mock-infected | sick line 3 | 1 | |
| P3 | axenic mock-infected | sick line 4 | 1 | |
| P3 | axenic mock-infected | sick line 5 | 1 | |
| P3 | axenic mock-infected | sick line 6 | 1 | |
| P4 | axenic mock-infected | healthy line 1 | 1 | |
| P4 | axenic mock-infected | healthy line 2 | 1 | |
| P4 | axenic mock-infected | healthy line 3 | 1 | |
| P4 | axenic mock-infected | healthy line 4 | 1 | |
| P4 | axenic mock-infected | healthy line 5 | 1 | |
| P4 | axenic mock-infected | healthy line 6 | 0.11006 | |
| P4 | axenic mock-infected | sick line 1 | 1 | |
| P4 | axenic mock-infected | sick line 2 | 1 | |
| P4 | axenic mock-infected | sick line 3 | 1 | |
| P4 | axenic mock-infected | sick line 4 | 1 | |
| P4 | axenic mock-infected | sick line 5 | 1 | |
| P4 | axenic mock-infected | sick line 6 | 1 | |
| P5 | axenic mock-infected | healthy line 1 | 1 | |
| P5 | axenic mock-infected | healthy line 2 | 1 | |
| P5 | axenic mock-infected | healthy line 3 | 1 | |
| P5 | axenic mock-infected | healthy line 4 | 1 | |

Supplemental Table 18 continued

| | | | | |
|---|---|---|---|---|
| P5 | axenic mock-infected | healthy line 5 | 1 | |
| P5 | axenic mock-infected | healthy line 6 | 1 | |
| P5 | axenic mock-infected | sick line 1 | 1 | |
| P5 | axenic mock-infected | sick line 2 | 1 | |
| P5 | axenic mock-infected | sick line 3 | 1 | |
| P5 | axenic mock-infected | sick line 4 | 1 | |
| P5 | axenic mock-infected | sick line 5 | 1 | |
| P5 | axenic mock-infected | sick line 6 | 1 | |
| P0 | axenic infected | parental line 1 | 1 | |
| P0 | axenic infected | parental line 2 | 1 | |
| P0 | axenic infected | parental line 3 | 1 | |
| P0 | axenic infected | parental line 4 | 1 | |
| P0 | axenic infected | parental line 5 | 1 | |
| P0 | axenic infected | parental line 6 | 1 | |
| P1 | axenic infected | healthy line 1 | 1 | |
| P1 | axenic infected | healthy line 2 | 0.22615 | |
| P1 | axenic infected | healthy line 3 | 1 | |
| P1 | axenic infected | healthy line 4 | 0.0003 | *** |
| P1 | axenic infected | healthy line 5 | 0.04156 | * |
| P1 | axenic infected | healthy line 6 | 1 | |
| P1 | axenic infected | sick line 1 | 0.00014 | *** |
| P1 | axenic infected | sick line 2 | 0.00223 | ** |
| P1 | axenic infected | sick line 3 | 1 | |
| P1 | axenic infected | sick line 4 | 1 | |
| P1 | axenic infected | sick line 5 | 0.06767 | |
| P1 | axenic infected | sick line 6 | 0.24935 | |
| P2 | axenic infected | healthy line 1 | 1 | |
| P2 | axenic infected | healthy line 2 | 1 | |
| P2 | axenic infected | healthy line 3 | 1 | |
| P2 | axenic infected | healthy line 4 | 1 | |
| P2 | axenic infected | healthy line 5 | 1 | |
| P2 | axenic infected | healthy line 6 | 1 | |
| P2 | axenic infected | sick line 1 | 1 | |
| P2 | axenic infected | sick line 2 | 1 | |
| P2 | axenic infected | sick line 3 | 1 | |
| P2 | axenic infected | sick line 4 | 1 | |
| P2 | axenic infected | sick line 5 | 1 | |
| P2 | axenic infected | sick line 6 | 1 | |
| P3 | axenic infected | healthy line 1 | 1 | |
| P3 | axenic infected | healthy line 2 | 1 | |
| P3 | axenic infected | healthy line 3 | 1 | |
| P3 | axenic infected | healthy line 4 | 1 | |
| P3 | axenic infected | healthy line 5 | 1 | |
| P3 | axenic infected | healthy line 6 | 1 | |
| P3 | axenic infected | sick line 1 | 1 | |
| P3 | axenic infected | sick line 2 | 1 | |
| P3 | axenic infected | sick line 3 | 1 | |
| P3 | axenic infected | sick line 4 | 1 | |
| P3 | axenic infected | sick line 5 | 0.40003 | |
| P3 | axenic infected | sick line 6 | 1 | |
| P4 | axenic infected | healthy line 1 | 1 | |
| P4 | axenic infected | healthy line 2 | 1 | |
| P4 | axenic infected | healthy line 3 | 1 | |
| P4 | axenic infected | healthy line 4 | 0.66221 | |
| P4 | axenic infected | healthy line 5 | 1 | |
| P4 | axenic infected | healthy line 6 | 1 | |
| P4 | axenic infected | sick line 1 | 1 | |

Supplemental Table 18 continued

| | | | | |
|---|---|---|---|---|
| P4 | axenic infected | sick line 2 | 0.03213 | * |
| P4 | axenic infected | sick line 3 | 0.87811 | |
| P4 | axenic infected | sick line 4 | 1 | |
| P4 | axenic infected | sick line 5 | 1 | |
| P4 | axenic infected | sick line 6 | 1 | |
| P5 | axenic infected | healthy line 1 | 1 | |
| P5 | axenic infected | healthy line 2 | 1 | |
| P5 | axenic infected | healthy line 3 | 1 | |
| P5 | axenic infected | healthy line 4 | 1 | |
| P5 | axenic infected | healthy line 5 | 1 | |
| P5 | axenic infected | healthy line 6 | 1 | |
| P5 | axenic infected | sick line 1 | 1 | |
| P5 | axenic infected | sick line 2 | 1 | |
| P5 | axenic infected | sick line 3 | 1 | |
| P5 | axenic infected | sick line 4 | 1 | |
| P5 | axenic infected | sick line 5 | 1 | |
| P5 | axenic infected | sick line 6 | 1 | |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 19:** Bonferroni-corrected p-values of pairwise Welch's t-tests of disease severity scores at 7 dpi of selective passaged communities in first passaging experiment. Note that data of passage 4 is missing.

| Comment | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|
| healthy vs sick selection | healthy line 1, P1 | sick line 1, P1 | 1 | |
| healthy vs sick selection | healthy line 1, P2 | sick line 1, P2 | 1 | |
| healthy vs sick selection | healthy line 1, P3 | sick line 1, P3 | 1 | |
| healthy vs sick selection | healthy line 1, P5 | sick line 1, P5 | 1 | |
| healthy vs sick selection | healthy line 2, P1 | sick line 2, P1 | 1 | |
| healthy vs sick selection | healthy line 2, P2 | sick line 2, P2 | 1 | |
| healthy vs sick selection | healthy line 2, P3 | sick line 2, P3 | 1 | |
| healthy vs sick selection | healthy line 2, P5 | sick line 2, P5 | 1 | |
| healthy vs sick selection | healthy line 3, P1 | sick line 3, P1 | 1 | |
| healthy vs sick selection | healthy line 3, P2 | sick line 3, P2 | 1 | |
| healthy vs sick selection | healthy line 3, P3 | sick line 3, P3 | 1 | |
| healthy vs sick selection | healthy line 3, P5 | sick line 3, P5 | 1 | |
| healthy vs sick selection | healthy line 4, P1 | sick line 4, P1 | 0.0263 | * |
| healthy vs sick selection | healthy line 4, P2 | sick line 4, P2 | 1 | |
| healthy vs sick selection | healthy line 4, P3 | sick line 4, P3 | 1 | |
| healthy vs sick selection | healthy line 4, P5 | sick line 4, P5 | 1 | |
| healthy vs sick selection | healthy line 5, P1 | sick line 5, P1 | 1 | |
| healthy vs sick selection | healthy line 5, P2 | sick line 5, P2 | 0.91975 | |
| healthy vs sick selection | healthy line 5, P3 | sick line 5, P3 | 0.59703 | |
| healthy vs sick selection | healthy line 5, P5 | sick line 5, P5 | 1 | |
| healthy vs sick selection | healthy line 6, P1 | sick line 6, P1 | 1 | |
| healthy vs sick selection | healthy line 6, P2 | sick line 6, P2 | 1 | |
| healthy vs sick selection | healthy line 6, P3 | sick line 6, P3 | 1 | |
| healthy vs sick selection | healthy line 6, P5 | sick line 6, P5 | 1 | |
| parental to healthy selection | parental line 1, P0 | healthy line 1, P1 | 1 | |
| healthy line 1 | P1 | P2 | 1 | |
| healthy line 1 | P2 | P3 | 1 | |
| healthy line 1 | P3 | P5 | 1 | |
| parental to healthy selection | parental line 2, P0 | healthy line 2, P1 | 1 | |
| healthy line 2 | P1 | P2 | 1 | |
| healthy line 2 | P2 | P3 | 1 | |
| healthy line 2 | P3 | P5 | 1 | |
| parental to healthy selection | parental line 3, P0 | healthy line 3, P1 | 0.01229 | * |
| healthy line 3 | P1 | P2 | 1 | |
| healthy line 3 | P2 | P3 | 1 | |
| healthy line 3 | P3 | P5 | 1 | |
| parental to healthy selection | parental line 4, P0 | healthy line 4, P1 | 1 | |
| healthy line 4 | P1 | P2 | 1 | |
| healthy line 4 | P2 | P3 | 1 | |
| healthy line 4 | P3 | P5 | 1 | |
| parental to healthy selection | parental line 5, P0 | healthy line 5, P1 | 1 | |
| healthy line 5 | P1 | P2 | 0.00009 | **** |
| healthy line 5 | P2 | P3 | 1 | |
| healthy line 5 | P3 | P5 | 1 | |
| parental to healthy selection | parental line 6, P0 | healthy line 6, P1 | 1 | |
| healthy line 6 | P1 | P2 | 0.91975 | |
| healthy line 6 | P2 | P3 | 1 | |
| healthy line 6 | P3 | P5 | 1 | |
| parental to sick selection | parental line 1, P0 | sick line 1, P1 | 1 | |
| sick line 1 | P1 | P2 | 1 | |
| sick line 1 | P2 | P3 | 1 | |
| sick line 1 | P3 | P5 | 1 | |
| parental to sick selection | parental line 2, P0 | sick line 2, P1 | 1 | |
| sick line 2 | P1 | P2 | 1 | |
| sick line 2 | P2 | P3 | 1 | |

Supplemental Table 19 continued

| | | | | |
|---|---|---|---|---|
| sick line 2 | P3 | P5 | 1 | |
| parental to sick selection | parental line 3, P0 | sick line 3, P1 | 0.00618 | ** |
| sick line 3 | P1 | P2 | 1 | |
| sick line 3 | P2 | P3 | 1 | |
| sick line 3 | P3 | P5 | 1 | |
| parental to sick selection | parental line 4, P0 | sick line 4, P1 | 1 | |
| sick line 4 | P1 | P2 | 1 | |
| sick line 4 | P2 | P3 | 1 | |
| sick line 4 | P3 | P5 | 1 | |
| parental to sick selection | parental line 5, P0 | sick line 5, P1 | 1 | |
| sick line 5 | P1 | P2 | 1 | |
| sick line 5 | P2 | P3 | 1 | |
| sick line 5 | P3 | P5 | 1 | |
| parental to sick selection | parental line 6, P0 | sick line 6, P1 | 1 | |
| sick line 6 | P1 | P2 | 1 | |
| sick line 6 | P2 | P3 | 1 | |
| sick line 6 | P3 | P5 | 1 | |
| P0 | start community infected | parental line 1 | 1 | |
| P0 | start community infected | parental line 2 | 1 | |
| P0 | start community infected | parental line 3 | 1 | |
| P0 | start community infected | parental line 4 | 1 | |
| P0 | start community infected | parental line 5 | 1 | |
| P0 | start community infected | parental line 6 | 1 | |
| P1 | start community infected | healthy line 1 | 1 | |
| P1 | start community infected | healthy line 2 | 1 | |
| P1 | start community infected | healthy line 3 | 1 | |
| P1 | start community infected | healthy line 4 | 1 | |
| P1 | start community infected | healthy line 5 | 1 | |
| P1 | start community infected | healthy line 6 | 1 | |
| P1 | start community infected | sick line 1 | 1 | |
| P1 | start community infected | sick line 2 | 1 | |
| P1 | start community infected | sick line 3 | 1 | |
| P1 | start community infected | sick line 4 | 1 | |
| P1 | start community infected | sick line 5 | 1 | |
| P1 | start community infected | sick line 6 | 1 | |
| P2 | start community infected | healthy line 1 | 1 | |
| P2 | start community infected | healthy line 2 | 1 | |
| P2 | start community infected | healthy line 3 | 1 | |
| P2 | start community infected | healthy line 4 | 1 | |
| P2 | start community infected | healthy line 5 | 0.00035 | *** |
| P2 | start community infected | healthy line 6 | 1 | |
| P2 | start community infected | sick line 1 | 1 | |
| P2 | start community infected | sick line 2 | 1 | |
| P2 | start community infected | sick line 3 | 1 | |
| P2 | start community infected | sick line 4 | 1 | |
| P2 | start community infected | sick line 5 | 1 | |
| P2 | start community infected | sick line 6 | 1 | |
| P3 | start community infected | healthy line 1 | 1 | |
| P3 | start community infected | healthy line 2 | 1 | |
| P3 | start community infected | healthy line 3 | 1 | |
| P3 | start community infected | healthy line 4 | 1 | |
| P3 | start community infected | healthy line 5 | 1 | |
| P3 | start community infected | healthy line 6 | 1 | |
| P3 | start community infected | sick line 1 | 1 | |
| P3 | start community infected | sick line 2 | 1 | |
| P3 | start community infected | sick line 3 | 1 | |
| P3 | start community infected | sick line 4 | 1 | |

Supplemental Table 19 continued

| | | | | |
|---|---|---|---|---|
| P3 | start community infected | sick line 5 | 1 | |
| P3 | start community infected | sick line 6 | 1 | |
| P5 | start community infected | healthy line 1 | 1 | |
| P5 | start community infected | healthy line 2 | 1 | |
| P5 | start community infected | healthy line 3 | 1 | |
| P5 | start community infected | healthy line 4 | 1 | |
| P5 | start community infected | healthy line 5 | 1 | |
| P5 | start community infected | healthy line 6 | 1 | |
| P5 | start community infected | sick line 1 | 1 | |
| P5 | start community infected | sick line 2 | 1 | |
| P5 | start community infected | sick line 3 | 1 | |
| P5 | start community infected | sick line 4 | 1 | |
| P5 | start community infected | sick line 5 | 1 | |
| P5 | start community infected | sick line 6 | 1 | |
| P0 | axenic mock-infected | parental line 1 | 1 | |
| P0 | axenic mock-infected | parental line 2 | 1 | |
| P0 | axenic mock-infected | parental line 3 | 1 | |
| P0 | axenic mock-infected | parental line 4 | 1 | |
| P0 | axenic mock-infected | parental line 5 | 1 | |
| P0 | axenic mock-infected | parental line 6 | 1 | |
| P1 | axenic mock-infected | healthy line 1 | 0.00237 | ** |
| P1 | axenic mock-infected | healthy line 2 | 0.58891 | |
| P1 | axenic mock-infected | healthy line 3 | 0.00021 | *** |
| P1 | axenic mock-infected | healthy line 4 | 1 | |
| P1 | axenic mock-infected | healthy line 5 | 1 | |
| P1 | axenic mock-infected | healthy line 6 | 1 | |
| P1 | axenic mock-infected | sick line 1 | 1 | |
| P1 | axenic mock-infected | sick line 2 | 1 | |
| P1 | axenic mock-infected | sick line 3 | 0.00009 | **** |
| P1 | axenic mock-infected | sick line 4 | 0 | **** |
| P1 | axenic mock-infected | sick line 5 | 1 | |
| P1 | axenic mock-infected | sick line 6 | 1 | |
| P2 | axenic mock-infected | healthy line 1 | 0.03033 | * |
| P2 | axenic mock-infected | healthy line 2 | 1 | |
| P2 | axenic mock-infected | healthy line 3 | 1 | |
| P2 | axenic mock-infected | healthy line 4 | 1 | |
| P2 | axenic mock-infected | healthy line 5 | 0 | **** |
| P2 | axenic mock-infected | healthy line 6 | 1 | |
| P2 | axenic mock-infected | sick line 1 | 0.01676 | * |
| P2 | axenic mock-infected | sick line 2 | 1 | |
| P2 | axenic mock-infected | sick line 3 | 1 | |
| P2 | axenic mock-infected | sick line 4 | 0.08195 | |
| P2 | axenic mock-infected | sick line 5 | 1 | |
| P2 | axenic mock-infected | sick line 6 | 1 | |
| P3 | axenic mock-infected | healthy line 1 | 0.01957 | * |
| P3 | axenic mock-infected | healthy line 2 | 1 | |
| P3 | axenic mock-infected | healthy line 3 | 0.36456 | |
| P3 | axenic mock-infected | healthy line 4 | 1 | |
| P3 | axenic mock-infected | healthy line 5 | 0.01957 | * |
| P3 | axenic mock-infected | healthy line 6 | 0.62413 | |
| P3 | axenic mock-infected | sick line 1 | 0.36456 | |
| P3 | axenic mock-infected | sick line 2 | 1 | |
| P3 | axenic mock-infected | sick line 3 | 0.09511 | |
| P3 | axenic mock-infected | sick line 4 | 1 | |
| P3 | axenic mock-infected | sick line 5 | 1 | |
| P3 | axenic mock-infected | sick line 6 | 1 | |
| P5 | axenic mock-infected | healthy line 1 | 0.36607 | |

Supplemental Table 19 continued

| | | | | |
|---|---|---|---|---|
| P5 | axenic mock-infected | healthy line 2 | 0.61204 | |
| P5 | axenic mock-infected | healthy line 3 | 0.61204 | |
| P5 | axenic mock-infected | healthy line 4 | 1 | |
| P5 | axenic mock-infected | healthy line 5 | 1 | |
| P5 | axenic mock-infected | healthy line 6 | 1 | |
| P5 | axenic mock-infected | sick line 1 | 1 | |
| P5 | axenic mock-infected | sick line 2 | 1 | |
| P5 | axenic mock-infected | sick line 3 | 1 | |
| P5 | axenic mock-infected | sick line 4 | 1 | |
| P5 | axenic mock-infected | sick line 5 | 1 | |
| P5 | axenic mock-infected | sick line 6 | 1 | |
| P0 | axenic infected | parental line 1 | 0 | **** |
| P0 | axenic infected | parental line 2 | 0 | **** |
| P0 | axenic infected | parental line 3 | 0 | **** |
| P0 | axenic infected | parental line 4 | 0.04604 | * |
| P0 | axenic infected | parental line 5 | 0 | **** |
| P0 | axenic infected | parental line 6 | 0 | **** |
| P1 | axenic infected | healthy line 1 | 0.37138 | |
| P1 | axenic infected | healthy line 2 | 0.00132 | ** |
| P1 | axenic infected | healthy line 3 | 1 | |
| P1 | axenic infected | healthy line 4 | 0 | **** |
| P1 | axenic infected | healthy line 5 | 0 | **** |
| P1 | axenic infected | healthy line 6 | 0.00026 | *** |
| P1 | axenic infected | sick line 1 | 0 | **** |
| P1 | axenic infected | sick line 2 | 0 | **** |
| P1 | axenic infected | sick line 3 | 1 | |
| P1 | axenic infected | sick line 4 | 1 | |
| P1 | axenic infected | sick line 5 | 0 | **** |
| P1 | axenic infected | sick line 6 | 0.00054 | *** |
| P2 | axenic infected | healthy line 1 | 1 | |
| P2 | axenic infected | healthy line 2 | 1 | |
| P2 | axenic infected | healthy line 3 | 1 | |
| P2 | axenic infected | healthy line 4 | 1 | |
| P2 | axenic infected | healthy line 5 | 1 | |
| P2 | axenic infected | healthy line 6 | 1 | |
| P2 | axenic infected | sick line 1 | 1 | |
| P2 | axenic infected | sick line 2 | 1 | |
| P2 | axenic infected | sick line 3 | 1 | |
| P2 | axenic infected | sick line 4 | 1 | |
| P2 | axenic infected | sick line 5 | 1 | |
| P2 | axenic infected | sick line 6 | 1 | |
| P3 | axenic infected | healthy line 1 | 1 | |
| P3 | axenic infected | healthy line 2 | 0.00046 | *** |
| P3 | axenic infected | healthy line 3 | 1 | |
| P3 | axenic infected | healthy line 4 | 1 | |
| P3 | axenic infected | healthy line 5 | 1 | |
| P3 | axenic infected | healthy line 6 | 1 | |
| P3 | axenic infected | sick line 1 | 1 | |
| P3 | axenic infected | sick line 2 | 0.03417 | * |
| P3 | axenic infected | sick line 3 | 1 | |
| P3 | axenic infected | sick line 4 | 0.20608 | |
| P3 | axenic infected | sick line 5 | 0.00012 | *** |
| P3 | axenic infected | sick line 6 | 0.24993 | |
| P5 | axenic infected | healthy line 1 | 1 | |
| P5 | axenic infected | healthy line 2 | 1 | |
| P5 | axenic infected | healthy line 3 | 1 | |
| P5 | axenic infected | healthy line 4 | 0.00012 | *** |

Supplemental Table 19 continued

| | | | | |
|---|---|---|---|---|
| P5 | axenic infected | healthy line 5 | 0.00357 | ** |
| P5 | axenic infected | healthy line 6 | 1 | |
| P5 | axenic infected | sick line 1 | 0.36607 | |
| P5 | axenic infected | sick line 2 | 0.0005 | *** |
| P5 | axenic infected | sick line 3 | 1 | |
| P5 | axenic infected | sick line 4 | 0.00006 | **** |
| P5 | axenic infected | sick line 5 | 0.02258 | * |
| P5 | axenic infected | sick line 6 | 0.00014 | *** |
| P1 | non-selective line 5 | healthy line 3 | 0.91975 | |
| P1 | non-selective line 1 | sick line 4 | 0.16585 | |
| P1 | non-selective line 3 | sick line 4 | 0.19345 | |
| P1 | non-selective line 4 | sick line 4 | 0.0752 | |
| P1 | non-selective line 5 | sick line 3 | 0.52405 | |
| P1 | non-selective line 5 | sick line 4 | 0.00004 | **** |
| P2 | non-selective line 2 | healthy line 5 | 0.00044 | |
| P2 | non-selective line 5 | healthy line 3 | 0.65878 | |
| P3 | non-selective line 4 | healthy 2 | 0.08463 | |
| P3 | non-selective line 4 | sick line 5 | 0.02427 | * |
| P5 | non-selective line 6 | sick line 4 | 0.91975 | |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 20:** Bonferroni-corrected p-values of pairwise Welch's t-tests of disease severity scores at 14 dpi of selective passaged communities in the first passaging experiment.

| Comment | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|
| healthy vs sick selection | healthy line 1, P1 | sick line 1, P1 | 1 | |
| healthy vs sick selection | healthy line 1, P2 | sick line 1, P2 | 1 | |
| healthy vs sick selection | healthy line 1, P3 | sick line 1, P3 | 1 | |
| healthy vs sick selection | healthy line 1, P4 | sick line 1, P4 | 1 | |
| healthy vs sick selection | healthy line 1, P5 | sick line 1, P5 | 1 | |
| healthy vs sick selection | healthy line 2, P1 | sick line 2, P1 | 1 | |
| healthy vs sick selection | healthy line 2, P2 | sick line 2, P2 | 1 | |
| healthy vs sick selection | healthy line 2, P3 | sick line 2, P3 | 1 | |
| healthy vs sick selection | healthy line 2, P4 | sick line 2, P4 | 1 | |
| healthy vs sick selection | healthy line 2, P5 | sick line 2, P5 | 1 | |
| healthy vs sick selection | healthy line 3, P1 | sick line 3, P1 | 1 | |
| healthy vs sick selection | healthy line 3, P2 | sick line 3, P2 | 1 | |
| healthy vs sick selection | healthy line 3, P3 | sick line 3, P3 | 1 | |
| healthy vs sick selection | healthy line 3, P4 | sick line 3, P4 | 1 | |
| healthy vs sick selection | healthy line 3, P5 | sick line 3, P5 | 1 | |
| healthy vs sick selection | healthy line 4, P1 | sick line 4, P1 | 0.00596 | ** |
| healthy vs sick selection | healthy line 4, P2 | sick line 4, P2 | 1 | |
| healthy vs sick selection | healthy line 4, P3 | sick line 4, P3 | 1 | |
| healthy vs sick selection | healthy line 4, P4 | sick line 4, P4 | 1 | |
| healthy vs sick selection | healthy line 4, P5 | sick line 4, P5 | 1 | |
| healthy vs sick selection | healthy line 5, P1 | sick line 5, P1 | 1 | |
| healthy vs sick selection | healthy line 5, P2 | sick line 5, P2 | 1 | |
| healthy vs sick selection | healthy line 5, P3 | sick line 5, P3 | 1 | |
| healthy vs sick selection | healthy line 5, P4 | sick line 5, P4 | 0.98529 | |
| healthy vs sick selection | healthy line 5, P5 | sick line 5, P5 | 1 | |
| healthy vs sick selection | healthy line 6, P1 | sick line 6, P1 | 1 | |
| healthy vs sick selection | healthy line 6, P2 | sick line 6, P2 | 1 | |
| healthy vs sick selection | healthy line 6, P3 | sick line 6, P3 | 1 | |
| healthy vs sick selection | healthy line 6, P4 | sick line 6, P4 | 1 | |
| healthy vs sick selection | healthy line 6, P5 | sick line 6, P5 | 1 | |
| parental to healthy selection | parental line 1, P0 | healthy line 1, P1 | 0.1755 | |
| healthy line 1 | P1 | P2 | 1 | |
| healthy line 1 | P2 | P3 | 1 | |
| healthy line 1 | P3 | P4 | 1 | |
| healthy line 1 | P4 | P5 | 1 | |
| parental to healthy selection | parental line 2, P0 | healthy line 2, P1 | 1 | |
| healthy line 2 | P1 | P2 | 1 | |
| healthy line 2 | P2 | P3 | 1 | |
| healthy line 2 | P3 | P4 | 1 | |
| healthy line 2 | P4 | P5 | 1 | |
| parental to healthy selection | parental line 3, P0 | healthy line 3, P1 | 0.32895 | |
| healthy line 3 | P1 | P2 | 1 | |
| healthy line 3 | P2 | P3 | 1 | |
| healthy line 3 | P3 | P4 | 1 | |
| healthy line 3 | P4 | P5 | 1 | |
| parental to healthy selection | parental line 4, P0 | healthy line 4, P1 | 1 | |
| healthy line 4 | P1 | P2 | 1 | |
| healthy line 4 | P2 | P3 | 1 | |
| healthy line 4 | P3 | P4 | 1 | |
| healthy line 4 | P4 | P5 | 1 | |
| parental to healthy selection | parental line 5, P0 | healthy line 5, P1 | 1 | |
| healthy line 5 | P1 | P2 | 0.09495 | |
| healthy line 5 | P2 | P3 | 1 | |
| healthy line 5 | P3 | P4 | 1 | |
| healthy line 5 | P4 | P5 | 1 | |
| parental to healthy selection | parental line 6, P0 | healthy line 6, P1 | 1 | |
| healthy line 6 | P1 | P2 | 1 | |
| healthy line 6 | P2 | P3 | 1 | |
| healthy line 6 | P3 | P4 | 1 | |
| healthy line 6 | P4 | P5 | 1 | |
| parental to sick selection | parental line 1, P0 | sick line 1, P1 | 1 | |
| sick line 1 | P1 | P2 | 0.61108 | |
| sick line 1 | P2 | P3 | 1 | |
| sick line 1 | P3 | P4 | 1 | |

Supplemental Table 20 continued

| | | | | |
|---|---|---|---|---|
| sick line 1 | P4 | P5 | 1 | |
| parental to sick selection | parental line 2, P0 | sick line 2, P1 | 1 | |
| sick line 2 | P1 | P2 | 1 | |
| sick line 2 | P2 | P3 | 1 | |
| sick line 2 | P3 | P4 | 1 | |
| sick line 2 | P4 | P5 | 1 | |
| parental to sick selection | parental line 3, P0 | sick line 3, P1 | 0.02262 | * |
| sick line 3 | P1 | P2 | 1 | |
| sick line 3 | P2 | P3 | 1 | |
| sick line 3 | P3 | P4 | 1 | |
| sick line 3 | P4 | P5 | 1 | |
| parental to sick selection | parental line 4, P0 | sick line 4, P1 | 1 | |
| sick line 4 | P1 | P2 | 1 | |
| sick line 4 | P2 | P3 | 1 | |
| sick line 4 | P3 | P4 | 1 | |
| sick line 4 | P4 | P5 | 1 | |
| parental to sick selection | parental line 5, P0 | sick line 5, P1 | 1 | |
| sick line 5 | P1 | P2 | 1 | |
| sick line 5 | P2 | P3 | 1 | |
| sick line 5 | P3 | P4 | 1 | |
| sick line 5 | P4 | P5 | 1 | |
| parental to sick selection | parental line 6, P0 | sick line 6, P1 | 1 | |
| sick line 6 | P1 | P2 | 1 | |
| sick line 6 | P2 | P3 | 1 | |
| sick line 6 | P3 | P4 | 1 | |
| sick line 6 | P4 | P5 | 1 | |
| P0 | start community infected | parental line 1 | 1 | |
| P0 | start community infected | parental line 2 | 1 | |
| P0 | start community infected | parental line 3 | 1 | |
| P0 | start community infected | parental line 4 | 1 | |
| P0 | start community infected | parental line 5 | 1 | |
| P0 | start community infected | parental line 6 | 1 | |
| P1 | start community infected | healthy line 1 | 1 | |
| P1 | start community infected | healthy line 2 | 1 | |
| P1 | start community infected | healthy line 3 | 1 | |
| P1 | start community infected | healthy line 4 | 1 | |
| P1 | start community infected | healthy line 5 | 1 | |
| P1 | start community infected | healthy line 6 | 1 | |
| P1 | start community infected | sick line 1 | 1 | |
| P1 | start community infected | sick line 2 | 1 | |
| P1 | start community infected | sick line 3 | 1 | |
| P1 | start community infected | sick line 4 | 0.01054 | * |
| P1 | start community infected | sick line 5 | 1 | |
| P1 | start community infected | sick line 6 | 1 | |
| P2 | start community infected | healthy line 1 | 0.02695 | * |
| P2 | start community infected | healthy line 2 | 1 | |
| P2 | start community infected | healthy line 3 | 1 | |
| P2 | start community infected | healthy line 4 | 1 | |
| P2 | start community infected | healthy line 5 | 0.00095 | *** |
| P2 | start community infected | healthy line 6 | 1 | |
| P2 | start community infected | sick line 1 | 0.05019 | |
| P2 | start community infected | sick line 2 | 1 | |
| P2 | start community infected | sick line 3 | 1 | |
| P2 | start community infected | sick line 4 | 0.55142 | |
| P2 | start community infected | sick line 5 | 1 | |
| P2 | start community infected | sick line 6 | 1 | |
| P3 | start community infected | healthy line 1 | 1 | |
| P3 | start community infected | healthy line 2 | 1 | |
| P3 | start community infected | healthy line 3 | 1 | |
| P3 | start community infected | healthy line 4 | 1 | |
| P3 | start community infected | healthy line 5 | 1 | |
| P3 | start community infected | healthy line 6 | 1 | |
| P3 | start community infected | sick line 1 | 1 | |
| P3 | start community infected | sick line 2 | 1 | |
| P3 | start community infected | sick line 3 | 1 | |
| P3 | start community infected | sick line 4 | 1 | |
| P3 | start community infected | sick line 5 | 1 | |

Supplemental Table 20 continued

| | | | | |
|---|---|---|---|---|
| P3 | start community infected | sick line 6 | 1 | |
| P4 | start community infected | healthy line 1 | 1 | |
| P4 | start community infected | healthy line 2 | 1 | |
| P4 | start community infected | healthy line 3 | 1 | |
| P4 | start community infected | healthy line 4 | 1 | |
| P4 | start community infected | healthy line 5 | 0.8852 | |
| P4 | start community infected | healthy line 6 | 0.01424 | * |
| P4 | start community infected | sick line 1 | 0.00389 | ** |
| P4 | start community infected | sick line 2 | 1 | |
| P4 | start community infected | sick line 3 | 1 | |
| P4 | start community infected | sick line 4 | 1 | |
| P4 | start community infected | sick line 5 | 1 | |
| P4 | start community infected | sick line 6 | 0.51482 | |
| P5 | start community infected | healthy line 1 | 1 | |
| P5 | start community infected | healthy line 2 | 1 | |
| P5 | start community infected | healthy line 3 | 1 | |
| P5 | start community infected | healthy line 4 | 1 | |
| P5 | start community infected | healthy line 5 | 1 | |
| P5 | start community infected | healthy line 6 | 1 | |
| P5 | start community infected | sick line 1 | 1 | |
| P5 | start community infected | sick line 2 | 1 | |
| P5 | start community infected | sick line 3 | 1 | |
| P5 | start community infected | sick line 4 | 1 | |
| P5 | start community infected | sick line 5 | 1 | |
| P5 | start community infected | sick line 6 | 1 | |
| P0 | axenic mock-infected | parental line 1 | 1 | |
| P0 | axenic mock-infected | parental line 2 | 1 | |
| P0 | axenic mock-infected | parental line 3 | 1 | |
| P0 | axenic mock-infected | parental line 4 | 0.23451 | |
| P0 | axenic mock-infected | parental line 5 | 1 | |
| P0 | axenic mock-infected | parental line 6 | 1 | |
| P1 | axenic mock-infected | healthy line 1 | 0.00994 | ** |
| P1 | axenic mock-infected | healthy line 2 | 1 | |
| P1 | axenic mock-infected | healthy line 3 | 0.0215 | * |
| P1 | axenic mock-infected | healthy line 4 | 1 | |
| P1 | axenic mock-infected | healthy line 5 | 1 | |
| P1 | axenic mock-infected | healthy line 6 | 1 | |
| P1 | axenic mock-infected | sick line 1 | 1 | |
| P1 | axenic mock-infected | sick line 2 | 1 | |
| P1 | axenic mock-infected | sick line 3 | 0.00086 | *** |
| P1 | axenic mock-infected | sick line 4 | 0 | **** |
| P1 | axenic mock-infected | sick line 5 | 1 | |
| P1 | axenic mock-infected | sick line 6 | 1 | |
| P2 | axenic mock-infected | healthy line 1 | 0.01029 | * |
| P2 | axenic mock-infected | healthy line 2 | 0.67642 | |
| P2 | axenic mock-infected | healthy line 3 | 1 | |
| P2 | axenic mock-infected | healthy line 4 | 1 | |
| P2 | axenic mock-infected | healthy line 5 | 0.00032 | *** |
| P2 | axenic mock-infected | healthy line 6 | 1 | |
| P2 | axenic mock-infected | sick line 1 | 0.01962 | * |
| P2 | axenic mock-infected | sick line 2 | 1 | |
| P2 | axenic mock-infected | sick line 3 | 1 | |
| P2 | axenic mock-infected | sick line 4 | 0.24303 | |
| P2 | axenic mock-infected | sick line 5 | 1 | |
| P2 | axenic mock-infected | sick line 6 | 1 | |
| P3 | axenic mock-infected | healthy line 1 | 0.09252 | |
| P3 | axenic mock-infected | healthy line 2 | 1 | |
| P3 | axenic mock-infected | healthy line 3 | 0.17146 | |
| P3 | axenic mock-infected | healthy line 4 | 1 | |
| P3 | axenic mock-infected | healthy line 5 | 0.02556 | * |
| P3 | axenic mock-infected | healthy line 6 | 0.09252 | |
| P3 | axenic mock-infected | sick line 1 | 1 | |
| P3 | axenic mock-infected | sick line 2 | 1 | |
| P3 | axenic mock-infected | sick line 3 | 0.22159 | |
| P3 | axenic mock-infected | sick line 4 | 1 | |
| P3 | axenic mock-infected | sick line 5 | 1 | |
| P3 | axenic mock-infected | sick line 6 | 1 | |

Supplemental Table 20 continued

| | | | | |
|---|---|---|---|---|
| P4 | axenic mock-infected | healthy line 1 | 1 | |
| P4 | axenic mock-infected | healthy line 2 | 1 | |
| P4 | axenic mock-infected | healthy line 3 | 1 | |
| P4 | axenic mock-infected | healthy line 4 | 1 | |
| P4 | axenic mock-infected | healthy line 5 | 0.06809 | |
| P4 | axenic mock-infected | healthy line 6 | 0.00066 | *** |
| P4 | axenic mock-infected | sick line 1 | 0.00016 | *** |
| P4 | axenic mock-infected | sick line 2 | 1 | |
| P4 | axenic mock-infected | sick line 3 | 1 | |
| P4 | axenic mock-infected | sick line 4 | 1 | |
| P4 | axenic mock-infected | sick line 5 | 1 | |
| P4 | axenic mock-infected | sick line 6 | 0.03685 | * |
| P5 | axenic mock-infected | healthy line 1 | 0.22159 | |
| P5 | axenic mock-infected | healthy line 2 | 0.06809 | |
| P5 | axenic mock-infected | healthy line 3 | 0.67642 | |
| P5 | axenic mock-infected | healthy line 4 | 1 | |
| P5 | axenic mock-infected | healthy line 5 | 1 | |
| P5 | axenic mock-infected | healthy line 6 | 0.00531 | ** |
| P5 | axenic mock-infected | sick line 1 | 1 | |
| P5 | axenic mock-infected | sick line 2 | 1 | |
| P5 | axenic mock-infected | sick line 3 | 1 | |
| P5 | axenic mock-infected | sick line 4 | 1 | |
| P5 | axenic mock-infected | sick line 5 | 1 | |
| P5 | axenic mock-infected | sick line 6 | 1 | |
| P0 | axenic infected | parental line 1 | 0 | **** |
| P0 | axenic infected | parental line 2 | 0 | **** |
| P0 | axenic infected | parental line 3 | 0 | **** |
| P0 | axenic infected | parental line 4 | 0.00004 | **** |
| P0 | axenic infected | parental line 5 | 0 | **** |
| P0 | axenic infected | parental line 6 | 0 | **** |
| P1 | axenic infected | healthy line 1 | 0.00001 | **** |
| P1 | axenic infected | healthy line 2 | 0 | **** |
| P1 | axenic infected | healthy line 3 | 0 | **** |
| P1 | axenic infected | healthy line 4 | 0 | **** |
| P1 | axenic infected | healthy line 5 | 0 | **** |
| P1 | axenic infected | healthy line 6 | 0 | **** |
| P1 | axenic infected | sick line 1 | 0 | **** |
| P1 | axenic infected | sick line 2 | 0 | **** |
| P1 | axenic infected | sick line 3 | 0.00019 | *** |
| P1 | axenic infected | sick line 4 | 1 | |
| P1 | axenic infected | sick line 5 | 0 | **** |
| P1 | axenic infected | sick line 6 | 0 | **** |
| P2 | axenic infected | healthy line 1 | 1 | |
| P2 | axenic infected | healthy line 2 | 0.05019 | |
| P2 | axenic infected | healthy line 3 | 0 | **** |
| P2 | axenic infected | healthy line 4 | 0 | **** |
| P2 | axenic infected | healthy line 5 | 1 | |
| P2 | axenic infected | healthy line 6 | 0.00379 | ** |
| P2 | axenic infected | sick line 1 | 1 | |
| P2 | axenic infected | sick line 2 | 0 | **** |
| P2 | axenic infected | sick line 3 | 0.00017 | *** |
| P2 | axenic infected | sick line 4 | 0.36765 | |
| P2 | axenic infected | sick line 5 | 0.00046 | *** |
| P2 | axenic infected | sick line 6 | 0.00002 | **** |
| P3 | axenic infected | healthy line 1 | 0.08879 | |
| P3 | axenic infected | healthy line 2 | 0 | **** |
| P3 | axenic infected | healthy line 3 | 0.04781 | * |
| P3 | axenic infected | healthy line 4 | 0.00016 | *** |
| P3 | axenic infected | healthy line 5 | 0.29135 | |
| P3 | axenic infected | healthy line 6 | 0.08879 | |
| P3 | axenic infected | sick line 1 | 0.00169 | ** |
| P3 | axenic infected | sick line 2 | 0 | **** |
| P3 | axenic infected | sick line 3 | 0.02279 | * |
| P3 | axenic infected | sick line 4 | 0 | **** |
| P3 | axenic infected | sick line 5 | 0 | **** |
| P3 | axenic infected | sick line 6 | 0 | **** |
| P4 | axenic infected | healthy line 1 | 0.00015 | |

Supplemental Table 20 continued

| | | | | |
|---|---|---|---|---|
| P4 | axenic infected | healthy line 2 | 0 | **** |
| P4 | axenic infected | healthy line 3 | 0 | **** |
| P4 | axenic infected | healthy line 4 | 0 | **** |
| P4 | axenic infected | healthy line 5 | 0.03685 | * |
| P4 | axenic infected | healthy line 6 | 1 | |
| P4 | axenic infected | sick line 1 | 1 | |
| P4 | axenic infected | sick line 2 | 0 | **** |
| P4 | axenic infected | sick line 3 | 0.00015 | *** |
| P4 | axenic infected | sick line 4 | 0.0003 | *** |
| P4 | axenic infected | sick line 5 | 0 | **** |
| P4 | axenic infected | sick line 6 | 0.06809 | |
| P5 | axenic infected | healthy line 1 | 0.00135 | ** |
| P5 | axenic infected | healthy line 2 | 0.00531 | ** |
| P5 | axenic infected | healthy line 3 | 0.00032 | *** |
| P5 | axenic infected | healthy line 4 | 0 | **** |
| P5 | axenic infected | healthy line 5 | 0 | **** |
| P5 | axenic infected | healthy line 6 | 0.06809 | |
| P5 | axenic infected | sick line 1 | 0 | **** |
| P5 | axenic infected | sick line 2 | 0 | **** |
| P5 | axenic infected | sick line 3 | 0 | **** |
| P5 | axenic infected | sick line 4 | 0 | **** |
| P5 | axenic infected | sick line 5 | 0 | **** |
| P5 | axenic infected | sick line 6 | 0 | **** |
| P1 | non-selective line 1 | sick line 4 | 0.04798 | * |
| P1 | non-selective line 2 | sick line 4 | 0.3263 | |
| P1 | non-selective line 3 | sick line 4 | 0.00046 | *** |
| P1 | non-selective line 4 | sick line 4 | 0.00787 | ** |
| P1 | non-selective line 5 | sick line 4 | 0.00013 | *** |
| P1 | non-selective line 6 | sick line 4 | 0.01217 | * |
| P2 | non-selective line 2 | healthy line 5 | 0.04679 | * |
| P4 | non-selective line 6 | sick line 5 | 0.15615 | |
| P5 | non-selective line 1 | healthy line 2 | 0.32895 | |
| P5 | non-selective line 1 | healthy line 6 | 0.02262 | * |
| P5 | non-selective line 4 | sick line 2 | 0.10044 | |
| P5 | non-selective line 6 | sick line 2 | 0.08977 | |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 21:** Bonferroni-corrected p-values of pairwise Welch's t-tests of bacterial colonization of selective passaged communities. Note that commensal data of passage 5 is missing.

| colonization by | Comment | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|---|
| *Pst* abundance | P5 | start community | non-selective | 1 | |
| *Pst* abundance | P5 | start community | healthy selection | 0.26004 | |
| *Pst* abundance | P5 | start community | sick selection | 1 | |
| *Pst* abundance | P5 | non-selective | healthy selection | 0.10754 | |
| *Pst* abundance | P5 | non-selective | sick selection | 1 | |
| *Pst* abundance | P5 | healthy selection | sick selection | 0.10392 | |
| *Pst* abundance | P5 | start community | non-selective | 1 | |
| *Pst* abundance | P5 | start community | healthy line 1 | 1 | |
| *Pst* abundance | P5 | start community | healthy line 2 | 0.93939 | |
| *Pst* abundance | P5 | start community | healthy line 3 | 1 | |
| *Pst* abundance | P5 | start community | healthy line 4 | 0.18364 | |
| *Pst* abundance | P5 | start community | healthy line 5 | 1 | |
| *Pst* abundance | P5 | start community | healthy line 6 | 1 | |
| *Pst* abundance | P5 | start community | sick line 1 | 1 | |
| *Pst* abundance | P5 | start community | sick line 2 | 1 | |
| *Pst* abundance | P5 | start community | sick line 3 | 1 | |
| *Pst* abundance | P5 | start community | sick line 4 | 1 | |
| *Pst* abundance | P5 | start community | sick line 5 | 1 | |
| *Pst* abundance | P5 | start community | sick line 6 | 1 | |
| *Pst* abundance | P5 | non-selective | healthy line 1 | 1 | |
| *Pst* abundance | P5 | non-selective | healthy line 2 | 0.9274 | |
| *Pst* abundance | P5 | non-selective | healthy line 3 | 1 | |
| *Pst* abundance | P5 | non-selective | healthy line 4 | 0.08841 | |
| *Pst* abundance | P5 | non-selective | healthy line 5 | 1 | |
| *Pst* abundance | P5 | non-selective | healthy line 6 | 1 | |
| *Pst* abundance | P5 | non-selective | sick line 1 | 1 | |
| *Pst* abundance | P5 | non-selective | sick line 2 | 1 | |
| *Pst* abundance | P5 | non-selective | sick line 3 | 1 | |
| *Pst* abundance | P5 | non-selective | sick line 4 | 1 | |
| *Pst* abundance | P5 | non-selective | sick line 5 | 1 | |
| *Pst* abundance | P5 | non-selective | sick line 6 | 1 | |
| *Pst* abundance | P5 | healthy line 2 | sick line 1 | 0.71373 | |
| *Pst* abundance | P5 | healthy line 2 | sick line 2 | 0.89254 | |
| *Pst* abundance | P5 | healthy line 3 | healthy line 4 | 0.49016 | |
| *Pst* abundance | P5 | healthy line 4 | healthy line 5 | 0.16279 | |
| *Pst* abundance | P5 | healthy line 4 | sick line 1 | 0.1108 | |
| *Pst* abundance | P5 | healthy line 4 | sick line 2 | 0.14178 | |
| *Pst* abundance | P5 | healthy line 4 | sick line 4 | 0.83349 | |
| *Pst* abundance | P5 | healthy line 4 | sick line 5 | 0.46483 | |
| *Pst* | P0 | axenic | healthy selection | 0 | **** |
| *Pst* | P0 | axenic | sick selection | 0 | **** |
| *Pst* | P0 | start community | healthy selection | 1 | |
| *Pst* | P0 | start community | sick selection | 1 | |
| *Pst* | P0 | non-selective with *Pst* | healthy selection | 1 | |
| *Pst* | P0 | non-selective with *Pst* | sick selection | 1 | |
| *Pst* | P0 | healthy selection | sick selection | 0.03259 | * |
| *Pst* | P1 | axenic | healthy selection | 0 | **** |
| *Pst* | P1 | axenic | sick selection | 0.00412 | ** |
| *Pst* | P1 | start community | healthy selection | 1 | |
| *Pst* | P1 | start community | sick selection | 0.00198 | ** |
| *Pst* | P1 | non-selective with *Pst* | healthy selection | 0.0009 | *** |
| *Pst* | P1 | non-selective with *Pst* | sick selection | 1 | |
| *Pst* | P1 | healthy selection | sick selection | 0 | **** |
| *Pst* | P2 | axenic | healthy selection | 0 | **** |
| *Pst* | P2 | axenic | sick selection | 1 | |
| *Pst* | P2 | start community | healthy selection | 1 | |
| *Pst* | P2 | start community | sick selection | 0 | **** |
| *Pst* | P2 | non-selective with *Pst* | healthy selection | 0.23787 | |
| *Pst* | P2 | non-selective with *Pst* | sick selection | 1 | |
| *Pst* | P2 | healthy selection | sick selection | 0.01826 | * |
| *Pst* | P3 | axenic | healthy selection | 0 | **** |
| *Pst* | P3 | axenic | sick selection | 1 | |
| *Pst* | P3 | start community | healthy selection | 1 | |
| *Pst* | P3 | start community | sick selection | 0 | **** |
| *Pst* | P3 | non-selective with *Pst* | healthy selection | 1 | |
| *Pst* | P3 | non-selective with *Pst* | sick selection | 1 | |
| *Pst* | P3 | healthy selection | sick selection | 0.00171 | ** |
| *Pst* | P4 | axenic | healthy selection | 0 | **** |
| *Pst* | P4 | axenic | sick selection | 0.16633 | |
| *Pst* | P4 | start community | healthy selection | 1 | |
| *Pst* | P4 | start community | sick selection | 0.00001 | **** |
| *Pst* | P4 | non-selective with *Pst* | healthy selection | 0.23848 | |
| *Pst* | P4 | non-selective with *Pst* | sick selection | 1 | |
| *Pst* | P4 | healthy selection | sick selection | 1 | |
| *Pst* | P5 | axenic | healthy selection | 0 | **** |
| *Pst* | P5 | axenic | sick selection | 0.47382 | |
| *Pst* | P5 | start community | healthy selection | 1 | |

1

Supplemental Table 21 continued

| | | | | | |
|---|---|---|---|---|---|
| *Pst* | P5 | start community | sick selection | 0.00266 | ** |
| *Pst* | P5 | non-selective with *Pst* | healthy selection | 0.57877 | |
| *Pst* | P5 | non-selective with *Pst* | sick selection | 1 | |
| *Pst* | P5 | healthy selection | sick selection | 0.00221 | ** |
| *Pst* | healthy selection | P0 | P1 | 1 | |
| *Pst* | healthy selection | P1 | P2 | 1 | |
| *Pst* | healthy selection | P2 | P3 | 1 | |
| *Pst* | healthy selection | P3 | P4 | 1 | |
| *Pst* | healthy selection | P4 | P5 | 1 | |
| *Pst* | healthy selection | P0 | P5 | 1 | |
| *Pst* | healthy selection | P1 | P5 | 1 | |
| *Pst* | sick selection | P0 | P1 | 0.68397 | |
| *Pst* | sick selection | P1 | P2 | 1 | |
| *Pst* | sick selection | P2 | P3 | 1 | |
| *Pst* | sick selection | P3 | P4 | 1 | |
| *Pst* | sick selection | P4 | P5 | 1 | |
| *Pst* | sick selection | P0 | P5 | 1 | |
| *Pst* | sick selection | P1 | P5 | 1 | |
| Commensals | P0 | start community | healthy selection | 1 | |
| Commensals | P0 | start community | sick selection | 1 | |
| Commensals | P0 | non-selective with *Pst* | healthy selection | 1 | |
| Commensals | P0 | non-selective with *Pst* | sick selection | 1 | |
| Commensals | P0 | healthy selection | sick selection | 1 | |
| Commensals | P1 | start community | healthy selection | 1 | |
| Commensals | P1 | start community | sick selection | 0.33816 | |
| Commensals | P1 | non-selective with *Pst* | healthy selection | 1 | |
| Commensals | P1 | non-selective with *Pst* | sick selection | 1 | |
| Commensals | P1 | healthy selection | sick selection | 0.00431 | ** |
| Commensals | P2 | start community | healthy selection | 1 | |
| Commensals | P2 | start community | sick selection | 1 | |
| Commensals | P2 | non-selective with *Pst* | healthy selection | 1 | |
| Commensals | P2 | non-selective with *Pst* | sick selection | 1 | |
| Commensals | P2 | healthy selection | sick selection | 1 | |
| Commensals | P3 | start community | healthy selection | 1 | |
| Commensals | P3 | start community | sick selection | 1 | |
| Commensals | P3 | non-selective with *Pst* | healthy selection | 1 | |
| Commensals | P3 | non-selective with *Pst* | sick selection | 1 | |
| Commensals | P3 | healthy selection | sick selection | 0.64689 | |
| Commensals | P4 | start community | healthy selection | 1 | |
| Commensals | P4 | start community | sick selection | 1 | |
| Commensals | P4 | non-selective with *Pst* | healthy selection | 1 | |
| Commensals | P4 | non-selective with *Pst* | sick selection | 1 | |
| Commensals | P4 | healthy selection | sick selection | 1 | |
| Commensals | healthy selection | P0 | P1 | 1 | |
| Commensals | healthy selection | P1 | P2 | 1 | |
| Commensals | healthy selection | P2 | P3 | 1 | |
| Commensals | healthy selection | P3 | P4 | 1 | |
| Commensals | healthy selection | P1 | P4 | 1 | |
| Commensals | sick selection | P0 | P1 | 1 | |
| Commensals | sick selection | P1 | P2 | 1 | |
| Commensals | sick selection | P2 | P3 | 1 | |
| Commensals | sick selection | P3 | P4 | 1 | |
| Commensals | sick selection | P1 | P4 | 1 | |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 22:** Changes in relative abundance of ASVs comparing healthy and sick selection passaging over passages.

| Passage | Group 1 | Group 2 | ASV Representative | fold change | p-value | significance [1] |
|---|---|---|---|---|---|---|
| P1 through P5 | healthy selection | sick selection | Leaf3 | 49.6 | 0.0143 | * |
| P1 through P5 | healthy selection | sick selection | Leaf179 | 0.677 | 0.0188 | * |
| P1 through P5 | healthy selection | sick selection | Leaf21 | 9.9 | 0.0188 | * |
| P1 through P5 | healthy selection | sick selection | Leaf231 | 0.0133 | 0.0000236 | **** |
| P1 through P5 | healthy selection | sick selection | Leaf16 | 0.11 | 0.0278 | * |
| P1 through P5 | healthy selection | sick selection | Leaf119 | 0.125 | 0.00668 | * |
| P1 through P5 | healthy selection | sick selection | Leaf311 | 29.5 | 7.62E-09 | **** |
| P1 through P5 | healthy selection | sick selection | Leaf280 | 56.4 | 0.0000122 | **** |
| P1 through P5 | healthy selection | sick selection | Leaf159 | 2940 | 0.00000277 | **** |
| P1 through P5 | healthy selection | sick selection | Leaf456 | 0.00383 | 0.0000906 | **** |
| P0 | healthy selection | sick selection | Leaf3 | 22.8 | 0.00000261 | **** |
| P0 | healthy selection | sick selection | Leaf127 | 13.3 | 0.0169 | * |
| P0 | healthy selection | sick selection | Leaf82 | 7.21 | 0.00175 | ** |
| P1 | healthy selection | sick selection | Leaf3 | 430 | 0.000493 | *** |
| P1 | healthy selection | sick selection | Leaf58 | 0.023 | 0.00237 | ** |
| P1 | healthy selection | sick selection | Leaf122 | 0.0173 | 0.00781 | ** |
| P1 | healthy selection | sick selection | Leaf231 | 0.00175 | 7.44E-13 | **** |
| P1 | healthy selection | sick selection | Leaf119 | 0.0219 | 0.00000746 | **** |
| P1 | healthy selection | sick selection | Leaf15 | 0.0503 | 0.0177 | * |
| P1 | healthy selection | sick selection | Leaf91 | 0.049 | 0.0126 | * |
| P1 | healthy selection | sick selection | Leaf98 | 0.0362 | 0.00781 | ** |
| P1 | healthy selection | sick selection | Leaf159 | 57.4 | 0.0266 | * |
| P1 | healthy selection | sick selection | Leaf201 | 51.8 | 0.0108 | * |
| P2 | healthy selection | sick selection | Leaf3 | 1220 | 0.0047 | ** |
| P2 | healthy selection | sick selection | Leaf122 | 0.0347 | 0.0105 | * |
| P2 | healthy selection | sick selection | Leaf233 | 110 | 0.0000896 | **** |
| P2 | healthy selection | sick selection | Leaf78 | 66.4 | 0.00261 | ** |
| P2 | healthy selection | sick selection | Leaf88 | 334 | 0.00486 | ** |
| P2 | healthy selection | sick selection | Leaf456 | 0.000000115 | 1.65E-17 | **** |
| P2 | healthy selection | sick selection | Leaf82 | 13900000 | 8.38E-18 | **** |
| P3 | healthy selection | sick selection | Leaf3 | 309 | 0.00189 | ** |
| P3 | healthy selection | sick selection | Leaf91 | 0.00444 | 0.00084 | **** |
| P3 | healthy selection | sick selection | Leaf361 | 0.000539 | 0.000307 | **** |
| P3 | healthy selection | sick selection | Leaf83 | 0.000000156 | 6.36E-14 | **** |
| P5 | healthy selection | sick selection | Leaf119 | 0.00802 | 0.00000685 | **** |
| P5 | healthy selection | sick selection | Leaf311 | 48.5 | 0.00236 | ** |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 23:** Bonferroni-corrected p-values of pairwise Welch's t-tests of community diversity scores of selectively passaged communities in first passaging experiment.

| Score | Comment | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|---|
| Shannon's Diversity | P0 | start community | unchallenged | 1 | |
| Shannon's Diversity | P1 | start community | unchallenged | 0 | **** |
| Shannon's Diversity | P2 | start community | unchallenged | 0 | **** |
| Shannon's Diversity | P3 | start community | unchallenged | 0 | **** |
| Shannon's Diversity | P4 | start community | unchallenged | 0 | **** |
| Shannon's Diversity | P5 | start community | unchallenged | 0 | **** |
| Shannon's Diversity | unchallenged | P0 | P1 | 0 | **** |
| Shannon's Diversity | P1 | non-selective | healthy selection | 1 | |
| Shannon's Diversity | P1 | non-selective | sick selection | 1 | |
| Shannon's Diversity | P1 | healthy selection | sick selection | 1 | |
| Shannon's Diversity | P2 | start community | healthy selection | 0 | **** |
| Shannon's Diversity | P2 | start community | sick selection | 0 | **** |
| Shannon's Diversity | P2 | non-selective | healthy selection | 1 | |
| Shannon's Diversity | P2 | non-selective | sick selection | 1 | |
| Shannon's Diversity | P2 | healthy selection | sick selection | 1 | |
| Shannon's Diversity | P3 | start community | healthy selection | 0 | **** |
| Shannon's Diversity | P3 | start community | sick selection | 0 | **** |
| Shannon's Diversity | P3 | non-selective | healthy selection | 1 | |
| Shannon's Diversity | P3 | non-selective | sick selection | 1 | |
| Shannon's Diversity | P3 | healthy selection | sick selection | 1 | |
| Shannon's Diversity | P5 | start community | healthy selection | 0 | **** |
| Shannon's Diversity | P5 | start community | sick selection | 0 | **** |
| Shannon's Diversity | P5 | non-selective | healthy selection | 1 | |
| Shannon's Diversity | P5 | non-selective | sick selection | 1 | |
| Shannon's Diversity | P5 | healthy selection | sick selection | 1 | |
| Shannon's Diversity | healthy selection | P0 | P1 | 0 | **** |
| Shannon's Diversity | healthy selection | P1 | P2 | 1 | |
| Shannon's Diversity | healthy selection | P2 | P3 | 1 | |
| Shannon's Diversity | healthy selection | P3 | P5 | 1 | |
| Shannon's Diversity | healthy selection | P1 | P5 | 0.00001 | **** |
| Shannon's Diversity | sick selection | P0 | P1 | 0 | **** |
| Shannon's Diversity | sick selection | P1 | P2 | 1 | |
| Shannon's Diversity | sick selection | P2 | P3 | 1 | |
| Shannon's Diversity | sick selection | P3 | P5 | 1 | |
| Shannon's Diversity | sick selection | P1 | P5 | 0.75661 | |
| Pielou's Evenness | P0 | start community | healthy selection | 1 | |
| Pielou's Evenness | P0 | start community | sick selection | 1 | |
| Pielou's Evenness | P0 | non-selective | healthy selection | 1 | |
| Pielou's Evenness | P0 | non-selective | sick selection | 1 | |
| Pielou's Evenness | P0 | healthy selection | sick selection | 1 | |
| Pielou's Evenness | P1 | start community | healthy selection | 1 | |
| Pielou's Evenness | P1 | start community | sick selection | 1 | |
| Pielou's Evenness | P1 | non-selective | healthy selection | 1 | |
| Pielou's Evenness | P1 | non-selective | sick selection | 1 | |
| Pielou's Evenness | P1 | healthy selection | sick selection | 1 | |
| Pielou's Evenness | P2 | start community | healthy selection | 1 | |
| Pielou's Evenness | P2 | start community | sick selection | 1 | |
| Pielou's Evenness | P2 | non-selective | healthy selection | 1 | |
| Pielou's Evenness | P2 | non-selective | sick selection | 1 | |
| Pielou's Evenness | P2 | healthy selection | sick selection | 1 | |
| Pielou's Evenness | P3 | start community | healthy selection | 1 | |
| Pielou's Evenness | P3 | start community | sick selection | 1 | |
| Pielou's Evenness | P3 | non-selective | healthy selection | 1 | |
| Pielou's Evenness | P3 | non-selective | sick selection | 1 | |
| Pielou's Evenness | P3 | healthy selection | sick selection | 1 | |
| Pielou's Evenness | P5 | start community | healthy selection | 1 | |
| Pielou's Evenness | P5 | start community | sick selection | 1 | |
| Pielou's Evenness | P5 | non-selective | healthy selection | 1 | |
| Pielou's Evenness | P5 | non-selective | sick selection | 1 | |
| Pielou's Evenness | P5 | healthy selection | sick selection | 1 | |
| Pielou's Evenness | healthy selection | P0 | P1 | 1 | |
| Pielou's Evenness | healthy selection | P1 | P2 | 1 | |
| Pielou's Evenness | healthy selection | P2 | P3 | 1 | |
| Pielou's Evenness | healthy selection | P3 | P5 | 1 | |
| Pielou's Evenness | healthy selection | P1 | P5 | 1 | |
| Pielou's Evenness | sick selection | P0 | P1 | 1 | |
| Pielou's Evenness | sick selection | P1 | P2 | 1 | |
| Pielou's Evenness | sick selection | P2 | P3 | 1 | |
| Pielou's Evenness | sick selection | P3 | P5 | 1 | |

Supplemental Table 23 continued

| | | | | | |
|---|---|---|---|---|---|
| Pielou's Evenness | sick selection | P1 | P5 | 1 | |
| Richness | P0 | start community | healthy selection | 1 | |
| Richness | P0 | start community | sick selection | 1 | |
| Richness | P0 | non-selective | healthy selection | 1 | |
| Richness | P0 | non-selective | sick selection | 1 | |
| Richness | P0 | healthy selection | sick selection | 1 | |
| Richness | P1 | start community | healthy selection | 0 | **** |
| Richness | P1 | start community | sick selection | 0 | **** |
| Richness | P1 | non-selective | healthy selection | 1 | |
| Richness | P1 | non-selective | sick selection | 1 | |
| Richness | P1 | healthy selection | sick selection | 1 | |
| Richness | P2 | start community | healthy selection | 0 | **** |
| Richness | P2 | start community | sick selection | 0 | **** |
| Richness | P2 | non-selective | healthy selection | 1 | |
| Richness | P2 | non-selective | sick selection | 1 | |
| Richness | P2 | healthy selection | sick selection | 1 | |
| Richness | P3 | start community | healthy selection | 0 | **** |
| Richness | P3 | start community | sick selection | 0 | **** |
| Richness | P3 | non-selective | healthy selection | 1 | |
| Richness | P3 | non-selective | sick selection | 1 | |
| Richness | P3 | healthy selection | sick selection | 1 | |
| Richness | P5 | start community | healthy selection | 0 | **** |
| Richness | P5 | start community | sick selection | 0 | **** |
| Richness | P5 | non-selective | healthy selection | 1 | |
| Richness | P5 | non-selective | sick selection | 1 | |
| Richness | P5 | healthy selection | sick selection | 1 | |
| Richness | healthy selection | P0 | P1 | 0 | **** |
| Richness | healthy selection | P1 | P2 | 0.00053 | *** |
| Richness | healthy selection | P2 | P3 | 1 | |
| Richness | healthy selection | P3 | P5 | 1 | |
| Richness | healthy selection | P1 | P5 | 0 | **** |
| Richness | sick selection | P0 | P1 | 0 | **** |
| Richness | sick selection | P1 | P2 | 1 | |
| Richness | sick selection | P2 | P3 | 1 | |
| Richness | sick selection | P3 | P5 | 1 | |
| Richness | sick selection | P1 | P5 | 0.37646 | |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 24:** Bonferroni-corrected p-values of pairwise Welch's t-tests of pathogen colonization of parental passage in the second passaging experiment.

| Treatment | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|
| SynCom-210 | 14dpi, healthy selection | 14dpi, sick selection | 1 | |
| SynCom-210 | 14dpi, healthy selection | 7dpi, healthy selection | 1 | |
| SynCom-210 | 14dpi, healthy selection | 7dpi, sick selection | 0.0847 | |
| SynCom-210 | 14dpi, sick selection | 7dpi, healthy selection | 1 | |
| SynCom-210 | 14dpi, sick selection | 7dpi, sick selection | 1 | |
| SynCom-210 | 7dpi, healthy selection | 7dpi, sick selection | 1 | |
| SynCom-48p | 14dpi, healthy selection | 14dpi, sick selection | 1 | |
| SynCom-48p | 14dpi, healthy selection | 7dpi, healthy selection | 1 | |
| SynCom-48p | 14dpi, healthy selection | 7dpi, sick selection | 1 | |
| SynCom-48p | 14dpi, sick selection | 7dpi, healthy selection | 1 | |
| SynCom-48p | 14dpi, sick selection | 7dpi, sick selection | 1 | |
| SynCom-48p | 7dpi, healthy selection | 7dpi, sick selection | 1 | |
| SynCom-15±5 | 14dpi, healthy selection | 14dpi, sick selection | 1 | |
| SynCom-15±5 | 14dpi, healthy selection | 7dpi, healthy selection | 1 | |
| SynCom-15±5 | 14dpi, healthy selection | 7dpi, sick selection | 0.22758 | |
| SynCom-15±5 | 14dpi, sick selection | 7dpi, healthy selection | 1 | |
| SynCom-15±5 | 14dpi, sick selection | 7dpi, sick selection | 1 | |
| SynCom-15±5 | 7dpi, healthy selection | 7dpi, sick selection | 1 | |
| SynCom-15 | 14dpi, healthy selection | 14dpi, sick selection | 1 | |
| SynCom-15 | 14dpi, healthy selection | 7dpi, healthy selection | 1 | |
| SynCom-15 | 14dpi, healthy selection | 7dpi, sick selection | 1 | |
| SynCom-15 | 14dpi, sick selection | 7dpi, healthy selection | 1 | |
| SynCom-15 | 14dpi, sick selection | 7dpi, sick selection | 1 | |
| SynCom-15 | 7dpi, healthy selection | 7dpi, sick selection | 1 | |
| 14 dpi (Early infection) | axenic infected | SynCom-210 | 0 | |
| 14 dpi (Early infection) | axenic infected | SynCom-48p | 0.00001 | **** |
| 14 dpi (Early infection) | axenic infected | SynCom-15±5 | 0.00027 | *** |
| 14 dpi (Early infection) | axenic infected | SynCom-15 | 0.06966 | |
| 14 dpi (Early infection) | SynCom-210 | SynCom-48p | 1 | |
| 14 dpi (Early infection) | SynCom-210 | SynCom-15±5 | 1 | |
| 14 dpi (Early infection) | SynCom-210 | SynCom-15 | 0.01336 | * |
| 14 dpi (Early infection) | SynCom-48p | SynCom-15±5 | 1 | |
| 14 dpi (Early infection) | SynCom-48p | SynCom-15 | 0.19675 | |
| 14 dpi (Early infection) | SynCom-15±5 | SynCom-15 | 1 | |
| axenic infected | 14 dpi (Early infection) | 7dpi (Late infection) | 1 | |
| SynCom-210 | 14 dpi (Early infection) | 7dpi (Late infection) | 1 | |
| SynCom-48p | 14 dpi (Early infection) | 7dpi (Late infection) | 1 | |
| SynCom-15±5 | 14 dpi (Early infection) | 7dpi (Late infection) | 1 | |
| SynCom-15 | 14 dpi (Early infection) | 7dpi (Late infection) | 1 | |
| 7dpi (Late infection) | axenic infected | SynCom-210 | 0.0021 | ** |
| 7dpi (Late infection) | axenic infected | SynCom-48p | 0.0016 | ** |
| 7dpi (Late infection) | axenic infected | SynCom-15±5 | 0.37858 | |
| 7dpi (Late infection) | axenic infected | SynCom-15 | 1 | |
| 7dpi (Late infection) | SynCom-210 | SynCom-48p | 1 | |
| 7dpi (Late infection) | SynCom-210 | SynCom-15±5 | 1 | |
| 7dpi (Late infection) | SynCom-210 | SynCom-15 | 0.32629 | |
| 7dpi (Late infection) | SynCom-48p | SynCom-15±5 | 1 | |
| 7dpi (Late infection) | SynCom-48p | SynCom-15 | 0.25839 | |
| 7dpi (Late infection) | SynCom-15±5 | SynCom-15 | 1 | |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 25:** Bonferroni-corrected p-values of pairwise Welch's t-tests of pathogen luminescence at 3 dpi in passage 1 of second passaging experiment. Note that early and late infection time points were measured on a different day.

| Infection Time | Inoculation | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|---|
| Early | SynCom-210 | healthy line 1 | healthy line 2 | 1 | |
| Early | SynCom-210 | healthy line 1 | healthy line 3 | 0.14791 | |
| Early | SynCom-210 | healthy line 1 | sick line 1 | 0.97002 | |
| Early | SynCom-210 | healthy line 1 | sick line 2 | 1 | |
| Early | SynCom-210 | healthy line 2 | healthy line 3 | 1 | |
| Early | SynCom-210 | healthy line 2 | sick line 1 | 1 | |
| Early | SynCom-210 | healthy line 2 | sick line 2 | 1 | |
| Early | SynCom-210 | healthy line 3 | sick line 1 | 1 | |
| Early | SynCom-210 | healthy line 3 | sick line 2 | 1 | |
| Early | SynCom-210 | sick line 1 | sick line 2 | 1 | |
| Early | SynCom-48p | healthy line 1 | healthy line 2 | 1 | |
| Early | SynCom-48p | healthy line 1 | healthy line 3 | 1 | |
| Early | SynCom-48p | healthy line 1 | sick line 1 | 1 | |
| Early | SynCom-48p | healthy line 1 | sick line 2 | 1 | |
| Early | SynCom-48p | healthy line 2 | healthy line 3 | 1 | |
| Early | SynCom-48p | healthy line 2 | sick line 1 | 1 | |
| Early | SynCom-48p | healthy line 2 | sick line 2 | 1 | |
| Early | SynCom-48p | healthy line 3 | sick line 1 | 1 | |
| Early | SynCom-48p | healthy line 3 | sick line 2 | 1 | |
| Early | SynCom-48p | sick line 1 | sick line 2 | 1 | |
| Early | SynCom-15Low | healthy line 1 | healthy line 2 | 1 | |
| Early | SynCom-15Low | healthy line 1 | healthy line 3 | 1 | |
| Early | SynCom-15Low | healthy line 1 | sick line 1 | 1 | |
| Early | SynCom-15Low | healthy line 1 | sick line 2 | 1 | |
| Early | SynCom-15Low | healthy line 2 | healthy line 3 | 1 | |
| Early | SynCom-15Low | healthy line 2 | sick line 1 | 1 | |
| Early | SynCom-15Low | healthy line 2 | sick line 2 | 1 | |
| Early | SynCom-15Low | healthy line 3 | sick line 1 | 1 | |
| Early | SynCom-15Low | healthy line 3 | sick line 2 | 1 | |
| Early | SynCom-15Low | sick line 1 | sick line 2 | 1 | |
| Early | SynCom-15 | healthy line 1 | healthy line 2 | 1 | |
| Early | SynCom-15 | healthy line 1 | healthy line 3 | 1 | |
| Early | SynCom-15 | healthy line 1 | sick line 1 | 1 | |
| Early | SynCom-15 | healthy line 1 | sick line 2 | 1 | |
| Early | SynCom-15 | healthy line 2 | healthy line 3 | 1 | |
| Early | SynCom-15 | healthy line 2 | sick line 1 | 1 | |
| Early | SynCom-15 | healthy line 2 | sick line 2 | 1 | |
| Early | SynCom-15 | healthy line 3 | sick line 1 | 1 | |
| Early | SynCom-15 | healthy line 3 | sick line 2 | 0.15123 | |
| Early | SynCom-15 | sick line 1 | sick line 2 | 1 | |
| Late | SynCom-210 | healthy line 1 | healthy line 2 | 1 | |
| Late | SynCom-210 | healthy line 1 | healthy line 3 | 1 | |
| Late | SynCom-210 | healthy line 1 | sick line 1 | 1 | |
| Late | SynCom-210 | healthy line 1 | sick line 2 | 1 | |
| Late | SynCom-210 | healthy line 2 | healthy line 3 | 1 | |
| Late | SynCom-210 | healthy line 2 | sick line 1 | 1 | |
| Late | SynCom-210 | healthy line 2 | sick line 2 | 1 | |
| Late | SynCom-210 | healthy line 3 | sick line 1 | 1 | |
| Late | SynCom-210 | healthy line 3 | sick line 2 | 1 | |
| Late | SynCom-210 | sick line 1 | sick line 2 | 1 | |
| Late | SynCom-48p | healthy line 1 | healthy line 2 | 1 | |
| Late | SynCom-48p | healthy line 1 | healthy line 3 | 1 | |
| Late | SynCom-48p | healthy line 1 | sick line 1 | 1 | |
| Late | SynCom-48p | healthy line 1 | sick line 2 | 1 | |
| Late | SynCom-48p | healthy line 2 | healthy line 3 | 1 | |
| Late | SynCom-48p | healthy line 2 | sick line 1 | 1 | |
| Late | SynCom-48p | healthy line 2 | sick line 2 | 1 | |
| Late | SynCom-48p | healthy line 3 | sick line 1 | 1 | |
| Late | SynCom-48p | healthy line 3 | sick line 2 | 1 | |
| Late | SynCom-48p | sick line 1 | sick line 2 | 1 | |
| Late | SynCom-15±5 | healthy line 1 | healthy line 2 | 1 | |
| Late | SynCom-15±5 | healthy line 1 | healthy line 3 | 1 | |
| Late | SynCom-15±5 | healthy line 1 | sick line 1 | 1 | |
| Late | SynCom-15±5 | healthy line 1 | sick line 2 | 1 | |
| Late | SynCom-15±5 | healthy line 2 | healthy line 3 | 1 | |
| Late | SynCom-15±5 | healthy line 2 | sick line 1 | 1 | |
| Late | SynCom-15±5 | healthy line 2 | sick line 2 | 1 | |
| Late | SynCom-15±5 | healthy line 3 | sick line 1 | 1 | |
| Late | SynCom-15±5 | healthy line 3 | sick line 2 | 1 | |
| Late | SynCom-15±5 | sick line 1 | sick line 2 | 1 | |

Supplemental Table 25 continued

| | | | | | |
|---|---|---|---|---|---|
| Late | SynCom-15 | healthy line 1 | healthy line 2 | 1 | |
| Late | SynCom-15 | healthy line 1 | healthy line 3 | 1 | |
| Late | SynCom-15 | healthy line 1 | sick line 1 | 1 | |
| Late | SynCom-15 | healthy line 1 | sick line 2 | 1 | |
| Late | SynCom-15 | healthy line 2 | healthy line 3 | 1 | |
| Late | SynCom-15 | healthy line 2 | sick line 1 | 1 | |
| Late | SynCom-15 | healthy line 2 | sick line 2 | 1 | |
| Late | SynCom-15 | healthy line 3 | sick line 1 | 1 | |
| Late | SynCom-15 | healthy line 3 | sick line 2 | 0.41001 | |
| Late | SynCom-15 | sick line 1 | sick line 2 | 1 | |
| Early | | ax_NI | SynCom-210, healthy 1 | 0.18022 | |
| Early | | ax_NI | SynCom-210, healthy 2 | 1 | |
| Early | | ax_NI | SynCom-210, healthy 3 | 1 | |
| Early | | ax_NI | SynCom-210, sick 1 | 1 | |
| Early | | ax_NI | SynCom-210, sick 2 | 1 | |
| Early | | ax_NI | SynCom-48p, healthy 1 | 1 | |
| Early | | ax_NI | SynCom-48p, healthy 2 | 1 | |
| Early | | ax_NI | SynCom-48p, healthy 3 | 1 | |
| Early | | ax_NI | SynCom-48p, sick 1 | 1 | |
| Early | | ax_NI | SynCom-48p, sick 2 | 1 | |
| Early | | ax_NI | SynCom-15±5, healthy 1 | 1 | |
| Early | | ax_NI | SynCom-15±5, healthy 2 | 1 | |
| Early | | ax_NI | SynCom-15±5, healthy 3 | 1 | |
| Early | | ax_NI | SynCom-15±5, sick 1 | 1 | |
| Early | | ax_NI | SynCom-15±5, sick 2 | 1 | |
| Early | | ax_NI | SynCom-15, healthy 1 | 1 | |
| Early | | ax_NI | SynCom-15, healthy 2 | 1 | |
| Early | | ax_NI | SynCom-15, healthy 3 | 1 | |
| Early | | ax_NI | SynCom-15, sick 1 | 1 | |
| Early | | ax_NI | SynCom-15, sick 2 | 0.17478 | |
| Early | | ax_pst | SynCom-210, healthy 1 | 0 | **** |
| Early | | ax_pst | SynCom-210, healthy 2 | 0.00002 | **** |
| Early | | ax_pst | SynCom-210, healthy 3 | 0 | **** |
| Early | | ax_pst | SynCom-210, sick 1 | 0 | **** |
| Early | | ax_pst | SynCom-210, sick 2 | 0 | **** |
| Early | | ax_pst | SynCom-48p, healthy 1 | 1 | |
| Early | | ax_pst | SynCom-48p, healthy 2 | 0.00015 | *** |
| Early | | ax_pst | SynCom-48p, healthy 3 | 0 | **** |
| Early | | ax_pst | SynCom-48p, sick 1 | 0 | **** |
| Early | | ax_pst | SynCom-48p, sick 2 | 0 | **** |
| Early | | ax_pst | SynCom-15±5, healthy 1 | 0 | **** |
| Early | | ax_pst | SynCom-15±5, healthy 2 | 0.00061 | *** |
| Early | | ax_pst | SynCom-15±5, healthy 3 | 0.00005 | **** |
| Early | | ax_pst | SynCom-15±5, sick 1 | 0.00022 | *** |
| Early | | ax_pst | SynCom-15±5, sick 2 | 0.00213 | ** |
| Early | | ax_pst | SynCom-15, healthy 1 | 0.00065 | *** |
| Early | | ax_pst | SynCom-15, healthy 2 | 0.00083 | *** |
| Early | | ax_pst | SynCom-15, healthy 3 | 0 | **** |
| Early | | ax_pst | SynCom-15, sick 1 | 0.00002 | **** |
| Early | | ax_pst | SynCom-15, sick 2 | 0.13013 | |
| Late | | ax_NI | SynCom-210, healthy 1 | 1 | |
| Late | | ax_NI | SynCom-210, healthy 2 | 1 | |
| Late | | ax_NI | SynCom-210, healthy 3 | 0.00392 | ** |
| Late | | ax_NI | SynCom-210, sick 1 | 0.02949 | * |
| Late | | ax_NI | SynCom-210, sick 2 | 1 | |
| Late | | ax_NI | SynCom-48p, healthy 1 | 1 | |
| Late | | ax_NI | SynCom-48p, healthy 2 | 0.14871 | |
| Late | | ax_NI | SynCom-48p, healthy 3 | 1 | |
| Late | | ax_NI | SynCom-48p, sick 1 | 1 | |
| Late | | ax_NI | SynCom-48p, sick 2 | 0.49064 | |
| Late | | ax_NI | SynCom-15±5, healthy 1 | 1 | |
| Late | | ax_NI | SynCom-15±5, healthy 2 | 0.84122 | |
| Late | | ax_NI | SynCom-15±5, healthy 3 | 0.0889 | |
| Late | | ax_NI | SynCom-15±5, sick 1 | 0.12496 | |
| Late | | ax_NI | SynCom-15±5, sick 2 | 0.02568 | * |
| Late | | ax_NI | SynCom-15, healthy 1 | 1 | |
| Late | | ax_NI | SynCom-15, healthy 2 | 1 | |
| Late | | ax_NI | SynCom-15, healthy 3 | 0.0933 | |
| Late | | ax_NI | SynCom-15, sick 1 | 1 | |
| Late | | ax_NI | SynCom-15, sick 2 | 1 | |
| Late | | ax_pst | SynCom-210, healthy 1 | 0 | **** |
| Late | | ax_pst | SynCom-210, healthy 2 | 0 | **** |
| Late | | ax_pst | SynCom-210, healthy 3 | 0.20331 | |

Supplemental Table 25 continued

| | | | | |
|---|---|---|---|---|
| Late | ax_pst | SynCom-210, sick 1 | 0.02133 | * |
| Late | ax_pst | SynCom-210, sick 2 | 0.00001 | **** |
| Late | ax_pst | SynCom-48p, healthy 1 | 0 | **** |
| Late | ax_pst | SynCom-48p, healthy 2 | 0.00234 | ** |
| Late | ax_pst | SynCom-48p, healthy 3 | 0 | **** |
| Late | ax_pst | SynCom-48p, sick 1 | 0.00002 | **** |
| Late | ax_pst | SynCom-48p, sick 2 | 0.00034 | *** |
| Late | ax_pst | SynCom-15±5, healthy 1 | 0 | **** |
| Late | ax_pst | SynCom-15±5, healthy 2 | 0.00009 | **** |
| Late | ax_pst | SynCom-15±5, healthy 3 | 0.00369 | ** |
| Late | ax_pst | SynCom-15±5, sick 1 | 0.00225 | ** |
| Late | ax_pst | SynCom-15±5, sick 2 | 0.01929 | * |
| Late | ax_pst | SynCom-15, healthy 1 | 0.00004 | **** |
| Late | ax_pst | SynCom-15, healthy 2 | 0.00001 | **** |
| Late | ax_pst | SynCom-15, healthy 3 | 0.00344 | ** |
| Late | ax_pst | SynCom-15, sick 1 | 0 | **** |
| Late | ax_pst | SynCom-15, sick 2 | 0 | **** |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 26:** Bonferroni-corrected p-values of pairwise Welch's t-tests of pathogen luminescence at 7 dpi in passage 1 of second passaging experiment. Note that early and late infection time points were measured on a different day.

| Infection Time | Inoculation | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|---|
| Early | SynCom-210 | healthy line 1 | healthy line 2 | 1 | |
| Early | SynCom-210 | healthy line 1 | healthy line 3 | 1 | |
| Early | SynCom-210 | healthy line 1 | sick line 1 | 1 | |
| Early | SynCom-210 | healthy line 1 | sick line 2 | 1 | |
| Early | SynCom-210 | healthy line 2 | healthy line 3 | 1 | |
| Early | SynCom-210 | healthy line 2 | sick line 1 | 1 | |
| Early | SynCom-210 | healthy line 2 | sick line 2 | 1 | |
| Early | SynCom-210 | healthy line 3 | sick line 1 | 1 | |
| Early | SynCom-210 | healthy line 3 | sick line 2 | 1 | |
| Early | SynCom-210 | sick line 1 | sick line 2 | 1 | |
| Early | SynCom-48p | healthy line 1 | healthy line 2 | 1 | |
| Early | SynCom-48p | healthy line 1 | healthy line 3 | 1 | |
| Early | SynCom-48p | healthy line 1 | sick line 1 | 1 | |
| Early | SynCom-48p | healthy line 1 | sick line 2 | 1 | |
| Early | SynCom-48p | healthy line 2 | healthy line 3 | 1 | |
| Early | SynCom-48p | healthy line 2 | sick line 1 | 1 | |
| Early | SynCom-48p | healthy line 2 | sick line 2 | 1 | |
| Early | SynCom-48p | healthy line 3 | sick line 1 | 1 | |
| Early | SynCom-48p | healthy line 3 | sick line 2 | 1 | |
| Early | SynCom-48p | sick line 1 | sick line 2 | 1 | |
| Early | SynCom-15±5 | healthy line 1 | healthy line 2 | 1 | |
| Early | SynCom-15±5 | healthy line 1 | healthy line 3 | 1 | |
| Early | SynCom-15±5 | healthy line 1 | sick line 1 | 1 | |
| Early | SynCom-15±5 | healthy line 1 | sick line 2 | 1 | |
| Early | SynCom-15±5 | healthy line 2 | healthy line 3 | 1 | |
| Early | SynCom-15±5 | healthy line 2 | sick line 1 | 1 | |
| Early | SynCom-15±5 | healthy line 2 | sick line 2 | 1 | |
| Early | SynCom-15±5 | healthy line 3 | sick line 1 | 1 | |
| Early | SynCom-15±5 | healthy line 3 | sick line 2 | 1 | |
| Early | SynCom-15±5 | sick line 1 | sick line 2 | 1 | |
| Early | SynCom-15 | healthy line 1 | healthy line 2 | 1 | |
| Early | SynCom-15 | healthy line 1 | healthy line 3 | 1 | |
| Early | SynCom-15 | healthy line 1 | sick line 1 | 0.0621 | |
| Early | SynCom-15 | healthy line 1 | sick line 2 | 1 | |
| Early | SynCom-15 | healthy line 2 | healthy line 3 | 1 | |
| Early | SynCom-15 | healthy line 2 | sick line 1 | 0.00457 | ** |
| Early | SynCom-15 | healthy line 2 | sick line 2 | 1 | |
| Early | SynCom-15 | healthy line 3 | sick line 1 | 1 | |
| Early | SynCom-15 | healthy line 3 | sick line 2 | 1 | |
| Early | SynCom-15 | sick line 1 | sick line 2 | 1 | |
| Late | SynCom-210 | healthy line 1 | healthy line 2 | 1 | |
| Late | SynCom-210 | healthy line 1 | healthy line 3 | 1 | |
| Late | SynCom-210 | healthy line 1 | sick line 1 | 1 | |
| Late | SynCom-210 | healthy line 1 | sick line 2 | 1 | |
| Late | SynCom-210 | healthy line 2 | healthy line 3 | 1 | |
| Late | SynCom-210 | healthy line 2 | sick line 1 | 1 | |
| Late | SynCom-210 | healthy line 2 | sick line 2 | 1 | |
| Late | SynCom-210 | healthy line 3 | sick line 1 | 1 | |
| Late | SynCom-210 | healthy line 3 | sick line 2 | 1 | |
| Late | SynCom-210 | sick line 1 | sick line 2 | 1 | |
| Late | SynCom-48p | healthy line 1 | healthy line 2 | 1 | |
| Late | SynCom-48p | healthy line 1 | healthy line 3 | 1 | |
| Late | SynCom-48p | healthy line 1 | sick line 1 | 1 | |
| Late | SynCom-48p | healthy line 1 | sick line 2 | 1 | |
| Late | SynCom-48p | healthy line 2 | healthy line 3 | 0.20528 | |
| Late | SynCom-48p | healthy line 2 | sick line 1 | 1 | |
| Late | SynCom-48p | healthy line 2 | sick line 2 | 1 | |
| Late | SynCom-48p | healthy line 3 | sick line 1 | 1 | |
| Late | SynCom-48p | healthy line 3 | sick line 2 | 1 | |
| Late | SynCom-48p | sick line 1 | sick line 2 | 1 | |
| Late | SynCom-15±5 | healthy line 1 | healthy line 2 | 1 | |
| Late | SynCom-15±5 | healthy line 1 | healthy line 3 | 1 | |
| Late | SynCom-15±5 | healthy line 1 | sick line 1 | 1 | |
| Late | SynCom-15±5 | healthy line 1 | sick line 2 | 1 | |
| Late | SynCom-15±5 | healthy line 2 | healthy line 3 | 1 | |
| Late | SynCom-15±5 | healthy line 2 | sick line 1 | 1 | |
| Late | SynCom-15±5 | healthy line 2 | sick line 2 | 1 | |
| Late | SynCom-15±5 | healthy line 3 | sick line 1 | 1 | |
| Late | SynCom-15±5 | healthy line 3 | sick line 2 | 1 | |
| Late | SynCom-15±5 | sick line 1 | sick line 2 | 1 | |
| Late | SynCom-15 | healthy line 1 | healthy line 2 | 1 | |

Supplemental Table 26 continued

| | | | | | |
|---|---|---|---|---|---|
| Late | SynCom-15 | healthy line 1 | healthy line 3 | 1 | |
| Late | SynCom-15 | healthy line 1 | sick line 1 | 0 | **** |
| Late | SynCom-15 | healthy line 1 | sick line 2 | 0.00054 | *** |
| Late | SynCom-15 | healthy line 2 | healthy line 3 | 1 | |
| Late | SynCom-15 | healthy line 2 | sick line 1 | 0.23866 | |
| Late | SynCom-15 | healthy line 2 | sick line 2 | 1 | |
| Late | SynCom-15 | healthy line 3 | sick line 1 | 0.00066 | *** |
| Late | SynCom-15 | healthy line 3 | sick line 2 | 0.0882 | |
| Late | SynCom-15 | sick line 1 | sick line 2 | 1 | |
| Early | | axenic mock-infected | SynCom-210, healthy 1 | 1 | |
| Early | | axenic mock-infected | SynCom-210, healthy 2 | 1 | |
| Early | | axenic mock-infected | SynCom-210, healthy 3 | 1 | |
| Early | | axenic mock-infected | SynCom-210, sick 1 | 1 | |
| Early | | axenic mock-infected | SynCom-210, sick 2 | 1 | |
| Early | | axenic mock-infected | SynCom-48p, healthy 1 | 1 | |
| Early | | axenic mock-infected | SynCom-48p, healthy 2 | 1 | |
| Early | | axenic mock-infected | SynCom-48p, healthy 3 | 1 | |
| Early | | axenic mock-infected | SynCom-48p, sick 1 | 1 | |
| Early | | axenic mock-infected | SynCom-48p, sick 2 | 1 | |
| Early | | axenic mock-infected | SynCom-15±5, healthy 1 | 1 | |
| Early | | axenic mock-infected | SynCom-15±5, healthy 2 | 1 | |
| Early | | axenic mock-infected | SynCom-15±5, healthy 3 | 1 | |
| Early | | axenic mock-infected | SynCom-15±5, sick 1 | 1 | |
| Early | | axenic mock-infected | SynCom-15±5, sick 2 | 1 | |
| Early | | axenic mock-infected | SynCom-15, healthy 1 | 0.04298 | * |
| Early | | axenic mock-infected | SynCom-15, healthy 2 | 0.00503 | ** |
| Early | | axenic mock-infected | SynCom-15, healthy 3 | 1 | |
| Early | | axenic mock-infected | SynCom-15, sick 1 | 1 | |
| Early | | axenic mock-infected | SynCom-15, sick 2 | 0.64544 | |
| Early | | axenic infected | SynCom-210, healthy 1 | 0.0011 | ** |
| Early | | axenic infected | SynCom-210, healthy 2 | 0 | **** |
| Early | | axenic infected | SynCom-210, healthy 3 | 0 | **** |
| Early | | axenic infected | SynCom-210, sick 1 | 0 | **** |
| Early | | axenic infected | SynCom-210, sick 2 | 0.00004 | **** |
| Early | | axenic infected | SynCom-48p, healthy 1 | 0 | **** |
| Early | | axenic infected | SynCom-48p, healthy 2 | 0 | **** |
| Early | | axenic infected | SynCom-48p, healthy 3 | 0 | **** |
| Early | | axenic infected | SynCom-48p, sick 1 | 0 | **** |
| Early | | axenic infected | SynCom-48p, sick 2 | 0 | |
| Early | | axenic infected | SynCom-15±5, healthy 1 | 0.00032 | *** |
| Early | | axenic infected | SynCom-15±5, healthy 2 | 0 | **** |
| Early | | axenic infected | SynCom-15±5, healthy 3 | 0 | **** |
| Early | | axenic infected | SynCom-15±5, sick 1 | 0 | **** |
| Early | | axenic infected | SynCom-15±5, sick 2 | 0 | **** |
| Early | | axenic infected | SynCom-15, healthy 1 | 0.57328 | |
| Early | | axenic infected | SynCom-15, healthy 2 | 1 | |
| Early | | axenic infected | SynCom-15, healthy 3 | 0.10201 | |
| Early | | axenic infected | SynCom-15, sick 1 | 0 | **** |
| Early | | axenic infected | SynCom-15, sick 2 | 1 | |
| Late | | axenic mock-infected | SynCom-210, healthy 1 | 1 | |
| Late | | axenic mock-infected | SynCom-210, healthy 2 | 0.20319 | |
| Late | | axenic mock-infected | SynCom-210, healthy 3 | 0.00563 | ** |
| Late | | axenic mock-infected | SynCom-210, sick 1 | 0.01259 | * |
| Late | | axenic mock-infected | SynCom-210, sick 2 | 0.48237 | |
| Late | | axenic mock-infected | SynCom-48p, healthy 1 | 0.30834 | |
| Late | | axenic mock-infected | SynCom-48p, healthy 2 | 0.00058 | *** |
| Late | | axenic mock-infected | SynCom-48p, healthy 3 | 1 | |
| Late | | axenic mock-infected | SynCom-48p, sick 1 | 0.56938 | |
| Late | | axenic mock-infected | SynCom-48p, sick 2 | 0.36541 | |
| Late | | axenic mock-infected | SynCom-15±5, healthy 1 | 0.02914 | * |
| Late | | axenic mock-infected | SynCom-15±5, healthy 2 | 0.05852 | |
| Late | | axenic mock-infected | SynCom-15±5, healthy 3 | 0.00004 | **** |
| Late | | axenic mock-infected | SynCom-15±5, sick 1 | 0.01801 | * |
| Late | | axenic mock-infected | SynCom-15±5, sick 2 | 0.00002 | **** |
| Late | | axenic mock-infected | SynCom-15, healthy 1 | 0 | **** |
| Late | | axenic mock-infected | SynCom-15, healthy 2 | 0.00001 | **** |
| Late | | axenic mock-infected | SynCom-15, healthy 3 | 0 | **** |
| Late | | axenic mock-infected | SynCom-15, sick 1 | 0.72907 | |
| Late | | axenic mock-infected | SynCom-15, sick 2 | 0.02304 | * |
| Late | | axenic infected | SynCom-210, healthy 1 | 0 | **** |
| Late | | axenic infected | SynCom-210, healthy 2 | 0 | **** |
| Late | | axenic infected | SynCom-210, healthy 3 | 0 | **** |
| Late | | axenic infected | SynCom-210, sick 1 | 0 | **** |

Supplemental Table 26 continued

| | | | | |
|---|---|---|---|---|
| Late | axenic infected | SynCom-210, sick 2 | 0 | **** |
| Late | axenic infected | SynCom-48p, healthy 1 | 0 | **** |
| Late | axenic infected | SynCom-48p, healthy 2 | 0 | **** |
| Late | axenic infected | SynCom-48p, healthy 3 | 0 | **** |
| Late | axenic infected | SynCom-48p, sick 1 | 0 | **** |
| Late | axenic infected | SynCom-48p, sick 2 | 0 | **** |
| Late | axenic infected | SynCom-15±5, healthy 1 | 0 | **** |
| Late | axenic infected | SynCom-15±5, healthy 2 | 0 | **** |
| Late | axenic infected | SynCom-15±5, healthy 3 | 0.00007 | **** |
| Late | axenic infected | SynCom-15±5, sick 1 | 0 | **** |
| Late | axenic infected | SynCom-15±5, sick 2 | 0.00012 | *** |
| Late | axenic infected | SynCom-15, healthy 1 | 1 | |
| Late | axenic infected | SynCom-15, healthy 2 | 0.00053 | *** |
| Late | axenic infected | SynCom-15, healthy 3 | 0.20089 | |
| Late | axenic infected | SynCom-15, sick 1 | 0 | **** |
| Late | axenic infected | SynCom-15, sick 2 | 0 | **** |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 27:** Bonferroni-corrected p-values of pairwise Welch's t-tests of pathogen abundance at 7 or 14 dpi in passage 1 of second passaging experiment.

| Infection Time | Inoculation | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|---|
| Early (14dpi) | SynCom-210 | healthy selection | sick selection | 1 | |
| Early (14dpi) | SynCom-48Low | healthy selection | sick selection | 1 | |
| Early (14dpi) | SynCom-15Low | healthy selection | sick selection | 1 | |
| Early (14dpi) | SynCom-15High | healthy selection | sick selection | 0.00037 | *** |
| Late (7dpi) | SynCom-210 | healthy selection | sick selection | 1 | |
| Late (7dpi) | SynCom-48Low | healthy selection | sick selection | 1 | |
| Late (7dpi) | SynCom-15Low | healthy selection | sick selection | 1 | |
| Late (7dpi) | SynCom-15High | healthy selection | sick selection | 0.35049 | |
| Early (14dpi) | SynCom-210 | healthy line 1 | healthy line 2 | 1 | |
| Early (14dpi) | SynCom-210 | healthy line 1 | healthy line 3 | 1 | |
| Early (14dpi) | SynCom-210 | healthy line 1 | sick line 1 | 1 | |
| Early (14dpi) | SynCom-210 | healthy line 1 | sick line 2 | 1 | |
| Early (14dpi) | SynCom-210 | healthy line 2 | healthy line 3 | 1 | |
| Early (14dpi) | SynCom-210 | healthy line 2 | sick line 1 | 1 | |
| Early (14dpi) | SynCom-210 | healthy line 2 | sick line 2 | 1 | |
| Early (14dpi) | SynCom-210 | healthy line 3 | sick line 1 | 1 | |
| Early (14dpi) | SynCom-210 | healthy line 3 | sick line 2 | 1 | |
| Early (14dpi) | SynCom-210 | sick line 1 | sick line 2 | 1 | |
| Early (14dpi) | SynCom-48Low | healthy line 1 | healthy line 2 | 1 | |
| Early (14dpi) | SynCom-48Low | healthy line 1 | healthy line 3 | 1 | |
| Early (14dpi) | SynCom-48Low | healthy line 1 | sick line 1 | 1 | |
| Early (14dpi) | SynCom-48Low | healthy line 1 | sick line 2 | 1 | |
| Early (14dpi) | SynCom-48Low | healthy line 2 | healthy line 3 | 1 | |
| Early (14dpi) | SynCom-48Low | healthy line 2 | sick line 1 | 1 | |
| Early (14dpi) | SynCom-48Low | healthy line 2 | sick line 2 | 1 | |
| Early (14dpi) | SynCom-48Low | healthy line 3 | sick line 1 | 1 | |
| Early (14dpi) | SynCom-48Low | healthy line 3 | sick line 2 | 1 | |
| Early (14dpi) | SynCom-48Low | sick line 1 | sick line 2 | 1 | |
| Early (14dpi) | SynCom-15Low | healthy line 1 | healthy line 2 | 1 | |
| Early (14dpi) | SynCom-15Low | healthy line 1 | healthy line 3 | 0.70714 | |
| Early (14dpi) | SynCom-15Low | healthy line 1 | sick line 1 | 1 | |
| Early (14dpi) | SynCom-15Low | healthy line 1 | sick line 2 | 1 | |
| Early (14dpi) | SynCom-15Low | healthy line 2 | healthy line 3 | 1 | |
| Early (14dpi) | SynCom-15Low | healthy line 2 | sick line 1 | 1 | |
| Early (14dpi) | SynCom-15Low | healthy line 2 | sick line 2 | 1 | |
| Early (14dpi) | SynCom-15Low | healthy line 3 | sick line 1 | 1 | |
| Early (14dpi) | SynCom-15Low | healthy line 3 | sick line 2 | 1 | |
| Early (14dpi) | SynCom-15Low | sick line 1 | sick line 2 | 1 | |
| Early (14dpi) | SynCom-15High | healthy line 1 | healthy line 2 | 0 | **** |
| Early (14dpi) | SynCom-15High | healthy line 1 | healthy line 3 | 0 | **** |
| Early (14dpi) | SynCom-15High | healthy line 1 | sick line 1 | 0 | **** |
| Early (14dpi) | SynCom-15High | healthy line 1 | sick line 2 | 0 | **** |
| Early (14dpi) | SynCom-15High | healthy line 2 | healthy line 3 | 1 | |
| Early (14dpi) | SynCom-15High | healthy line 2 | sick line 1 | 0.01139 | * |
| Early (14dpi) | SynCom-15High | healthy line 2 | sick line 2 | 1 | |
| Early (14dpi) | SynCom-15High | healthy line 3 | sick line 1 | 0.30867 | |
| Early (14dpi) | SynCom-15High | healthy line 3 | sick line 2 | 1 | |
| Early (14dpi) | SynCom-15High | sick line 1 | sick line 2 | 0.02508 | * |
| Late (7dpi) | SynCom-210 | healthy line 1 | healthy line 2 | 1 | |
| Late (7dpi) | SynCom-210 | healthy line 1 | healthy line 3 | NA | |
| Late (7dpi) | SynCom-210 | healthy line 1 | sick line 1 | 1 | |
| Late (7dpi) | SynCom-210 | healthy line 1 | sick line 2 | 1 | |
| Late (7dpi) | SynCom-210 | healthy line 2 | healthy line 3 | NA | |
| Late (7dpi) | SynCom-210 | healthy line 2 | sick line 1 | 1 | |
| Late (7dpi) | SynCom-210 | healthy line 2 | sick line 2 | 1 | |
| Late (7dpi) | SynCom-210 | healthy line 3 | sick line 1 | NA | |
| Late (7dpi) | SynCom-210 | healthy line 3 | sick line 2 | NA | |
| Late (7dpi) | SynCom-210 | sick line 1 | sick line 2 | 1 | |
| Late (7dpi) | SynCom-48Low | healthy line 1 | healthy line 2 | 1 | |
| Late (7dpi) | SynCom-48Low | healthy line 1 | healthy line 3 | 1 | |
| Late (7dpi) | SynCom-48Low | healthy line 1 | sick line 1 | 1 | |
| Late (7dpi) | SynCom-48Low | healthy line 1 | sick line 2 | 1 | |
| Late (7dpi) | SynCom-48Low | healthy line 2 | healthy line 3 | 0.64801 | |
| Late (7dpi) | SynCom-48Low | healthy line 2 | sick line 1 | 1 | |
| Late (7dpi) | SynCom-48Low | healthy line 2 | sick line 2 | 1 | |
| Late (7dpi) | SynCom-48Low | healthy line 3 | sick line 1 | 1 | |
| Late (7dpi) | SynCom-48Low | healthy line 3 | sick line 2 | 1 | |
| Late (7dpi) | SynCom-48Low | sick line 1 | sick line 2 | 1 | |
| Late (7dpi) | SynCom-15Low | healthy line 1 | healthy line 2 | 1 | |
| Late (7dpi) | SynCom-15Low | healthy line 1 | healthy line 3 | 1 | |
| Late (7dpi) | SynCom-15Low | healthy line 1 | sick line 1 | 1 | |

Supplemental Table 27 continued

| | | | | |
|---|---|---|---|---|
| Late (7dpi) | SynCom-15Low | healthy line 1 | sick line 2 | 1 |
| Late (7dpi) | SynCom-15Low | healthy line 2 | healthy line 3 | 1 |
| Late (7dpi) | SynCom-15Low | healthy line 2 | sick line 1 | 1 |
| Late (7dpi) | SynCom-15Low | healthy line 2 | sick line 2 | 1 |
| Late (7dpi) | SynCom-15Low | healthy line 3 | sick line 1 | 1 |
| Late (7dpi) | SynCom-15Low | healthy line 3 | sick line 2 | 1 |
| Late (7dpi) | SynCom-15Low | sick line 1 | sick line 2 | 1 |
| Late (7dpi) | SynCom-15High | healthy line 1 | healthy line 2 | 1 |
| Late (7dpi) | SynCom-15High | healthy line 1 | healthy line 3 | 1 |
| Late (7dpi) | SynCom-15High | healthy line 1 | sick line 1 | 1 |
| Late (7dpi) | SynCom-15High | healthy line 1 | sick line 2 | 1 |
| Late (7dpi) | SynCom-15High | healthy line 2 | healthy line 3 | 1 |
| Late (7dpi) | SynCom-15High | healthy line 2 | sick line 1 | 1 |
| Late (7dpi) | SynCom-15High | healthy line 2 | sick line 2 | 1 |
| Late (7dpi) | SynCom-15High | healthy line 3 | sick line 1 | 1 |
| Late (7dpi) | SynCom-15High | healthy line 3 | sick line 2 | 1 |
| Late (7dpi) | SynCom-15High | sick line 1 | sick line 2 | 1 |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 28:** Bonferroni-corrected p-values of pairwise Welch's t-tests comparison Shannon's diversity, Pielou's evenness and species richness within synthetic communities at passage 1 of second passaging experiment.

| Diversity Score | General Group | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|---|
| Shannon's Diversity | SynCom-210 | 7 dpi | 14 dpi | 1 | |
| Shannon's Diversity | SynCom-210 | 7 dpi, healthy | 7 dpi, sick | 1 | |
| Shannon's Diversity | SynCom-210 | 7 dpi, healthy | 14 dpi, healthy | 1 | |
| Shannon's Diversity | SynCom-210 | 7 dpi, healthy | 14 dpi, sick | 0.53302 | |
| Shannon's Diversity | SynCom-210 | 7 dpi, sick | 14 dpi, healthy | 1 | |
| Shannon's Diversity | SynCom-210 | 7 dpi, sick | 14 dpi, sick | 1 | |
| Shannon's Diversity | SynCom-210 | 14 dpi, healthy | 14 dpi, sick | 0.44878 | |
| Shannon's Diversity | SynCom-Protective | 7 dpi | 14 dpi | 1 | |
| Shannon's Diversity | SynCom-Protective | 7 dpi, healthy | 7 dpi, sick | 1 | |
| Shannon's Diversity | SynCom-Protective | 7 dpi, healthy | 14 dpi, healthy | 1 | |
| Shannon's Diversity | SynCom-Protective | 7 dpi, healthy | 14 dpi, sick | 1 | |
| Shannon's Diversity | SynCom-Protective | 7 dpi, sick | 14 dpi, healthy | 1 | |
| Shannon's Diversity | SynCom-Protective | 7 dpi, sick | 14 dpi, sick | 1 | |
| Shannon's Diversity | SynCom-Protective | 14 dpi, healthy | 14 dpi, sick | 1 | |
| Shannon's Diversity | SynCom-15 | 7 dpi | 14 dpi | 1 | |
| Shannon's Diversity | SynCom-15 | 7 dpi, healthy | 7 dpi, sick | 1 | |
| Shannon's Diversity | SynCom-15 | 7 dpi, healthy | 14 dpi, healthy | 1 | |
| Shannon's Diversity | SynCom-15 | 7 dpi, healthy | 14 dpi, sick | 1 | |
| Shannon's Diversity | SynCom-15 | 7 dpi, sick | 14 dpi, healthy | 1 | |
| Shannon's Diversity | SynCom-15 | 7 dpi, sick | 14 dpi, sick | 1 | |
| Shannon's Diversity | SynCom-15 | 14 dpi, healthy | 14 dpi, sick | 1 | |
| Shannon's Diversity | SynCom-15±5 | 7 dpi | 14 dpi | 1 | |
| Shannon's Diversity | SynCom-15±5 | 7 dpi, healthy | 7 dpi, sick | 1 | |
| Shannon's Diversity | SynCom-15±5 | 7 dpi, healthy | 14 dpi, healthy | 1 | |
| Shannon's Diversity | SynCom-15±5 | 7 dpi, healthy | 14 dpi, sick | 1 | |
| Shannon's Diversity | SynCom-15±5 | 7 dpi, sick | 14 dpi, healthy | 0.98299 | |
| Shannon's Diversity | SynCom-15±5 | 7 dpi, sick | 14 dpi, sick | 1 | |
| Shannon's Diversity | SynCom-15±5 | 14 dpi, healthy | 14 dpi, sick | 1 | |
| Pielou's Evenness | SynCom-210 | 7 dpi | 14 dpi | 1 | |
| Pielou's Evenness | SynCom-210 | 7 dpi, healthy | 7 dpi, sick | 1 | |
| Pielou's Evenness | SynCom-210 | 7 dpi, healthy | 14 dpi, healthy | 1 | |
| Pielou's Evenness | SynCom-210 | 7 dpi, healthy | 14 dpi, sick | 1 | |
| Pielou's Evenness | SynCom-210 | 7 dpi, sick | 14 dpi, healthy | 1 | |
| Pielou's Evenness | SynCom-210 | 7 dpi, sick | 14 dpi, sick | 1 | |
| Pielou's Evenness | SynCom-210 | 14 dpi, healthy | 14 dpi, sick | 1 | |
| Pielou's Evenness | SynCom-Protective | 7 dpi | 14 dpi | 1 | |
| Pielou's Evenness | SynCom-Protective | 7 dpi, healthy | 7 dpi, sick | 1 | |
| Pielou's Evenness | SynCom-Protective | 7 dpi, healthy | 14 dpi, healthy | 1 | |
| Pielou's Evenness | SynCom-Protective | 7 dpi, healthy | 14 dpi, sick | 1 | |
| Pielou's Evenness | SynCom-Protective | 7 dpi, sick | 14 dpi, healthy | 1 | |
| Pielou's Evenness | SynCom-Protective | 7 dpi, sick | 14 dpi, sick | 1 | |
| Pielou's Evenness | SynCom-Protective | 14 dpi, healthy | 14 dpi, sick | 1 | |
| Pielou's Evenness | SynCom-15 | 7 dpi | 14 dpi | 1 | |
| Pielou's Evenness | SynCom-15 | 7 dpi, healthy | 7 dpi, sick | 0.07188 | |
| Pielou's Evenness | SynCom-15 | 7 dpi, healthy | 14 dpi, healthy | 1 | |
| Pielou's Evenness | SynCom-15 | 7 dpi, healthy | 14 dpi, sick | 1 | |
| Pielou's Evenness | SynCom-15 | 7 dpi, sick | 14 dpi, healthy | 0.0048 | ** |
| Pielou's Evenness | SynCom-15 | 7 dpi, sick | 14 dpi, sick | 1 | |
| Pielou's Evenness | SynCom-15 | 14 dpi, healthy | 14 dpi, sick | 1 | |
| Pielou's Evenness | SynCom-15±5 | 7 dpi | 14 dpi | 1 | |
| Pielou's Evenness | SynCom-15±5 | 7 dpi, healthy | 7 dpi, sick | 1 | |
| Pielou's Evenness | SynCom-15±5 | 7 dpi, healthy | 14 dpi, healthy | 1 | |
| Pielou's Evenness | SynCom-15±5 | 7 dpi, healthy | 14 dpi, sick | 1 | |
| Pielou's Evenness | SynCom-15±5 | 7 dpi, sick | 14 dpi, healthy | 0.06328 | |
| Pielou's Evenness | SynCom-15±5 | 7 dpi, sick | 14 dpi, sick | 1 | |
| Pielou's Evenness | SynCom-15±5 | 14 dpi, healthy | 14 dpi, sick | 0.01411 | * |
| Richness | SynCom-210 | 7 dpi | 14 dpi | 1 | |
| Richness | SynCom-210 | 7 dpi, healthy | 7 dpi, sick | 1 | |
| Richness | SynCom-210 | 7 dpi, healthy | 14 dpi, healthy | 1 | |
| Richness | SynCom-210 | 7 dpi, healthy | 14 dpi, sick | 1 | |
| Richness | SynCom-210 | 7 dpi, sick | 14 dpi, healthy | 1 | |
| Richness | SynCom-210 | 7 dpi, sick | 14 dpi, sick | 1 | |
| Richness | SynCom-210 | 14 dpi, healthy | 14 dpi, sick | 0.42865 | |

Supplemental Table 28 continued

| | | | | | |
|---|---|---|---|---|---|
| Richness | SynCom-Protective | 7 dpi | 14 dpi | 0.81672 | |
| Richness | SynCom-Protective | 7 dpi, healthy | 7 dpi, sick | 1 | |
| Richness | SynCom-Protective | 7 dpi, healthy | 14 dpi, healthy | 1 | |
| Richness | SynCom-Protective | 7 dpi, healthy | 14 dpi, sick | 0.48842 | |
| Richness | SynCom-Protective | 7 dpi, sick | 14 dpi, healthy | 1 | |
| Richness | SynCom-Protective | 7 dpi, sick | 14 dpi, sick | 1 | |
| Richness | SynCom-Protective | 14 dpi, healthy | 14 dpi, sick | 1 | |
| Richness | SynCom-15 | 7 dpi | 14 dpi | 1 | |
| Richness | SynCom-15 | 7 dpi, healthy | 7 dpi, sick | 1 | |
| Richness | SynCom-15 | 7 dpi, healthy | 14 dpi, healthy | 1 | |
| Richness | SynCom-15 | 7 dpi, healthy | 14 dpi, sick | 1 | |
| Richness | SynCom-15 | 7 dpi, sick | 14 dpi, healthy | 1 | |
| Richness | SynCom-15 | 7 dpi, sick | 14 dpi, sick | 1 | |
| Richness | SynCom-15 | 14 dpi, healthy | 14 dpi, sick | 1 | |
| Richness | SynCom-15±5 | 7 dpi | 14 dpi | 1 | |
| Richness | SynCom-15±5 | 7 dpi, healthy | 7 dpi, sick | 1 | |
| Richness | SynCom-15±5 | 7 dpi, healthy | 14 dpi, healthy | 1 | |
| Richness | SynCom-15±5 | 7 dpi, healthy | 14 dpi, sick | 1 | |
| Richness | SynCom-15±5 | 7 dpi, sick | 14 dpi, healthy | 1 | |
| Richness | SynCom-15±5 | 7 dpi, sick | 14 dpi, sick | 1 | |
| Richness | SynCom-15±5 | 14 dpi, healthy | 14 dpi, sick | 1 | |
| Shannon's Diversity | 7 dpi | SynCom-210 | SynCom-Protective | 1 | |
| Shannon's Diversity | 7 dpi | SynCom-210 | SynCom-15 | 0 | **** |
| Shannon's Diversity | 7 dpi | SynCom-210 | SynCom-15±5 | 0 | **** |
| Shannon's Diversity | 14 dpi | SynCom-210 | SynCom-Protective | 1 | |
| Shannon's Diversity | 14 dpi | SynCom-210 | SynCom-15 | 0 | **** |
| Shannon's Diversity | 14 dpi | SynCom-210 | SynCom-15±5 | 0 | **** |
| Shannon's Diversity | 7 dpi | SynCom-15 | SynCom-15±5 | 0.00463 | ** |
| Shannon's Diversity | 14 dpi | SynCom-15 | SynCom-15±5 | 0.04775 | * |
| Pielou's Evenness | 7 dpi | SynCom-210 | SynCom-Protective | 1 | |
| Pielou's Evenness | 7 dpi | SynCom-210 | SynCom-15 | 1 | |
| Pielou's Evenness | 7 dpi | SynCom-210 | SynCom-15±5 | 0.00005 | **** |
| Pielou's Evenness | 14 dpi | SynCom-210 | SynCom-Protective | 1 | |
| Pielou's Evenness | 14 dpi | SynCom-210 | SynCom-15 | 0.00863 | ** |
| Pielou's Evenness | 14 dpi | SynCom-210 | SynCom-15±5 | 0 | **** |
| Richness | 7 dpi | SynCom-210 | SynCom-Protective | 0 | **** |
| Richness | 7 dpi | SynCom-210 | SynCom-15 | 0 | **** |
| Richness | 7 dpi | SynCom-210 | SynCom-15±5 | 0 | **** |
| Richness | 14 dpi | SynCom-210 | SynCom-Protective | 0.00003 | **** |
| Richness | 14 dpi | SynCom-210 | SynCom-15 | 0 | **** |
| Richness | 14 dpi | SynCom-210 | SynCom-15±5 | 0 | **** |

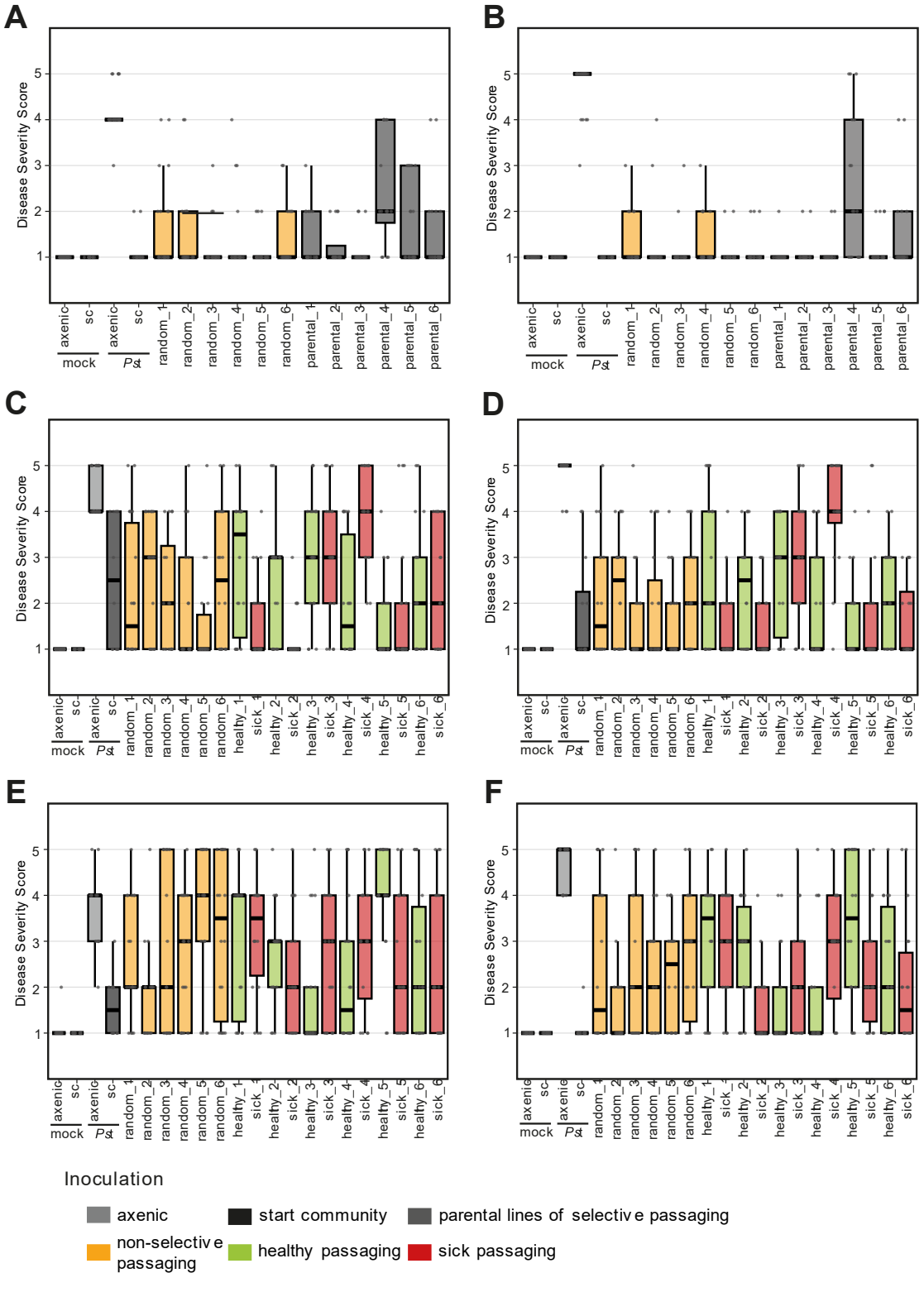
[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 29:** Changes in relative abundance of ASVs comparing healthy and sick selection within synthetic communities at passage 1 in second passaging experiment.

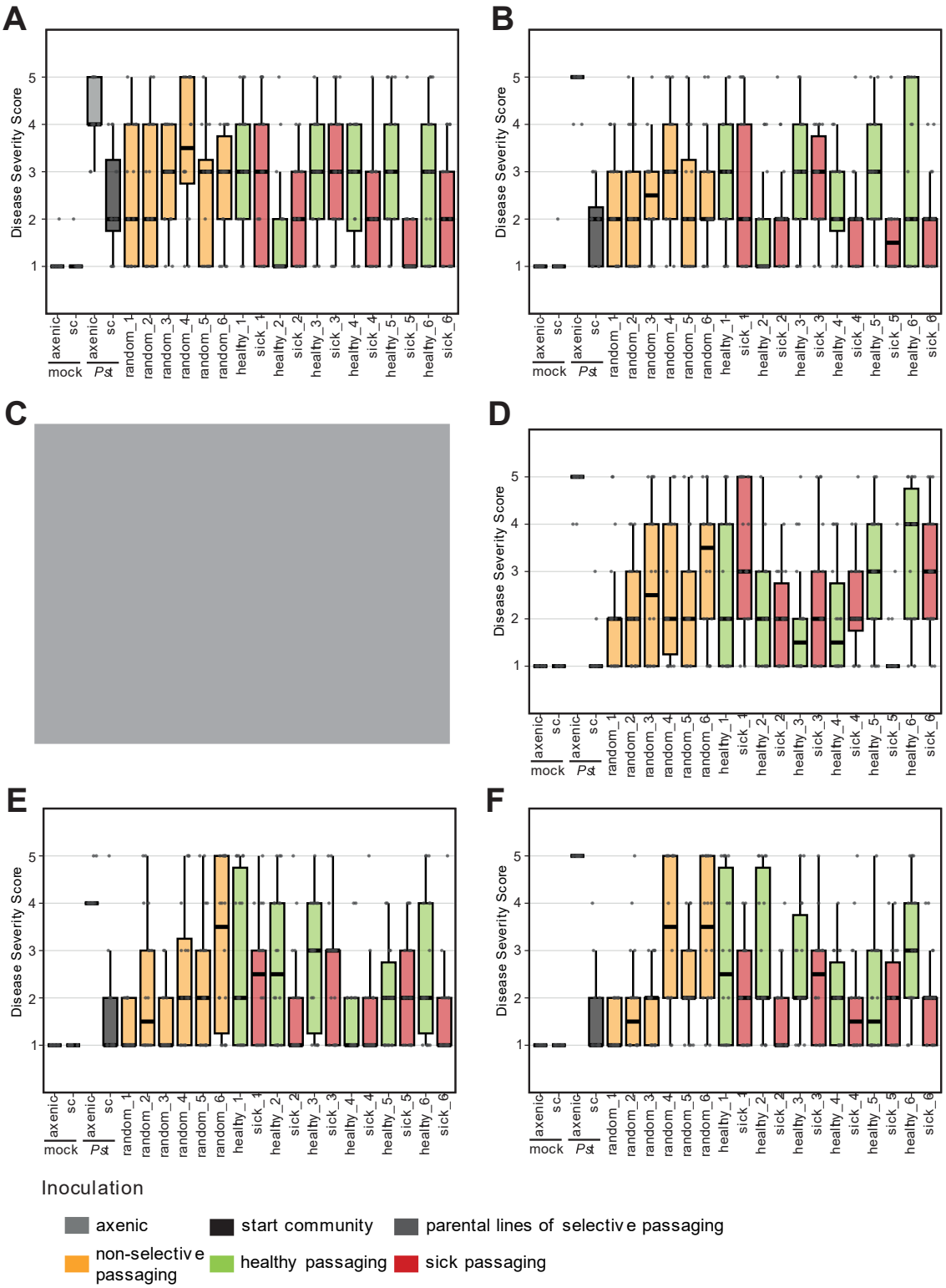
| Comparison of | Community | infection time | Group 1 | Group 2 | ASV representative StrainID | fold change | p-value | significance [1] | Comment |
|---|---|---|---|---|---|---|---|---|---|
| Selection | SynCom-210 | 14 dpi (early) | healthy lines | sick lines | Leaf191 | 50600000 | 9.47E-37 | **** | undetected in healthy lines |
| Selection | SynCom-210 | 7 dpi (late) | healthy lines | sick lines | Leaf272 | 0.0216 | 0.00499 | ** | not detected in most sick lines |
| Selection | SynCom-210 | 7 dpi (late) | healthy lines | sick lines | Leaf262 | 0.0135 | 0.002 | ** | not detected in most sick lines |
| Selection | SynCom-48p | 14 dpi (early) | healthy lines | sick lines | Leaf145 | 0.51 | 0.015 | * | |
| Selection | SynCom-48p | 14 dpi (early) | healthy lines | sick lines | Leaf257 | 0.109 | 0.0326 | * | |
| Selection | SynCom-48p | 7 dpi (late) | healthy lines | sick lines | Leaf15 | 0.0153 | 0.0131 | * | not detected in 5/6 samples of sick lines |
| Selection | SynCom-48p | 7 dpi (late) | healthy lines | sick lines | Leaf98 | 0.00703 | 0.0101 | * | completely not detected in sick lines |
| Phenotype | SynCom-210 | 14 dpi (early) | healthy phenotype | sick phenotype | Leaf16 | 95.6 | 0.0172 | * | |
| Phenotype | SynCom-210 | 14 dpi (early) | healthy phenotype | sick phenotype | Leaf405 | 59 | 0.00000164 | **** | |
| Phenotype | SynCom-210 | 14 dpi (early) | healthy phenotype | sick phenotype | Leaf404 | 91.7 | 0.00852 | ** | |
| Phenotype | SynCom-210 | 14 dpi (early) | healthy phenotype | sick phenotype | Leaf82 | 0.0356 | 0.016 | * | |
| Phenotype | SynCom-210 | 7 dpi (late) | healthy phenotype | sick phenotype | Leaf371 | 26.9 | 0.00984 | ** | |
| Phenotype | SynCom-48p | 14 dpi (early) | healthy phenotype | sick phenotype | Leaf151 | 0.00239 | 0.0122 | * | not detected in most sick phenotypes |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

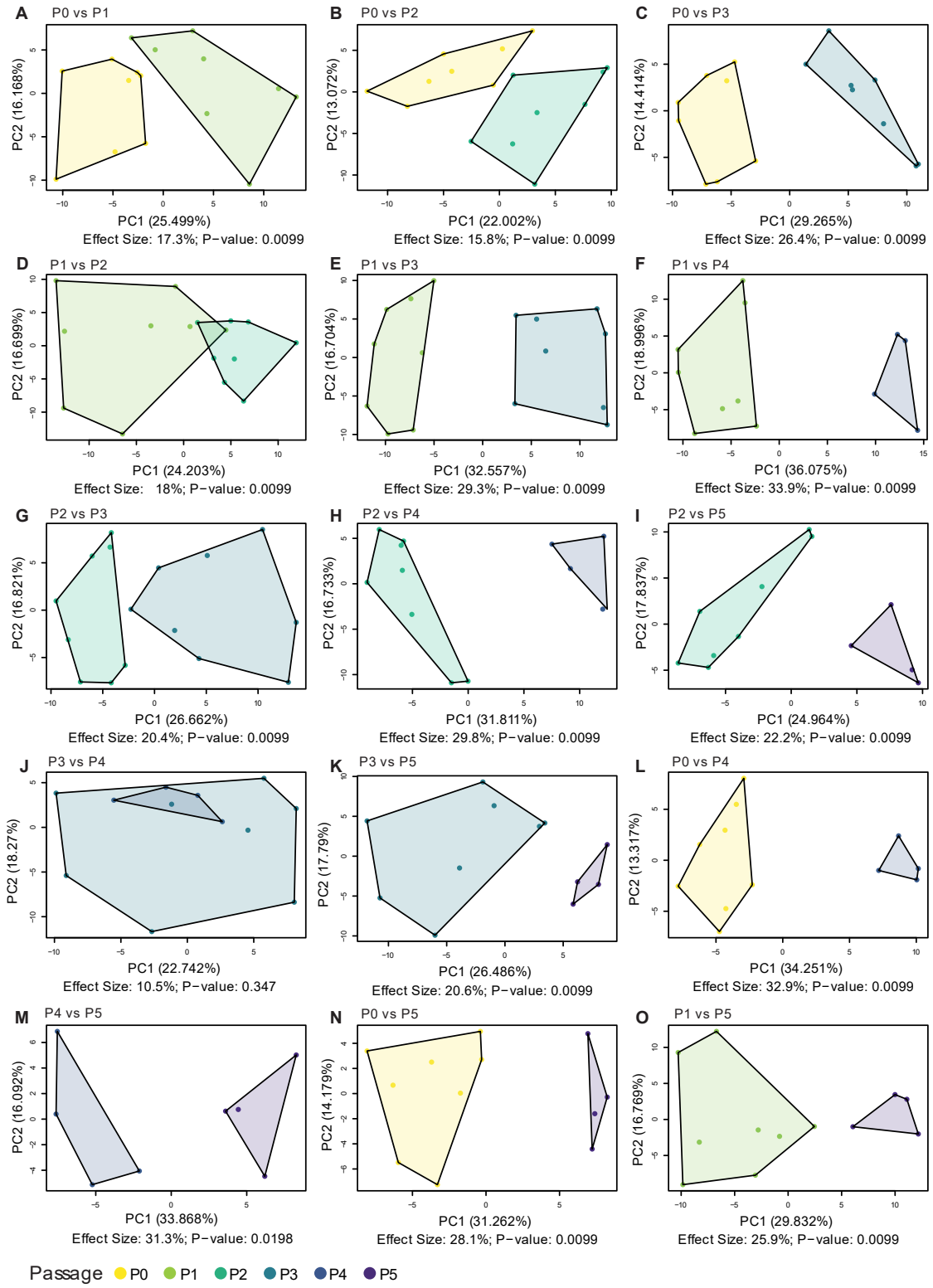
# Supplemental Figures



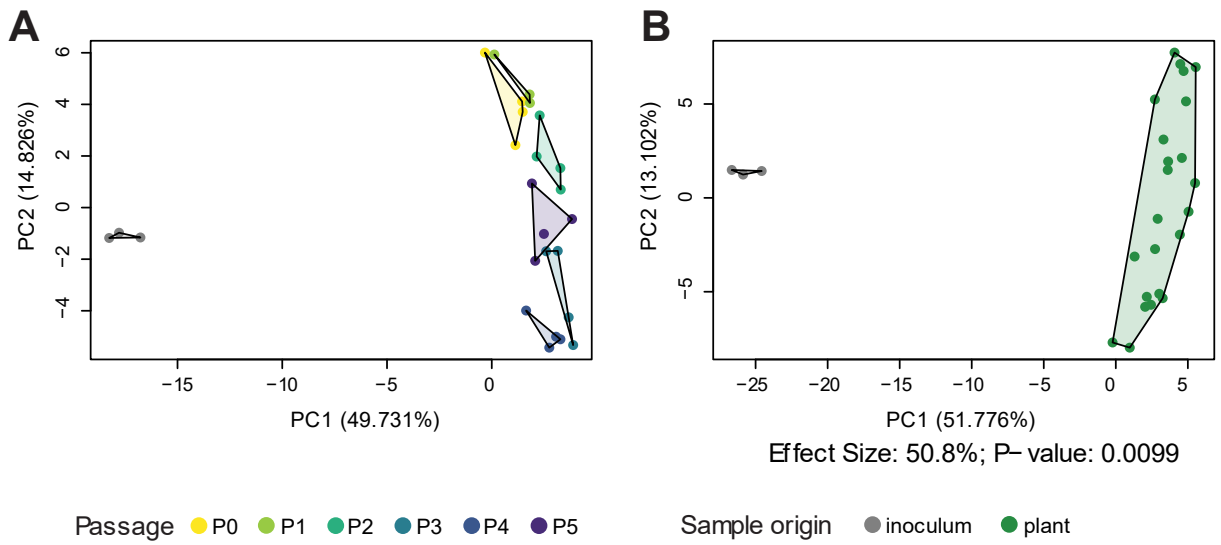

**Supplemental Figure 1: Disease severity scores of the first passaging experiments at 7 and 14 dpi.** The disease severity scores of parental passage (P0) (A,B), passage 1 (C,D) and passage 2 (E,F) are shown. Left column shows disease severity scores at 7 dpi (A,C,E), on the right column at 14 dpi (B,D,F). The plants were scored for disease symptoms from 1 (no visible symptoms, healthy) to 5 (diseased plant).



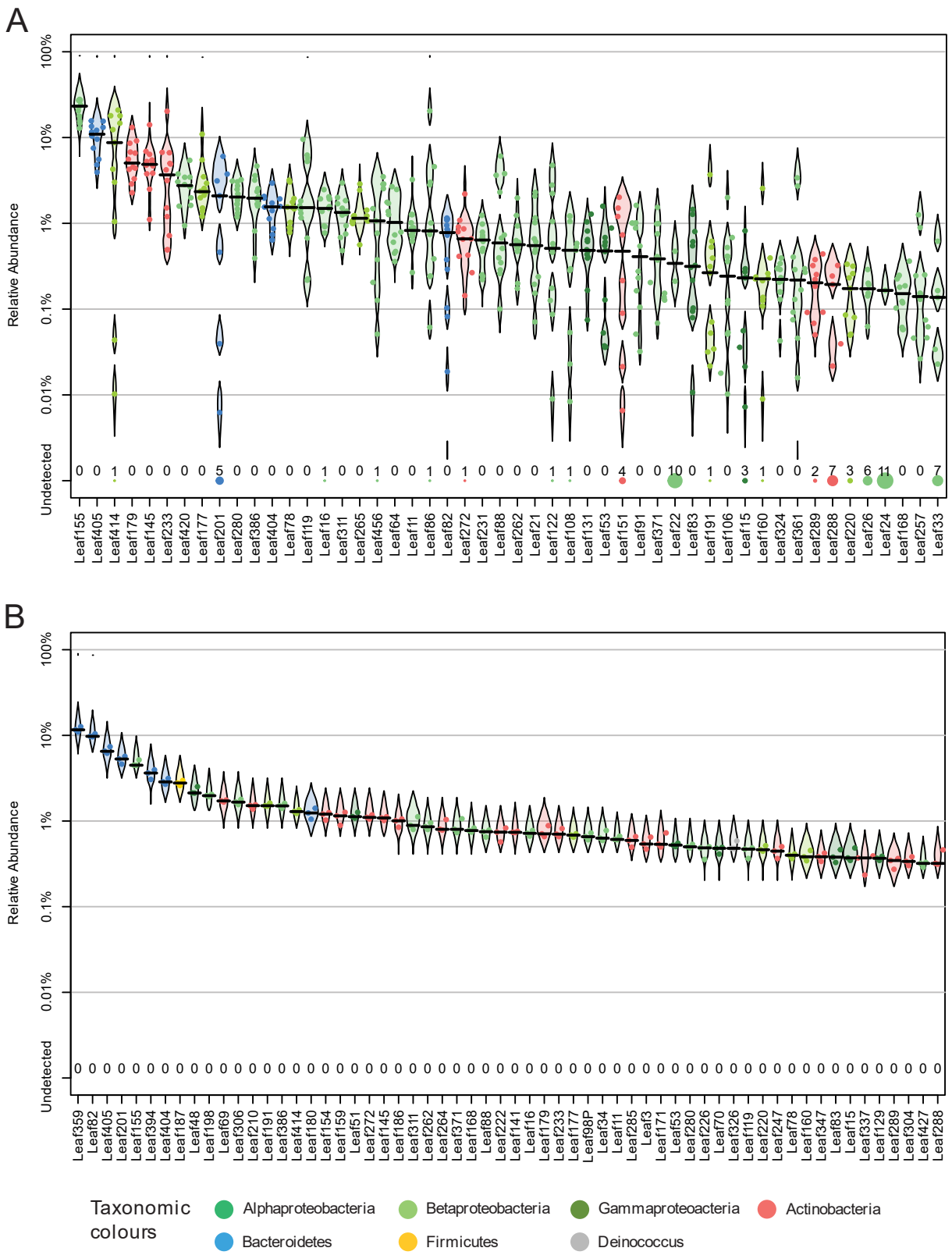

**Supplemental Figure 2: Disease severity scores of the first passaging experiments at 7 and 14 dpi.** The disease severity scores of passage 3 (A,B), passage 4 (C,D) and passage 5 (E,F) are shown. Left column shows disease severity scores at 7 dpi (A,C,E), on the right column at 14 dpi (B,D,F). The plants were scored for disease symptoms from 1 (no visible symptoms, healthy) to 5 (diseased plant). Data for passage 4 at 7 dpi was lost (grey rectangle).



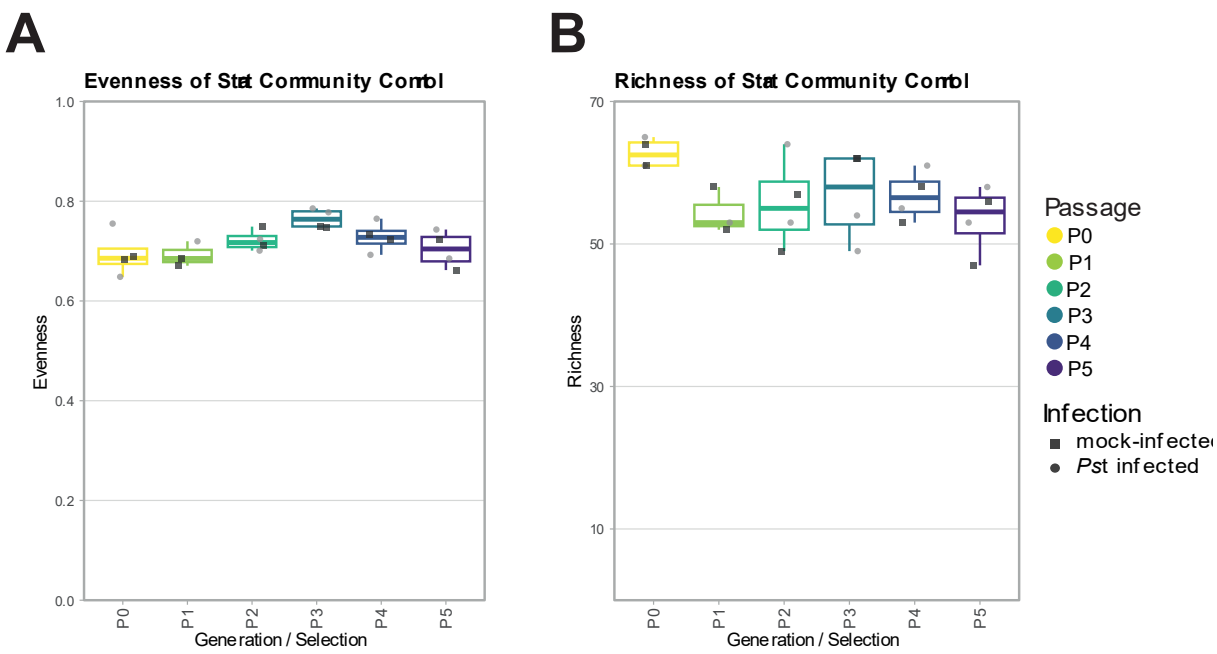

**Supplemental Figure 3: Community composition comparison of start community controls.** To get an overview of how much community establishment was different between the passages (abbreviation P), we compared the start community controls of each passage with all other passages by PCA and PERMANOVA analysis. The parental passage (P0) was compared to P1 (A), P2 (B), P3 (C), P4 (L), P5 (N). P1 was additionally compared to P2 (D), P3 (E), P4 (F) and P5 (O). P2 was additionally compared to P3 (G), P4 (H) and P5 (I). P3 was additionally compared to P4 (J) and P5 (K). M. Comparison of P4 and P5.



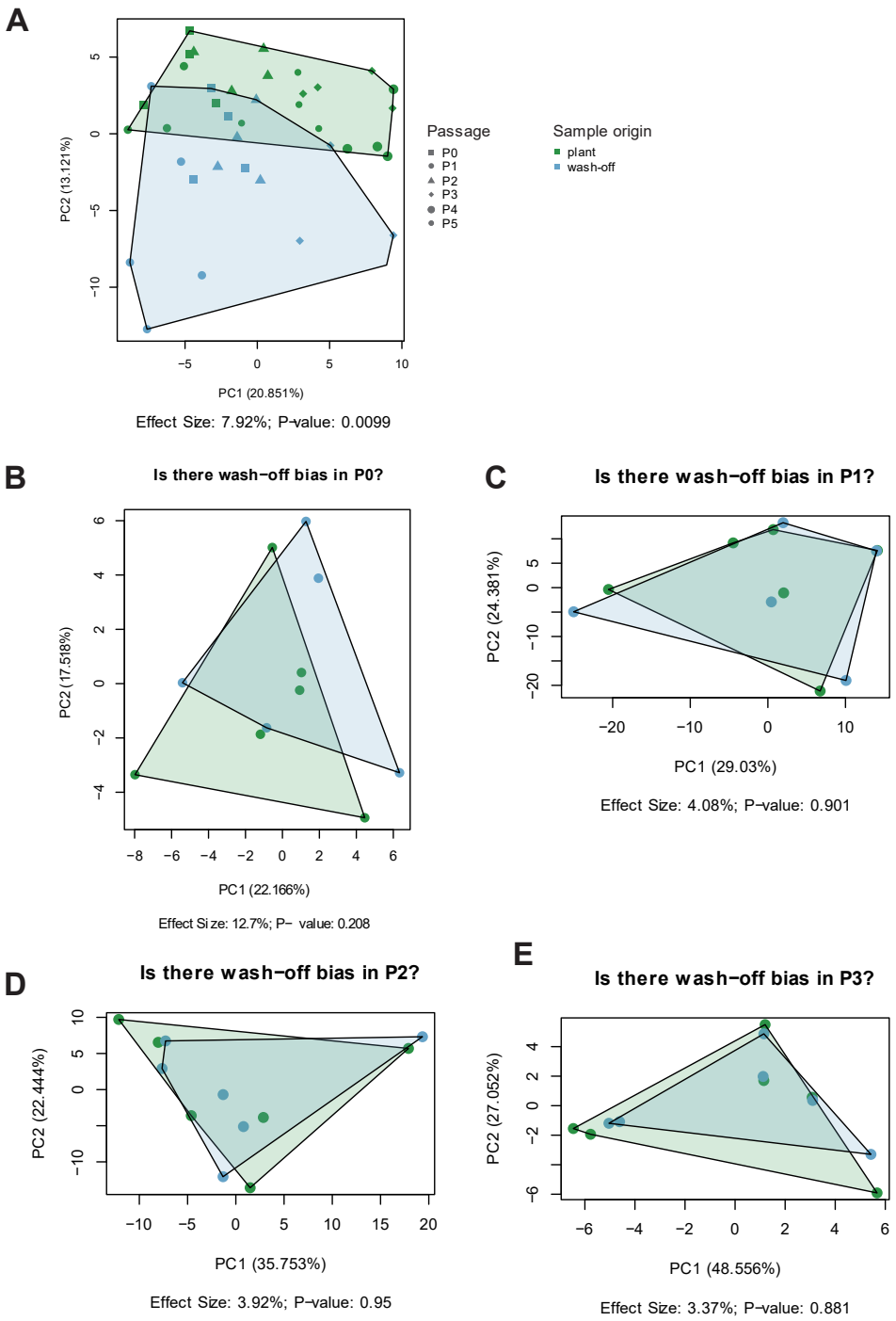

**Supplemental Figure 4: Comparison of start community controls *in planta* versus inoculum composition.** A. PCA of start community controls of inoculum (grey) and *in planta* communities (P0-P5). B. PCA of start community controls with PERMANOVA comparing the effect of composition differences between inoculum (grey) and *in planta* samples (green). The *in planta* samples are composed of the combination of start community control in each passage.



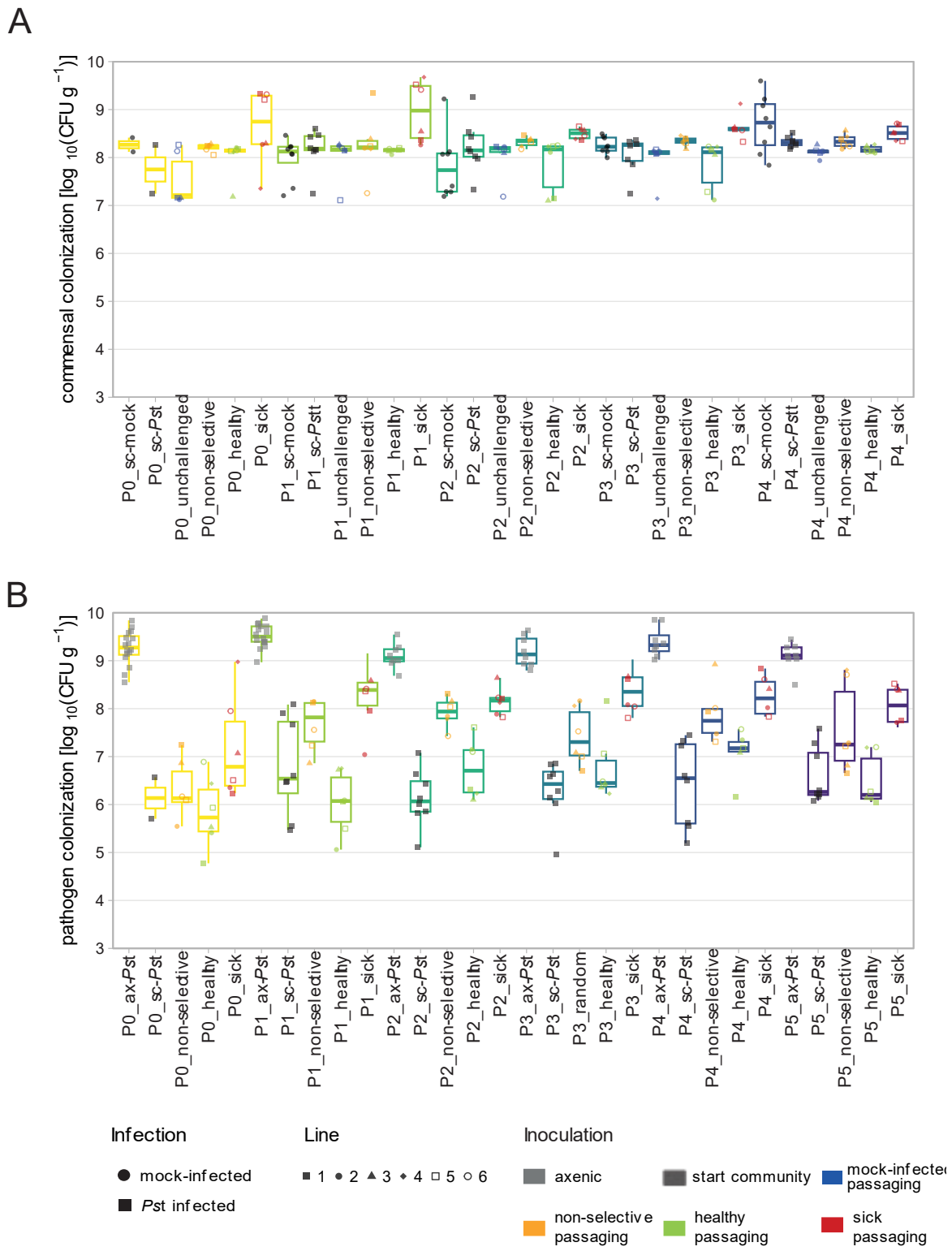

**Supplemental Figure 5: Community composition of start community controls.** The relative abundances of the top 50 ASVs are depicted (for readability purposes cut-off at 50). The ASVs are coloured based on their phylogeny. A. The *in planta* composition of mock-infected start community controls combined over all passaged (n=12). B. The inoculum composition of SynCom-210 (n=3).



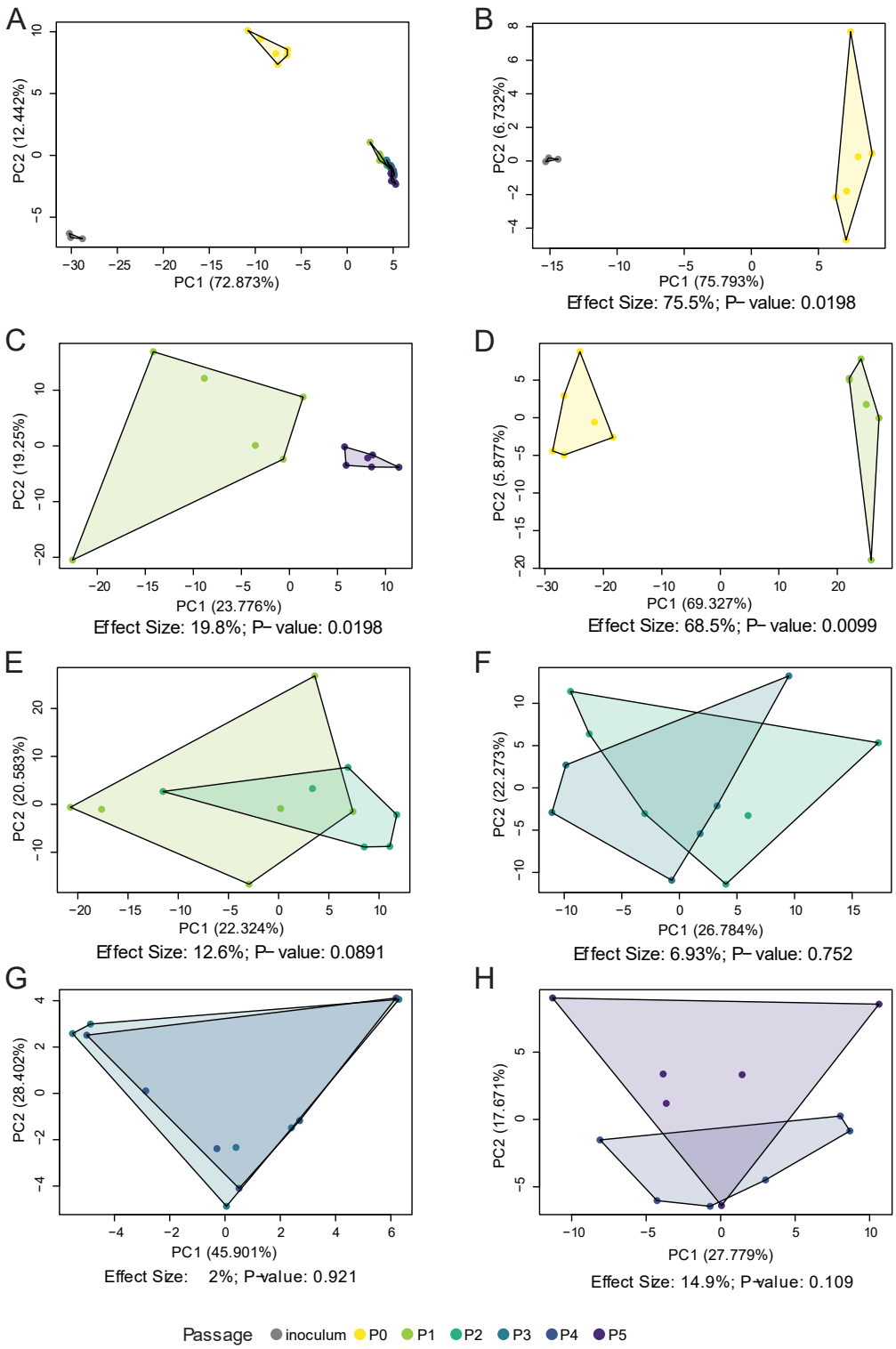

**Supplemental Figure 6: Pielou's evenness and species richness of start community controls in each passage.** The boxplot colour is indicative of the passage. The symbols of mock-infected start community controls are circles and light grey, *Pst*-infected are squares and dark grey. A. Pielou's evenness in each passage (n=2). B. Species richness in each passage (n=2).



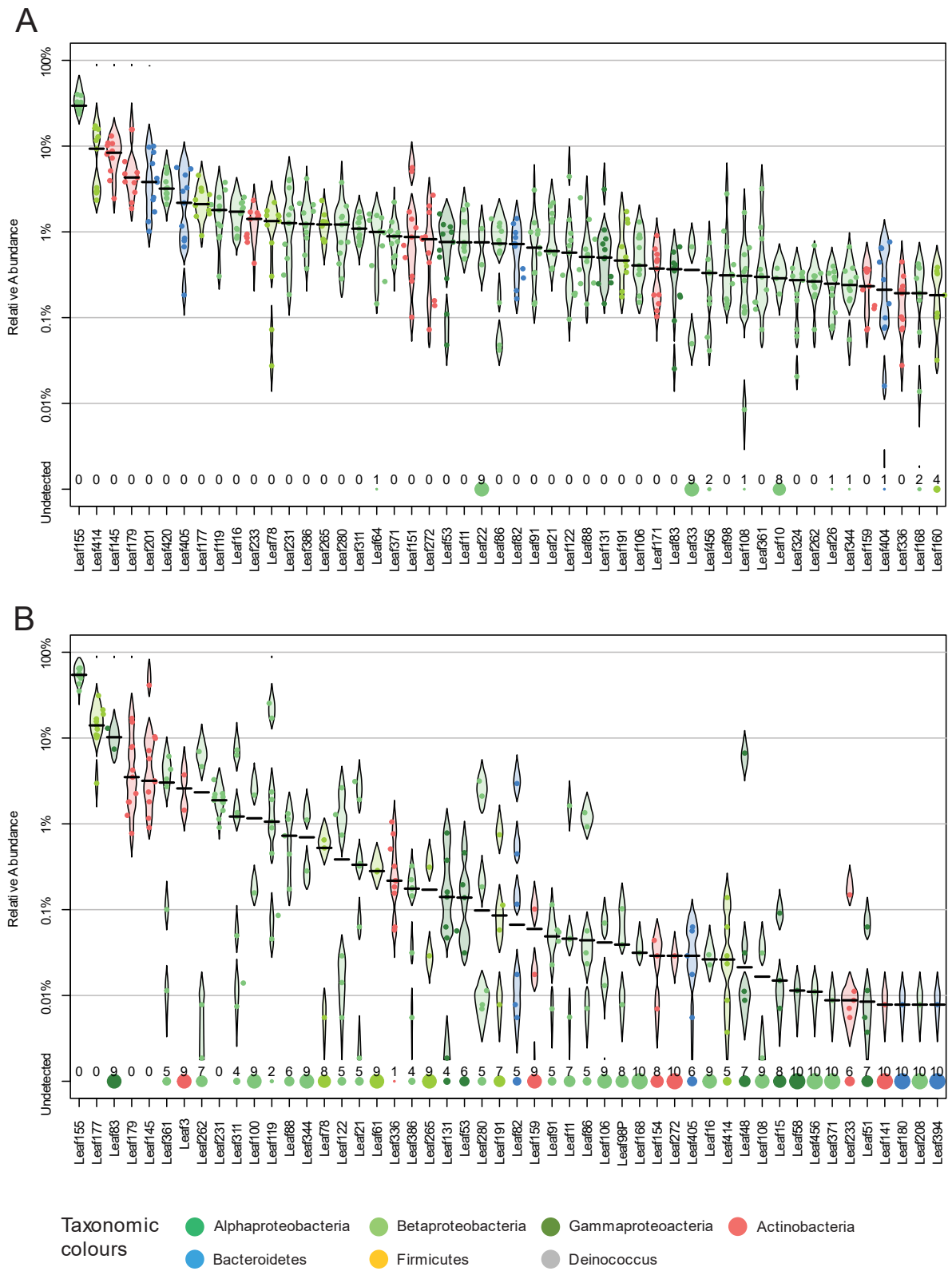

**Supplemental Figure 7: Comparing community composition of *in planta* samples and washed-off community samples.** To control whether our wash-off introduces biases into the community composition and therefore affects the microbiota passaging, we compared the *in planta* composition (green) to the washed-off composition (blue) trough PCA and PERMANOVA analysis. A. The combined start community controls (all passages). B. Samples of the unchallenged passaging in the parental passage. C. Samples of the unchallenged passaging in passage 1. D. Samples of the unchallenged passaging in passage 2. E. Samples of the unchallenged passaging in passage 3. Wash-off samples of passage 4 and 5 were not sequenced due to lack of space in the library.



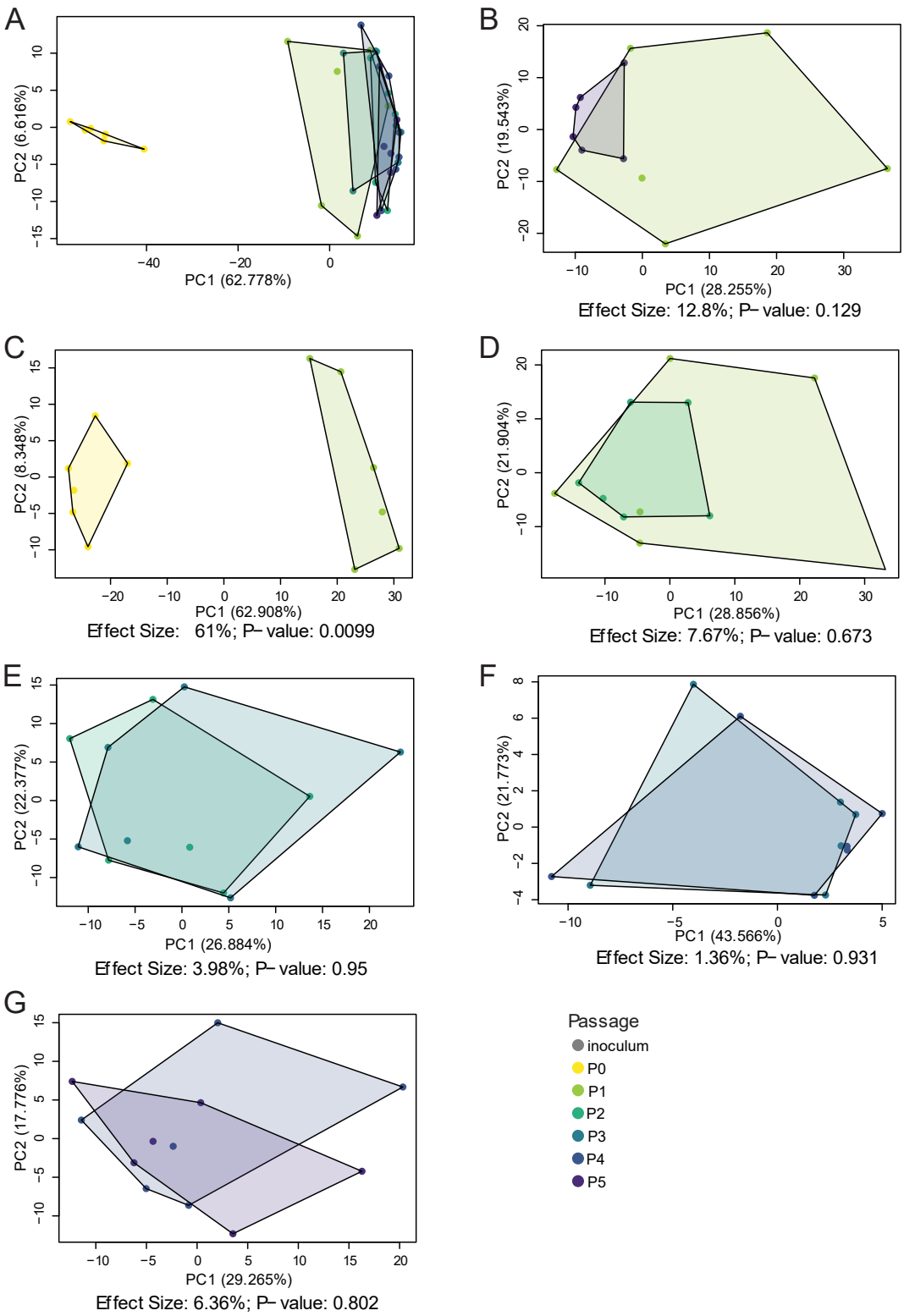

**Supplemental Figure 8: Bacterial colonization of all conditions in first passaging experiment.** A. Total commensal colonization. B. Pathogen colonization. The passage is indicated through boxplot colours, the symbol shape represents infection state for start community controls or selection line for passaging conditions. Commensal colonization of passage 5 is missing. Abbreviations: sc-mock, mock-infected start community control. sc-*Pst*, *Pst*-infected start community controls. ax-*Pst*, axenic *Pst*-infected controls.



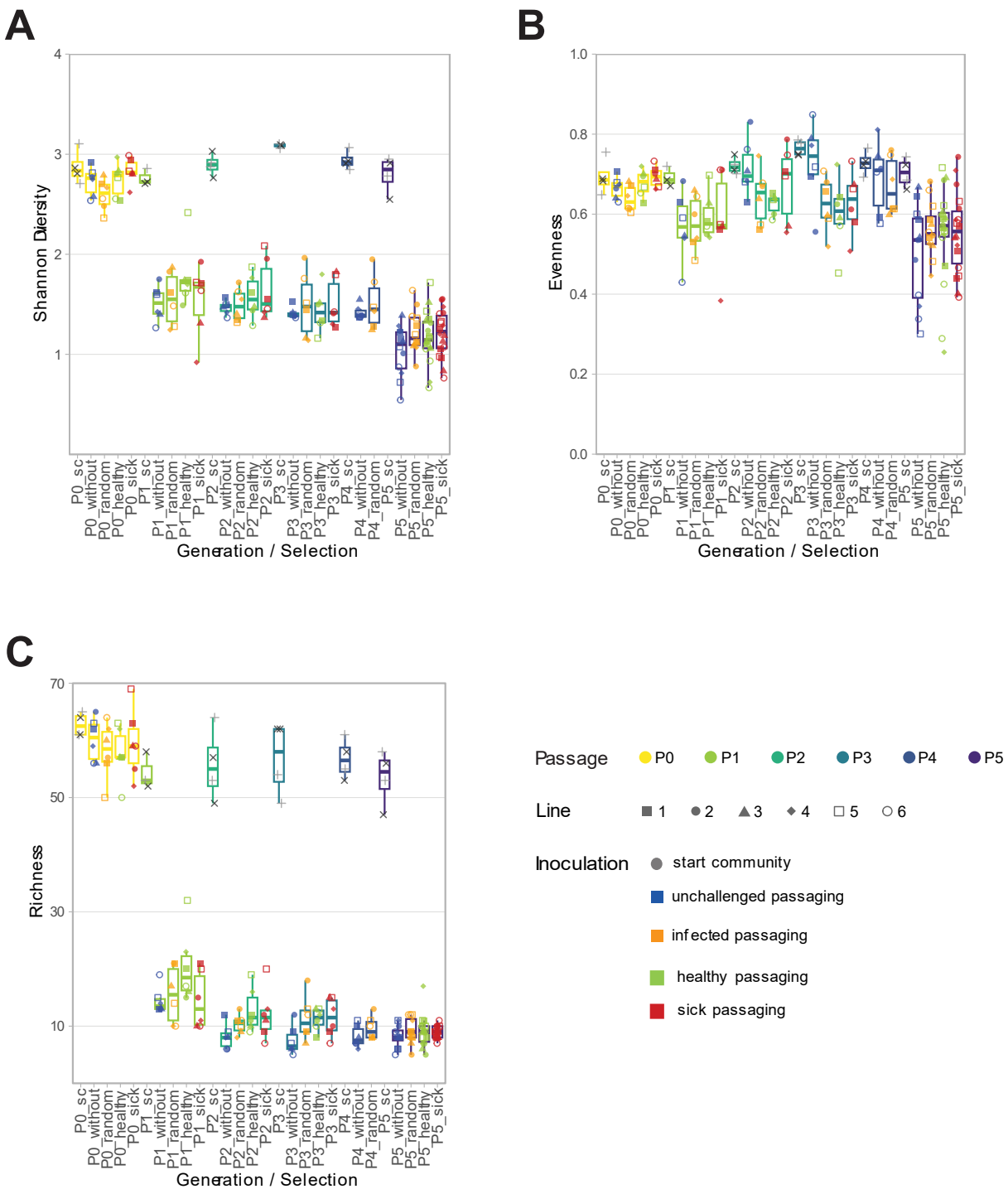

**Supplemental Figure 9: Comparing community composition in passages of unchallenged passaging.** To analyse the global effect on community composition, the samples of each passage was compared to the following passage with a PCA and PERMANOVA analysis A. PCA of samples from inoculum (grey) and all passages (colours). PCA and PERMANOVA comparisons: B. between inoculum (grey) and parental passage (P0) (yellow). C. between passage 1 (light green) and 5 (purple). D. between passage 0 (yellow) and 1 (light green) E. between passage 1 (light green) and 2 (green). F. between 2 (green) and 3 (blue). G. between passage 3 (blue) and 4 (dark blue). H. between passage 4 (dark blue) and 5 (purple).



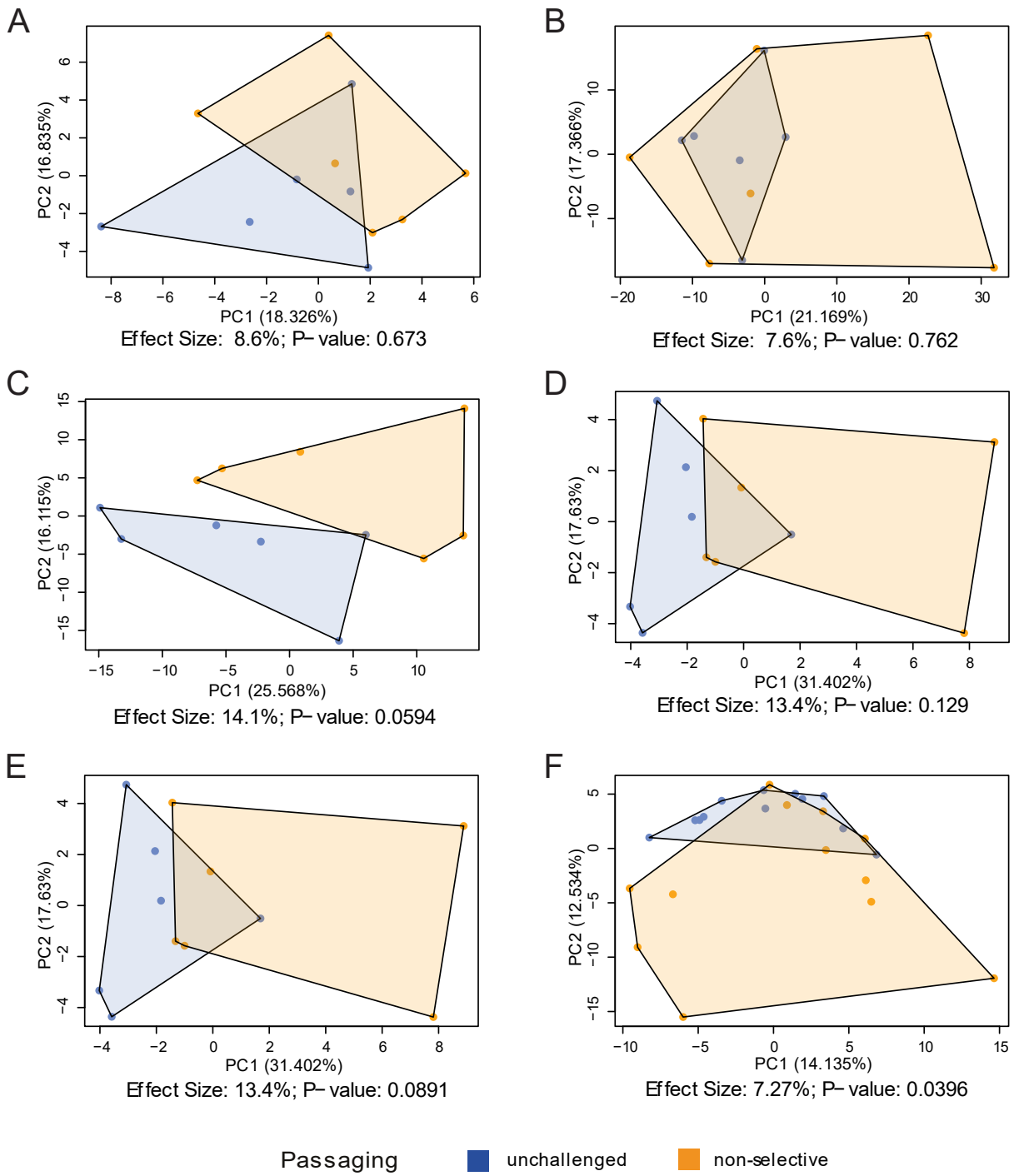

**Supplemental Figure 10: Community composition of unchallenged passaging.** The relative abundances of the top 50 ASVs are depicted (for readability purposes cut-off at 50). The ASVs are coloured based on their phylogeny. A. The community composition of unchallenged passaging in parental passage (P0) (n=12). B. The community composition of unchallenged passaging in passage 1 (n=12).



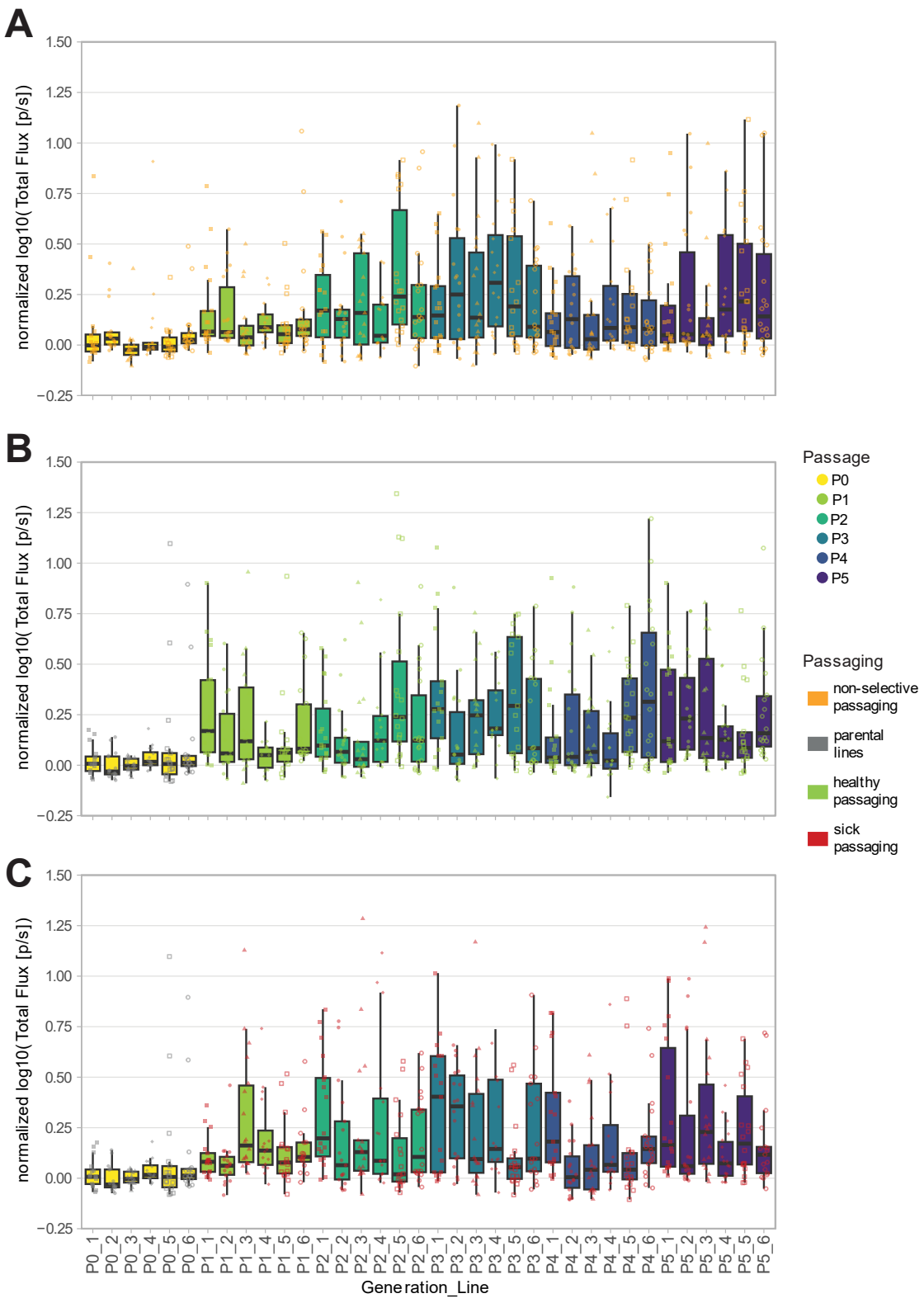

**Supplemental Figure 11: Comparing community composition in passages of non-selective passaging.** To analyse the global effect of passaging on community composition, the samples of each passage was compared to the following passage with a PCA and PERMANOVA analysis A. PCA of all passages (colours). PCA and PERMANOVA comparisons: B. between passage 1 (light green) and 5 (purple). C. between passage 0 (yellow) and 1 (light green) D. between passage 1 (light green) and 2 (green). E. between 2 (green) and 3 (blue). F. between passage 3 (blue) and 4 (dark blue). G. between passage 4 (dark blue) and 5 (purple).



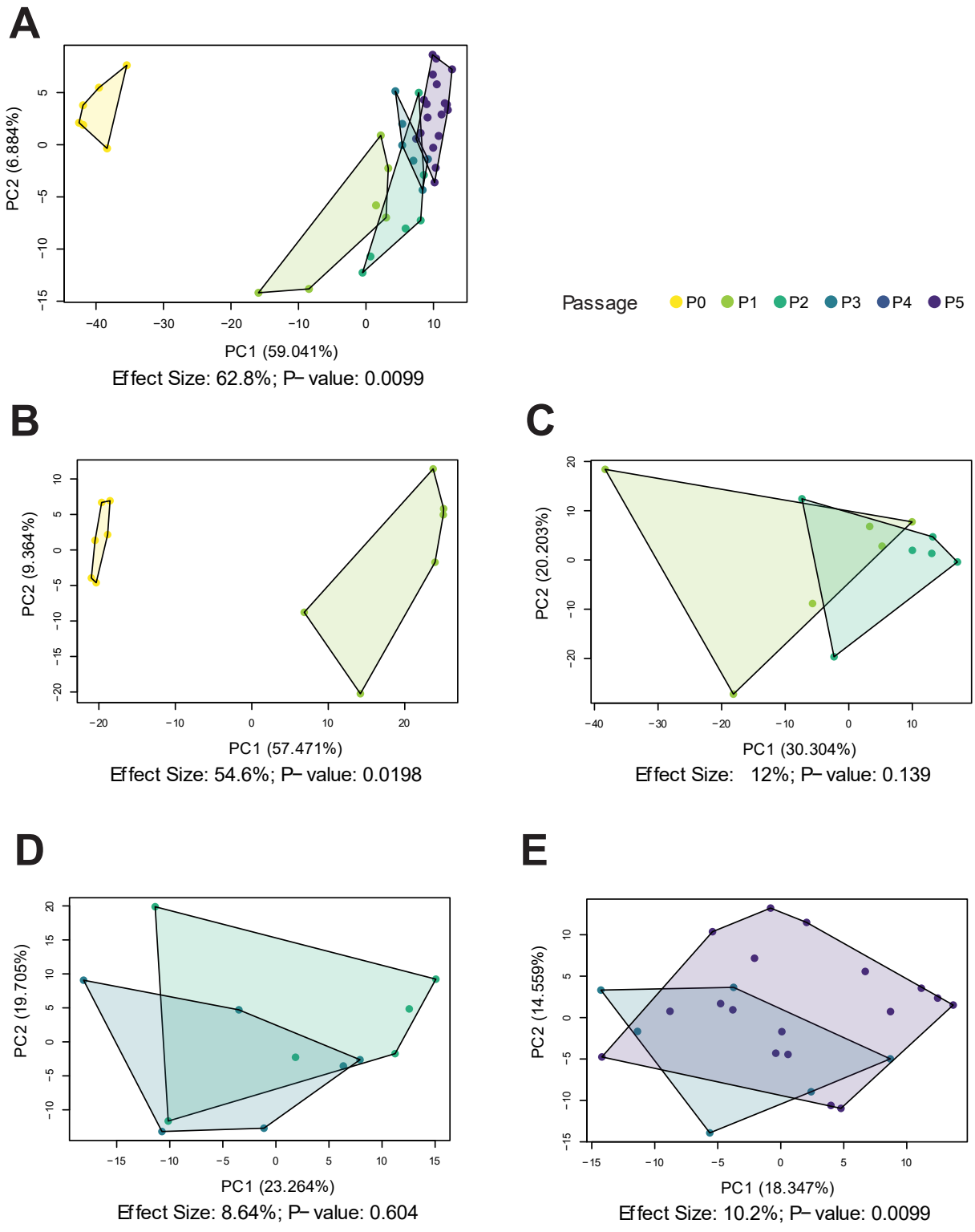

**Supplemental Figure 12: Diversity scores of all conditions in first passaging experiment.** Shannon's diversity, Pielou's evenness and species richness were calculated on rarefied counts (see methods). Passage is indicated by colour of the boxplot. Symbol shape and colour represent infection of start community controls (plus sign for mock-infected, x-shape for *Pst*-infected) and selection lines of passaging. A. Shannon's diversity. B. Pielou's evenness. C. Species richness.



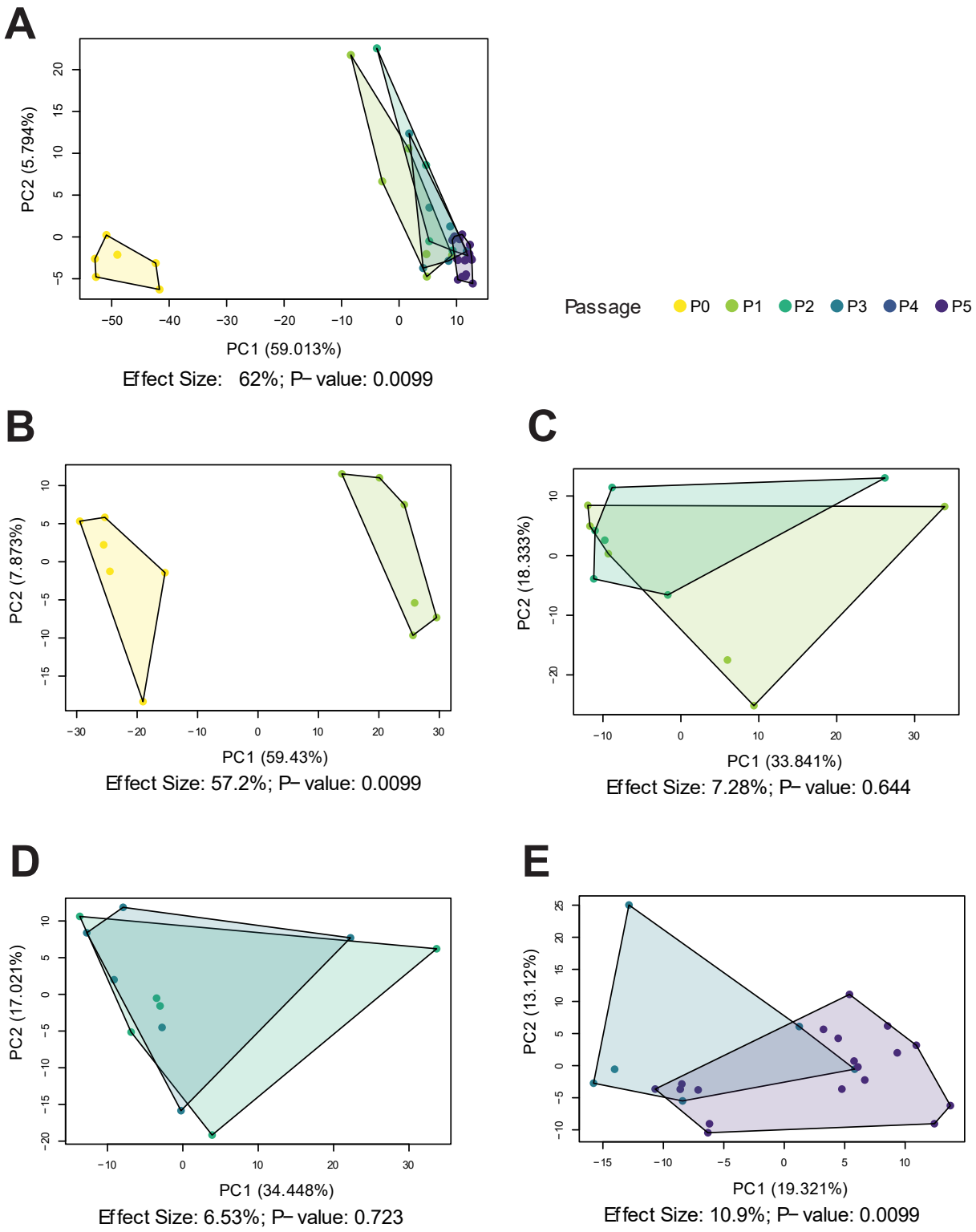

**Supplemental Figure 13: Comparing community composition of unchallenged versus non-selective passaging in each passage.** To analyse the global effect of pathogen challenge on community composition, the unchallenged passaging (blue) was compared to the non-selective passaging (orange) in each passage with a PCA and PERMANOVA analysis. A. Parental passage (P0). B. Passage 1. C. Passage 2. D. Passage 3. E. Passage 4. F. Passage 5.



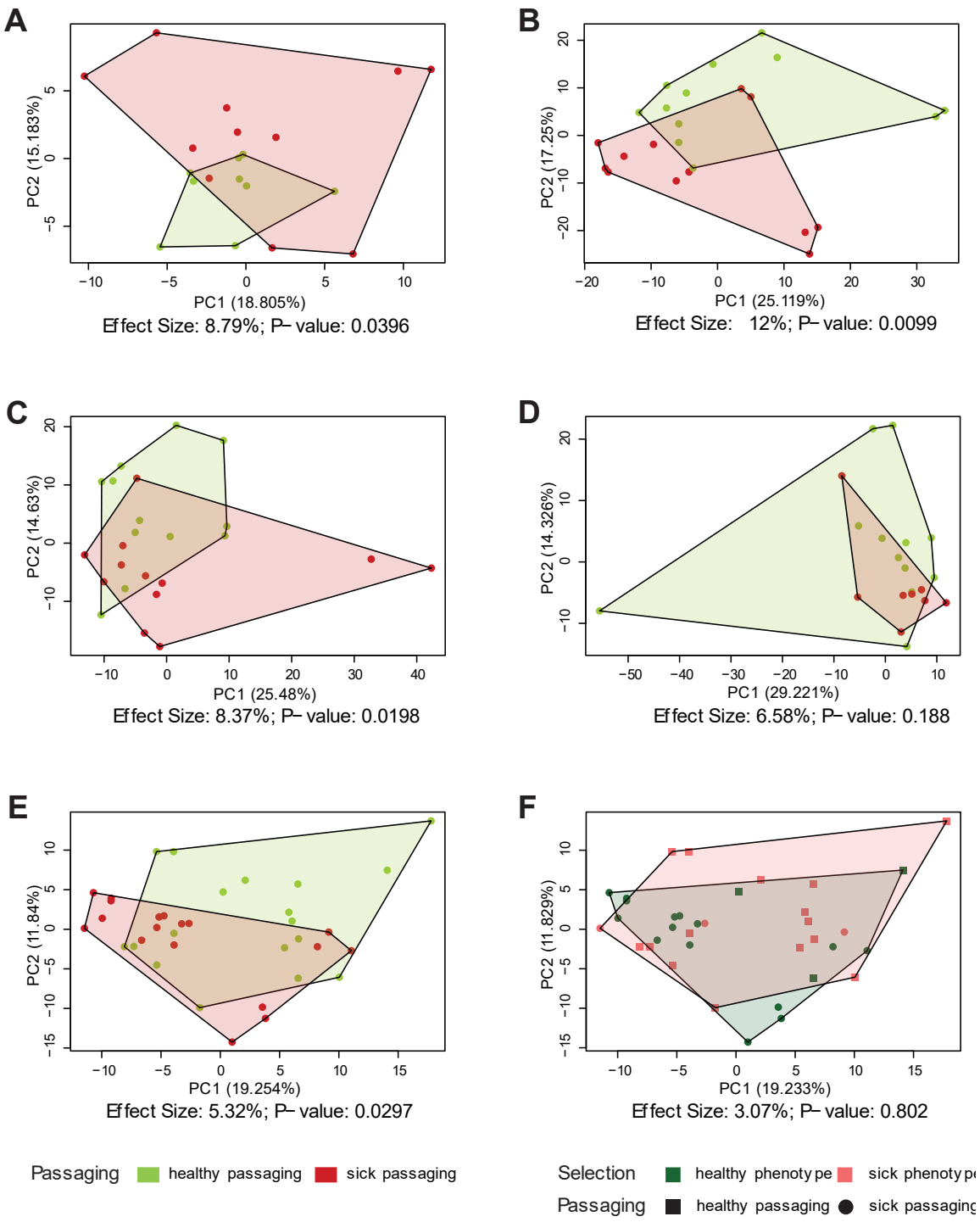

**Supplemental Figure 14: Normalized pathogen luminescence of infected passaging.** The normalized pathogen luminescence at 3dpi is shown for each selection that was infected. Boxplot colours refer to the passage number (see legend). The x-axis is sorted by passage followed by selection line number (1-6). In panels B and C, data for parental lines (grey) are repeated. A. non-selective passaging (orange). B. parental lines (grey) and healthy selection lines (green). C. parental lines (grey) and sick selection lines (red).



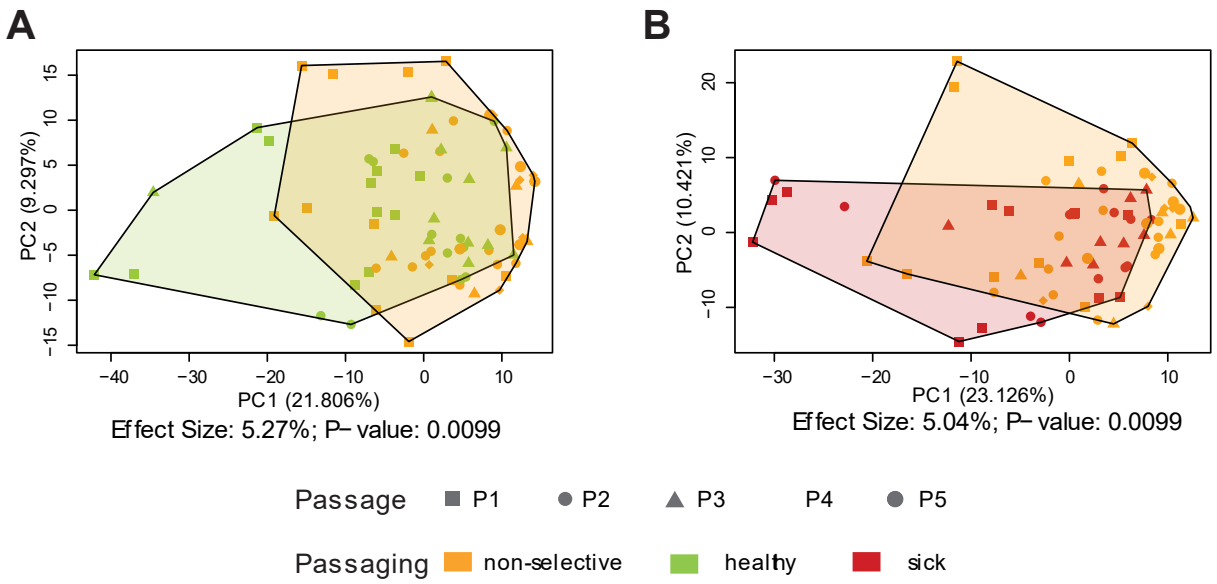

**Supplemental Figure 15: Comparing community composition in passages of healthy selection passaging.** To analyse the global effect of passaging on community composition, the samples of each passage was compared to the following passage with a PCA and PERMANOVA analysis A. PCA of all passages (see colour legend). PCA and PERMANOVA comparisons: B. between passage 0 (yellow) and 1 (light green) C. between passage 1 (light green) and 2 (green). D. between 2 (green) and 3 (blue). E. between passage 3 (blue) and 4 (dark blue). F. between passage 4 (dark blue) and 5 (purple).



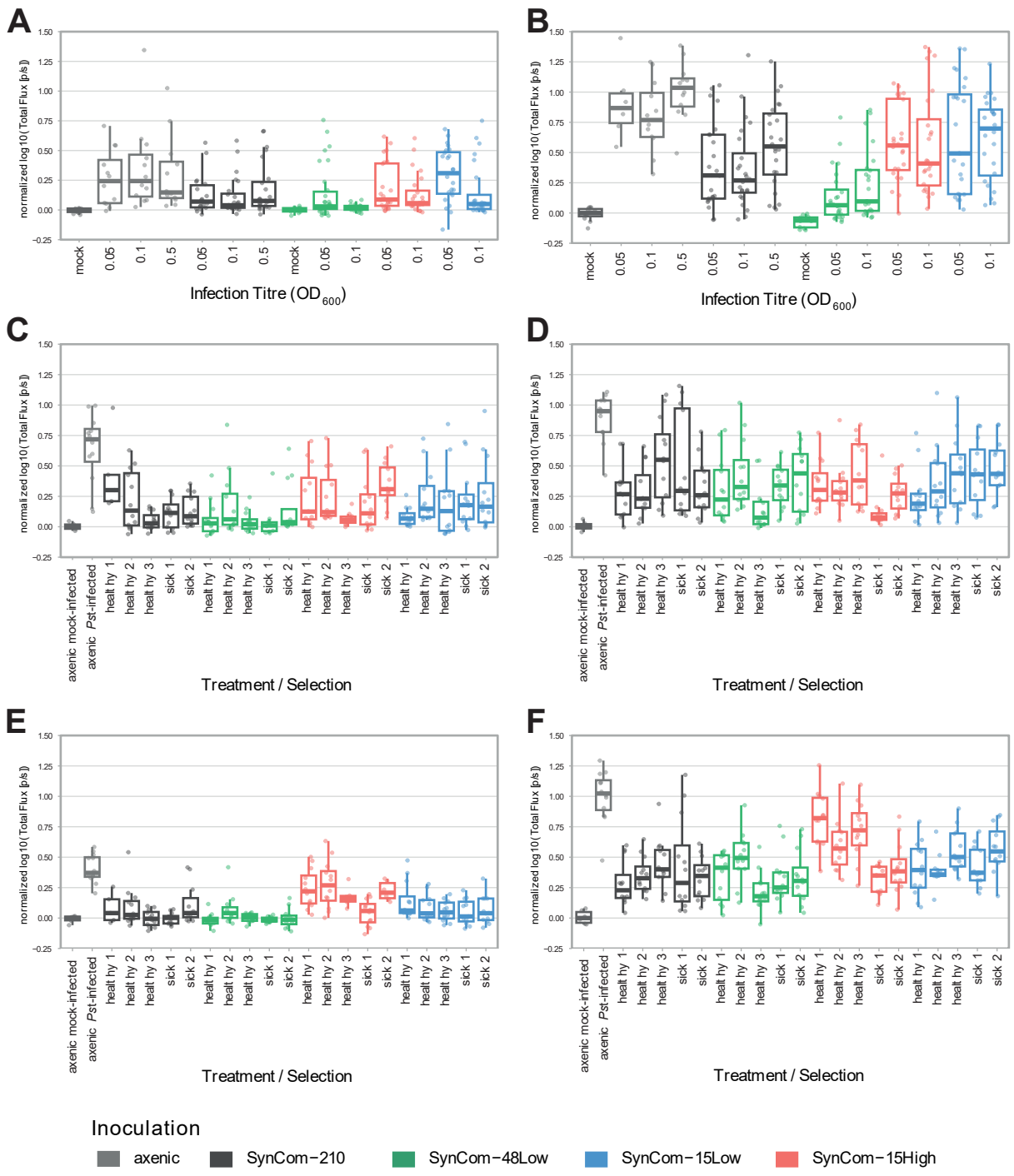

**Supplemental Figure 16: Comparing community composition in passages of sick selection passaging.** To analyse the global effect of passaging on community composition, the samples of each passage was compared to the following passage with a PCA and PERMANOVA analysis A. PCA of all passages (see colour legend). PCA and PERMANOVA comparisons: B. between passage 0 (yellow) and 1 (light green) C. between passage 1 (light green) and 2 (green). D. between 2 (green) and 3 (blue). E. between passage 3 (blue) and 4 (dark blue). F. between passage 4 (dark blue) and 5 (purple).



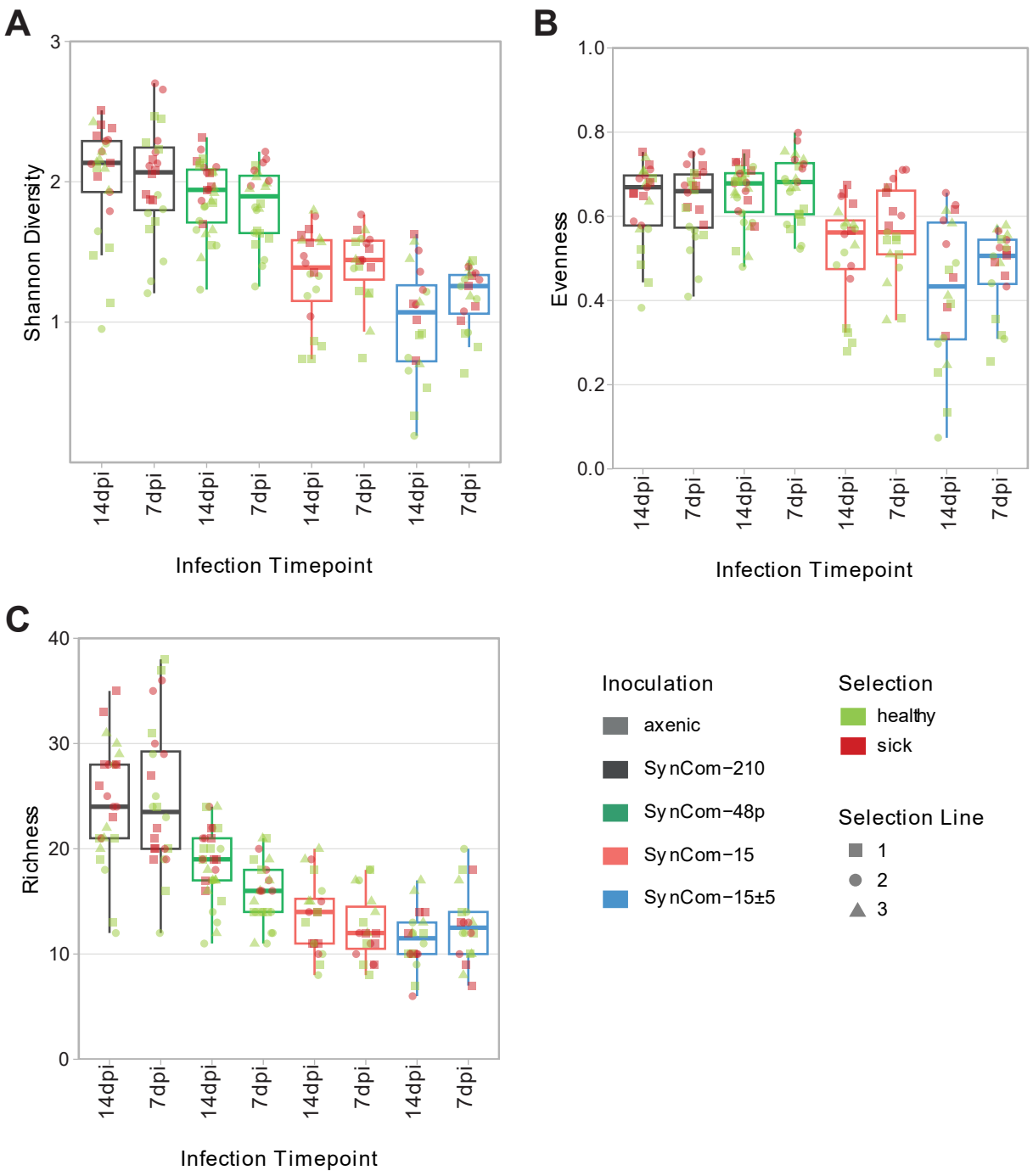

**Supplemental Figure 17: Comparing community composition of healthy versus sick selection passaging in each passage.** To analyse the global effect of phenotypic selection on community composition, the healthy selection passaging (green) was compared to the sick selection passaging (red) in each passage with a PCA and PERMANOVA analysis. A. Parental passage (P0). B. Passage 1. C. Passage 2. D. Passage 3. E. Passage 5. F. Comparison of healthy (dark green) versus sick (dark red) phenotype, regardless of selection type with a PCA and PERMANOVA analysis. To accommodate for the difference in community composition between healthy and sick selection types, it was included as an "batch effect". The symbol shape represents healthy selection (square) and sick selection (circle).



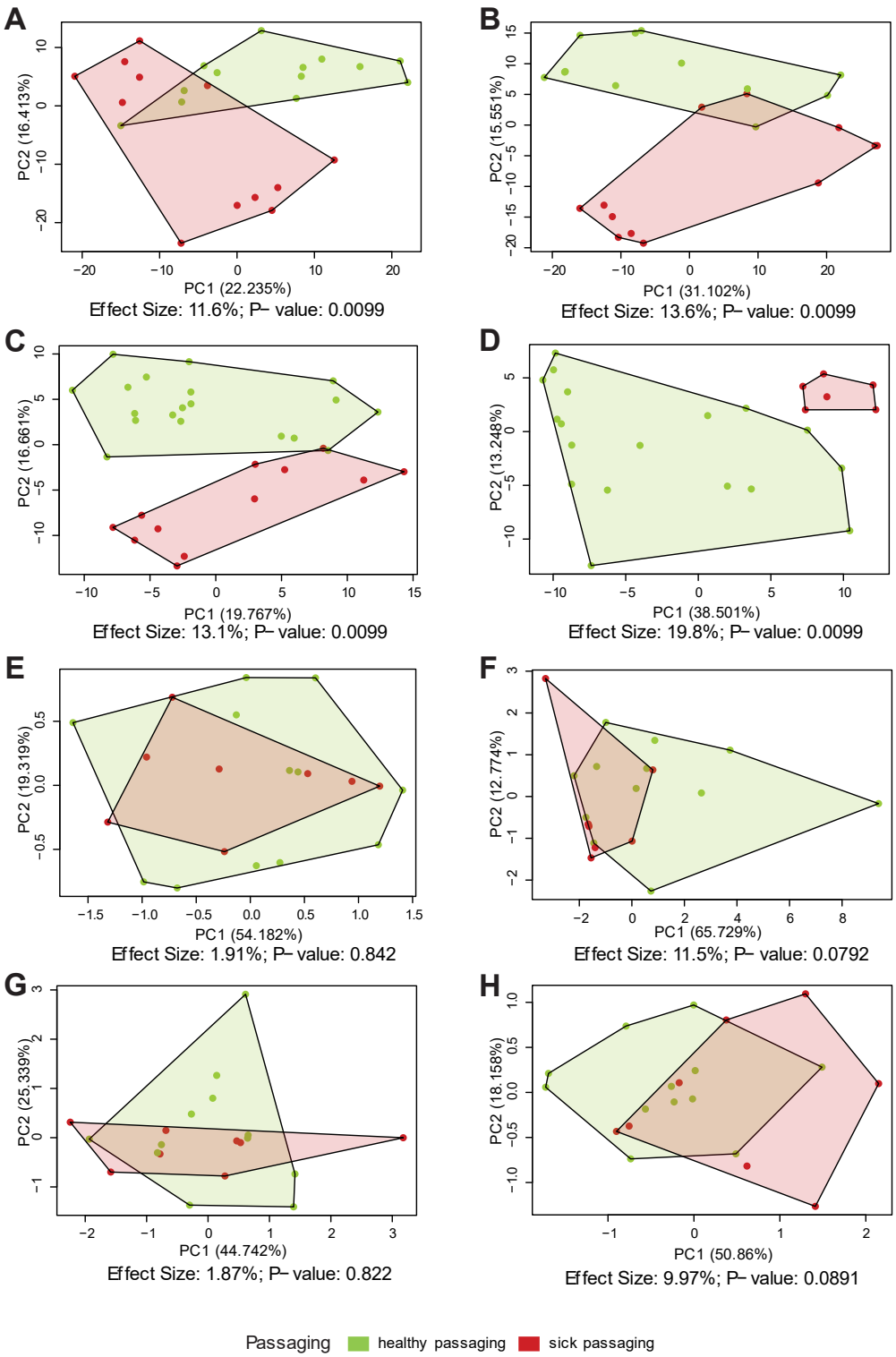

**Supplemental Figure 18: Comparison of selective versus non-selective passaging.** To analyse the global effect of selection type on community composition, the non-selective passaging (orange) was compared to the selective passaging in combined passage 1 through 5 with a PCA and PERMANOVA analysis. A. Non-selective passaging versus healthy selection passaging (green). B. Non-selective passaging versus sick selection passaging (red).



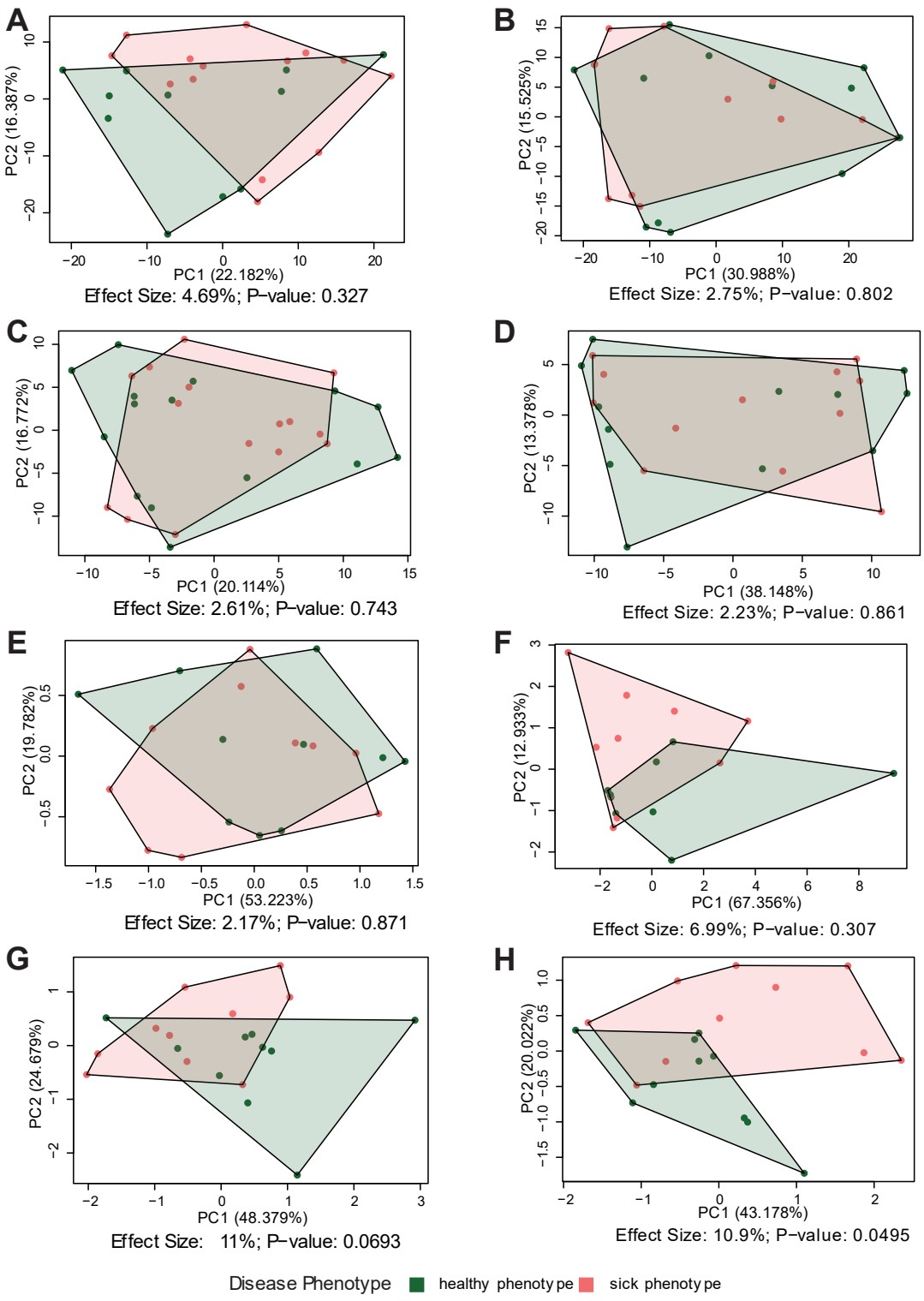

**Supplemental Figure 19: Pathogen luminescence of the second passaging experiment.** A. Normalized pathogen luminescence at 3 dpi in parental passage (P0) of the early infection (21 days old plants). B. Normalized pathogen luminescence at 3 dpi in parental passage (P0) of the late infection (28 days old plants). C. Normalized pathogen luminescence at 3 dpi in the first passage (P1) of the early infection (21 days old plants). D. Normalized pathogen luminescence at 3 dpi in the first passage (P1) of the late infection (28 days old plants). E. Normalized pathogen luminescence at 7 dpi in the first passage (P1) of the early infection (21 days old plants). F. Normalized pathogen luminescence at 7 dpi in the first passage (P1) of the late infection (28 days old plants).




**Supplemental Figure 20: Diversity scores of all conditions in the first passage of the second passaging experiment.** Shannon's diversity, Pielou's evenness and species richness were calculated on rarefied counts (see methods). Community composition is indicated by colour of the boxplot. Symbol colour represent selection type and symbol shape represents selection lines. A. Shannon's diversity. B. Pielou's evenness. C. Species richness.




**Supplemental Figure 21: Comparing community composition of healthy versus sick selection passaging in passage 1 of second passaging experiment.** To analyse the global effect of phenotypic selection on community composition, the healthy selection passaging (green) was compared to the sick selection passaging (red) with a PCA and PERMANOVA analysis. A. SynCom-210, early infection (14 dpi). B. SynCom-210, late infection (7 dpi). C. SynCom-48p, early infection (14 dpi). D. SynCom-48p, late infection (7 dpi). E. SynCom-15, early infection (14 dpi). F. SynCom-15, late infection (7 dpi). G. SynCom-15±5, early infection (14 dpi). H. SynCom-15±5, late infection (7 dpi).




**Supplemental Figure 22: Comparing community composition of different plant phenotypes passaging in passage 1 of second passaging experiment.** To analyse the global effect of plant phenotype on community composition, healthy plants (green) were compared to the sick plants (red) with a PCA and PERMANOVA analysis. A. SynCom-210, early infection (14 dpi). B. SynCom-210, late infection (7 dpi). C. SynCom-48p, early infection (14 dpi). D. SynCom-48p, late infection (7 dpi). E. SynCom-15, early infection (14 dpi). F. SynCom-15, late infection (7 dpi). G. SynCom-15±5, early infection (14 dpi). H. SynCom-15±5, late infection (7 dpi).

# Chapter III

# Correlating microbiota composition and disease outcomes using synthetic community experiments

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**Author contributions**

B.E. and J.A.V. designed the study. B.E. performed experimental laboratory work. M.B.M., B.A.M. and C.I.C contributed to the plant experiments. B.E. performed the data analysis. B.E. and J.A.V. wrote the manuscript.

# Abstract

The host-associated microbiota can alleviate abiotic and biotic stresses. Individual members of the plant-associated microbiota were shown to protect against pathogens. However, their protective effect may depend on the context of the microbiota, which may lead to different or even inconsistent outcomes, for example as a result of higher order interactions. This highlights the importance of considering the interactions of the entire microbiota on disease suppression. To link community composition to disease outcome, we conducted synthetic community experiments in which we linked pathogen colonization to changes in strain abundance and the presence of the leaf-associated native microbiota of *Arabidopsis thaliana*. We found the synthetic community establishment to be resilient against perturbation of initial strain abundances and pathogen invasion. This suggests that the microbiota harbours diverse and redundant mechanisms to control pathogen colonization. Drop-out experiments of the main bacterial phyla of the phyllosphere revealed that the capacity to prevent pathogen colonization was most pronounced in the Proteobacteria. Experiments with synthetic communities in which strains were replaced with others showed that synthetic community can be altered towards different disease outcomes, suggesting that presence of a limited number of members of the microbiota to be crucial for plant protection. We found that strains with higher levels of protection by themselves are generally more abundant *in planta*. The competitive trait might lead to a positive feedback on plant fitness and indirectly of these strains, providing an example how ecological interactions can drive evolutionary processes in the long term.


# Introduction

Complex multicellular organisms, including animals and plants, are hosts to diverse microbes, collectively called the microbiota [1,2]. Research has uncovered parts of the microbiota that can confer beneficial or harmful effects on the host – or generally – its phenotype. Members of the microbiota were shown to help with digestion of food [3,4], crosstalk with immunity and help with its development [5-7] and infers protection against pathogens [8,9], called colonization resistance [10]. Like the gut microbiota, members of the plant-associated impact their host-state by increasing nutrient availability [11,12], priming the plant immune system [13,14], and alleviating biotic and abiotic stresses [15-17] - or impacting flowering time [18,19].

Plant health is of global interest to ensure food safety [20] and to meet growing demands for food, feed and fibre [21]. However, crop health and yields are under threat from re-emergent and spread of disease following human activities [22]. The traditional way to manage pathogens and reduce disease outbreak was to apply chemicals that limit pathogen growth; however, such treatments come with a risk of negatively impacting beneficial microbes [23] [24]. The importance of keeping a healthy microbiome to control disease has been highlighted, though we lack the understanding of how to retain and restore them [25,26]. Sustaining a healthy

microbiome through management practices and applying knowledge of the beneficial components of the microbiota will help to move agriculture towards more sustainable and safe practices [27,28].

Major efforts are pursued to discover plant-associated beneficial microbes and their mechanisms to improve plant health [13,15,29-31]. It has been suggested that exploitation of the beneficial native microbes might close that gap, while having a lessened impact on the underlying microbiota [32]. However. application of these microbial inoculants as biocontrol strains have been shown with inconsistent success [33]. While knowledge about behaviour and mechanisms of individual strains in association with their respective targets (plant and pathogen species) are well researched, their behaviour when in contact and competition with the microbiota is not well understood. While several instances of emergent synergisms of consortia of strains over their individual components were shown [34-36], an inhibition of individually beneficial strains can also occur [37]. Strains in competition with each other may also display character displacement, where they utilize different metabolic pathways for growth upon competition with each other [38], the same is likely true for plant protection mechanisms – be it for better or worse outcomes.

The fundamental understanding of which parts of the microbiota confer protection, and how the behaviour of individual strains affects community properties is currently not well understood. While most studies focus on correlating disease prevalence and pathogen abundance with changes in community composition or co-occurrence networks of OTUs with the pathogen[16,39,40], the causal link between the two is lacking. Correlations of the pathogen with OTU changes alone will not provide information about underlying mechanisms driving observed patterns of community compositions, and do not guarantee an interaction between members of the community with each other or with the pathogen [41]. It was shown that traits in mono-association with the host can partially inform the design of synthetic communities, and be used to predict plant phenotype [42]. Additionally, it was suggested that initial composition of the microbiota is crucial for plant health outcome [43], while others suggested that abundance of microbiota members might be more important than their presence alone [16].

Here, we wanted to investigate the relationship between community composition and disease outcome by linking the strain presence to pathogen colonization. To do so, we took advantage of a previously established gnotobiotic system with *Arabidopsis thaliana* Col-0 as the plant host [44-46], a representative native bacterial collection, called *At*-LPSHERE [47], and the pathogen *Pseudomonas syringae* pathovar *tomato* DC300 (*Pst*) to conduct synthetic community experiments. Using synthetic communities and manipulating the inoculum composition gives the advantage of inferring altering the community composition in a targeted manner to establish causal relationships [44,45].

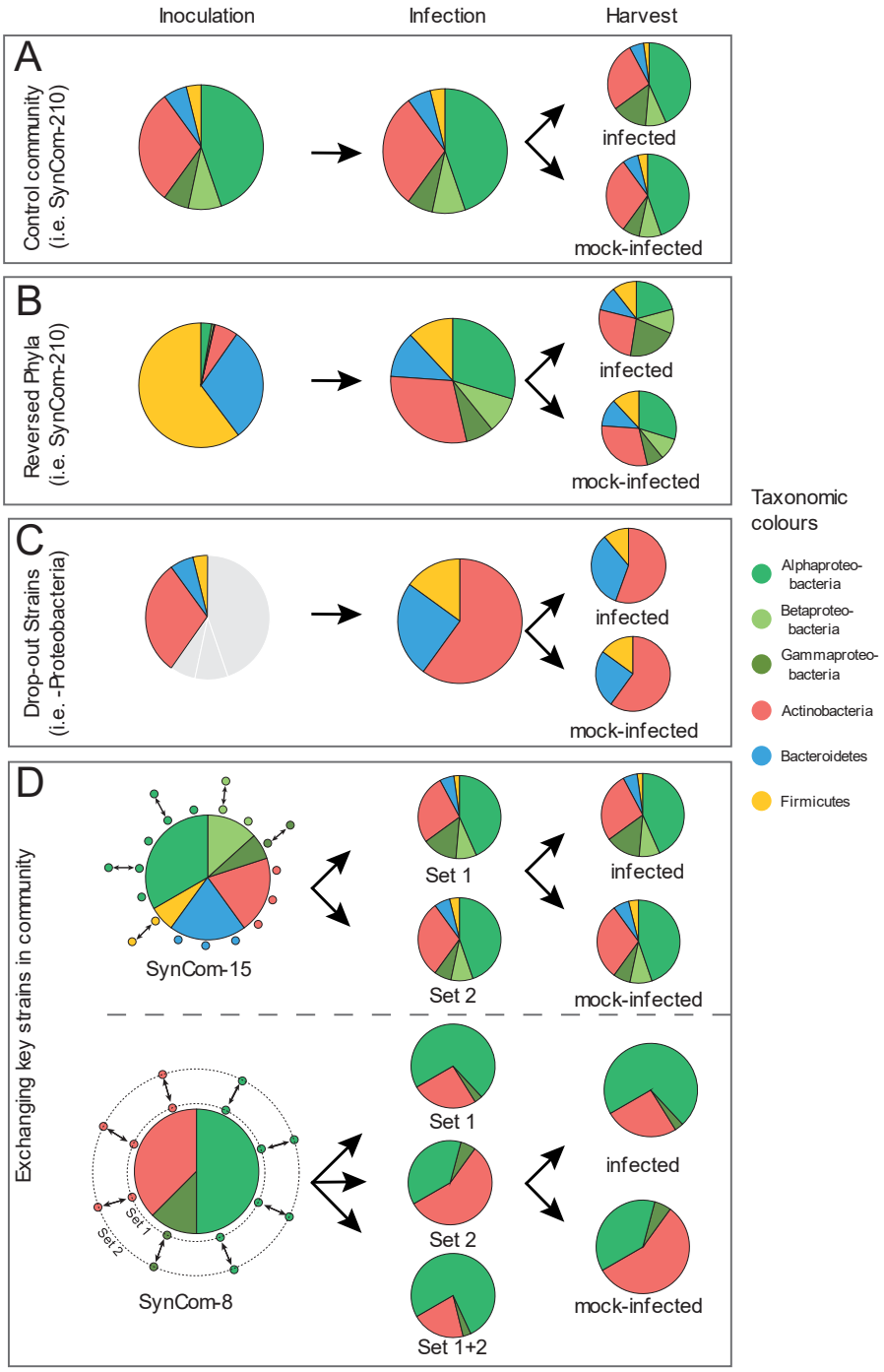


**Figure 1: Schematic overview of experimental designs of synthetic community experiments carried out in Chapter 3.** In each synthetic community experiment, plants were inoculated with varied inocula, infected and then harvested after 7 or 14 days post infection. A. The "default" composition of the SynCom-210 is composed of the main phyla Proteobacteria (126 strains), Actinobacteria (63), Bacteroidetes (13) and Firmicutes (8). B. In the first approach, strain abundances were changed. The largest shift introduced was to reverse the volume of each phylum, making the Firmicutes the most abundant in the inoculum. C. In the second approach, groups of strains were dropped out of the inoculum, here shown on the example of the Proteobacteria drop-out. D. In the third approach, strains were exchanged for others in the inoculum. Two communities were used for this, with different amounts of strains removed. Once, the SynCom-15 was altered by exchanging a few strains. Second, two sets of SynCom-8 were set up with completely distinct strains.

# Results

To investigate the effect of the microbiota composition on plant protection outcomes we tested different inocula. We used a previously established gnotobiotic growth system [44,46] that involved bacterial strains from the *At*-LSPHERE collection [47], *Arabidopsis thaliana* as a model plant, and the foliar pathogen *Pseudomonas syringae* (*Ps*t, pathogen) [31]. In a first approach, the relative abundance of the *At*-LSPHERE strains of the input inoculum was changed, either using an imbalanced microbiota composition, corresponding to a reversed phyla representation - or by reducing the percentage of specific genera, i.e. *Rhizobium*, *Methylophilus, Devosia* (Figure 1A,B). In an additional experiment, phylogenetic groups were omitted from the inoculum, for example the phylum Proteobacteria (Figure 1C). Finally, specific strains in the community were exchanged and the phenotypic outcomes of the two sets of strains compared (Figure 1D).

**Investigation of the impact of strain abundances in the inoculum on microbiota establishment and on plant protection**

To introduce microbiota perturbations that may lead to different plant protection outcomes, we changed the strain abundances in the inoculum. The SynCom-210 consists of 126 strains of the phylum Proteobacteria (94 Alpha-, 18 Beta-, 14 Gammaproteobacteria) 63 Actinobacteria, 13 Bacteroidetes and 8 Firmicutes (Figure 1A, Table 1) and this distribution was used as the default (SynCom-210). The default inoculum was generated by mixing all strains in a 1:1 ratio, resulting in Proteobacteria being most abundantly represented and Firmicutes the least. To perturb the inoculum in relative abundance the percentage of the phyla when mixing the inoculum was reversed (Figure 1B). To do so, the strains were mixed in a 1:1 ratio according to their phyla, and the phyla mixtures were then combined in the appropriate ratios to generate the "reversed phyla" composition of the SynCom-210. In parallel, we reduced the abundance of strains belonging to the genera *Rhizobium*, *Methylophilus* and *Devosia*, which were the most abundant genera in synthetic community experiments [44] (Chapter 2). To test the impact of these abundant genera, the amount of the genera mixed into the default inoculum was reduced tenfold, and the generated inocula are referred to as as "low *Rhizobium*" and "low *Methylophilus*, "low *Devosia*" inoculum variation. In a separate treatment, referred to as "with pathogen" *Serratia* Leaf50 and the model pathogen *P. syringae* pv. *tomato* DC3000 (*Ps*t) were included in the inoculum at the same level as any other strains of the microbiota (for details, see methods). After 14 and 21 days, all inocula variations were treated with the pathogen to analyse the pathogen colonization at 7 and 14 dpi, respectively. In both cases, 14 days after the first infection event, plants were harvested to analyse pathogen colonization and *in planta* community compositions.

First, we analyzed the differences of community compositions of the perturbed inocula compared to the default (SynCom-210). The *in planta* community composition seemed to be less distinct than the inocula communities, though due to only one inoculum sample, no statistical analysis was performed (Figure 2A).

As expected, *in planta*, the largest shift in community composition compared to the "default" SynCom-210 composition, was the "reversed phyla" treatment (size effect 19.3 %, p-value = 0.0099) (Supplemental Figure 1A). A surprising small number of ASVs significantly changed in relative abundance when comparing the default treatment to the "reversed phyla". Of the 12 ASVs that were significantly different in abundance, ten belonged to the Proteobacterium phyla, all decreased in abundance, while 2 ASVs belonging to the Actinobacteria phylum (*Agreia* Leaf210, *Microbacterium* Leaf159) increased in relative abundance by a fold change over 10 (Supplemental Table 2). Most notably of the decreased ASVs, ASV *Methylophilus* Leaf141 decreased from rank 4 in the default treatment to below rank 50 in "reversed phyla" treatment (Supplemental Figure 2A,B). However, the most abundant ASVs (*Rhizobium* Leaf155) remained the same and did not change substantially in relative abundance despite being reduced in the inoculum (Supplemental Figure 2A,B). Apart from the changed ASV abundances, we observed a reduction in bacterial diversity and species richness of the community upon plant colonization (Supplemental Figure 3A,B, Supplemental Table 3). Community evenness was not significantly affected (Supplemental Figure 3C, Supplemental Table 3). The dissimilarity of community composition of infected plants of the "reversed phyla" treatment compared to default were smaller, more so at 7 dpi (PERMANOVA effect size 11.8%, p-value 0.0099), then at 14 dpi (PERMANOVA effect size 16.4%, p-value 0.0099) (Supplemental Figure 1BC). Comparing the mock-infected community composition to infected samples within the default treatment, no significant differences were found (Supplemental Figure 4A-C). In contrast, the "reversed phyla" treatment significantly differed between mock-infected and 7 dpi (PERMANOVA effect size 8.68%, p-value 0.0099) (Supplemental Figure 4E), but not between mock and 14 dpi or 7 and 14 dpi (Supplemental Figure DF).

The reduction of the *Rhizobium* genus in the inoculum had no significant effect in community composition *in planta* compared to default (PERMANOVA effect size 6.09%, p-value 0.198) (Supplemental Figure 1D). Two *Rhizobium* ASVs, Leaf306 and Leaf311 were significantly decreased in the "low *Rhizobium*" treatment, but no other ASVs were significantly affected (Supplemental Table 2). The most abundant ASVs remained rather stable (Supplemental Figure 2A,C), despite the most abundant ASV belonging to the *Rhizobium* genus. Pathogen infection resulted in differences between the "low *Rhizobium*" treatment and the default at 7 dpi (PERMANOVA effect size 8.88%, p-value 0.0099) (Supplemental Figure 1E) but returned to a similar non-significant effect size as seen in mock-infected samples (PERMANOVA effect size 6.41%, p-value 0.139) (Supplemental Figure 1F). Comparing community composition of mock-infected plants compared to infected ones within "low *Rhizobium*" treatment, the composition is affected significantly at 7 dpi compared to mock-infected (PERMANOVA effect size 7.54%, p-value 0.0495) (Supplemental Figure 4H), but not at 14 dpi or between 7 and 14 dpi (Supplemental Figure 4G,I). Community diversity, evenness and species richness were not significantly affected by the "low *Rhizobium*" treatment compared to default, nor by pathogen infection (Supplemental Figure 3, Supplemental Table 3).

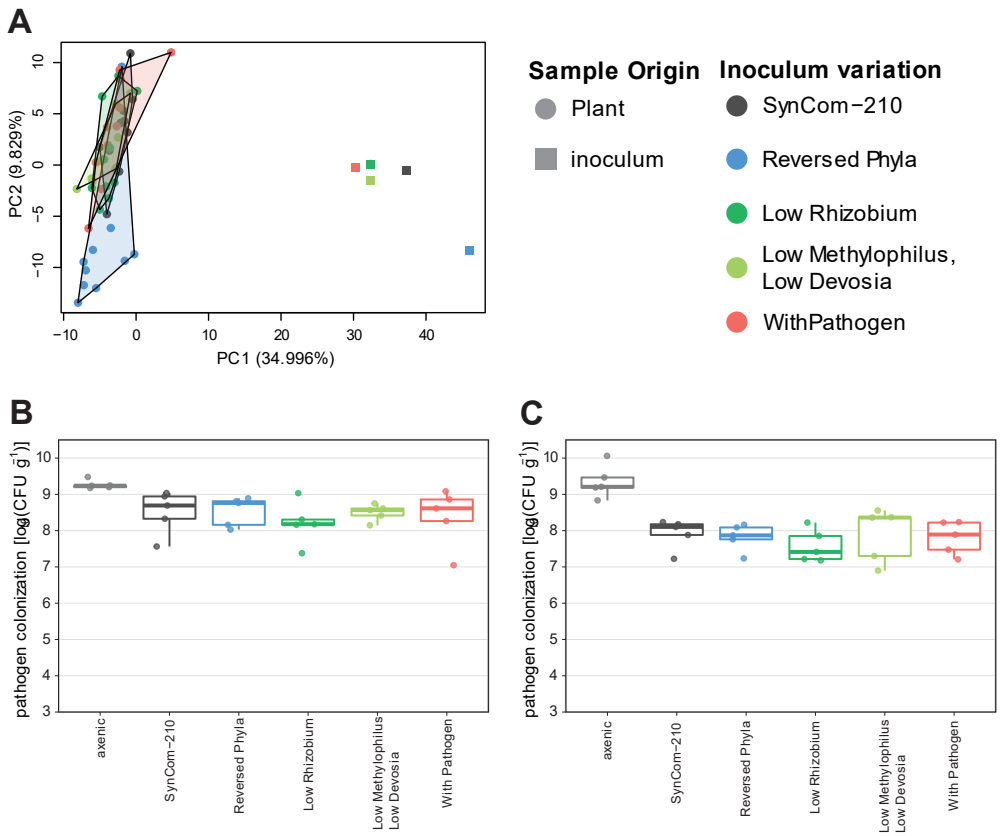


**Figure 2: Impact of strain abundances in the inoculum on microbiota establishment and on plant protection.** A. Principal component analysis of inoculum (squares) and *in planta* (circles) community composition of different inocula treatments. B. Pathogen colonization at 7 days post infection (n=5). C. Pathogen colonization at 14 days post infection (n=5).

Reducing *Methylophilus* and *Devosia* genera in the inoculum affected the community composition significantly when compared to default (PERMANOVA effect size 10.3%, p-value 0.0099) (Supplemental Figure 1G). Of the four ASVs affected by the perturbation, three belonged to the perturbed genera, as well as one *Arthrobacter* ASV (Leaf337), all were reduced in relative abundance in "low *Methylophilus*, low *Devosia*" compared to default (Supplemental Table 2). As seen in the other treatments, the most abundant ASVs that did not belong to *Methylophilus* or *Devosia* were unchanged by the perturbation (Supplemental Figure 2A,D). Community composition of infected plants of the "low *Methylophilus*, low *Devosia*" treatment compared to default differed at 7 dpi (PERMANOVA effect size 9.31%, p-value 0.0099) (Supplemental Figure 1H), but not at 14 dpi (PERMANOVA effect size 6.48%, p-value 0.188) (Supplemental Figure 1I). Pathogen infection significantly affected community composition when comparing the mock-infected samples of the "low *Methylophilus*, low *Devosia*" treatment compared to 14 dpi (PERMANOVA effect size 11%, p-value 0.0099) (Supplemental Figure 4K), and between 7 and 14 dpi (PERMANOVA effect size 8.74%, p-value 0.0099) (Supplemental Figure 4L). This shift could be due to the higher species richness seen at 14 dpi compared to mock-infected (Supplemental Figure 3B,

Supplemental Table 3). Community diversity and evenness was comparable to default conditions (Supplemental Figure 3A,C, Supplemental Table 3).

Addition of the pathogenic strains in the inoculum did not affect the overall community composition compared to default treatment, nor were any other ASVs changed significantly, other than the added strains (*Serratia* Leaf50, *Pst*) (Supplemental Figure 1J, Supplemental Table 2). Upon pathogen infection, the community composition becomes significantly different at 7dpi (PERMANOVA effect size 14.5%, p-value 0.0099) (Supplemental Figure 1K), before returning to the composition caused by the default inoculum at 14 dpi without significant difference (PERMANOVA effect size 6.19%, p-value 0.188) (Supplemental Figure 1L). Comparing the infection types within "with pathogen" treatment, all comparisons are significantly different (Supplemental Figure 4M-O), suggesting that the added strains did have an effect on community composition upon infection. Community diversity, evenness and species richness of the "with pathogen" treatment was not affected compared to default inoculum, not affected by infection (Supplemental Figure 3, Supplemental Table 3).

Despite some differences in community composition, none of the inoculum variations resulted in significant differences of the pathogen colonization compared to the default composition, neither at 7 dpi (Figure 2B) nor at14 dpi (Figure 2C) (Supplemental Table 4). At 7 dpi, none of the communities were significantly different from axenic infected samples either, which could be due to low sample size (n=5). The median pathogen colonization ranged from 1.5 to 5.8 x $10^8$ at 7 dpi, which was slightly but non-significantly reduced to a range of 2.6 x$10^7$ to 2.3 x $10^8$ cfu $g^{-1}$ plant fresh weight at 14 dpi (Supplemental Table 4,5). Pathogen colonization in axenic infected remained at a similar level, suggesting that the plants inoculated with synthetic communities were restricting pathogen growth over time.

In conclusion, despite having introduced perturbation at the inoculum level, and having seen these perturbations affected the *in planta* composition to various degrees, none of the perturbations resulted in differences in the pathogen colonization. This could be due to the fact that these strains were still present on the plant despite reduced strain abundances. Additionally, our observations highlight the redundancy of protection mechanisms conferred by the SynCom-210.

**Investigation of phyla and class drop-outs on plant protection**

To introduce a more severe perturbation in the plant microbiota, we conducted drop out experiments using the *At*-LSPHERE (SynCom-223). Namely we left out each of the phyla, i.e. Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes as well as Proteobacteria classes from the inoculum (Figure 1C). To increase statistical power of the pathogen colonization, focus was put on collecting samples for pathogen colonization instead of investigating the community composition. Effect of removal of Proteobacteria and the individual Proteobacteria classes on community composition and strain abundances was analysed in a previous study [44].

The pathogen colonization at 14 dpi revealed that most communities were fully protective, highlighting again the redundancy in the trait of plant protection. The only exception was the drop-out of the Proteobacteria, which showed partial loss of protection through higher pathogen colonization (Figure 3, Supplemental Table 6). The median pathogen colonization was reduced 9000 times by SynCom-223 compared to axenic infected, while Proteobacteria drop-out showed 150 times higher pathogen colonization compared to SynCom-223 (Supplemental Table 5). Over the infection time course, the pathogen luminescence also only differed for the Proteobacteria drop-out from both background (axenic mock-infected) and SynCom-223 (Supplemental Figure 5A-C, Supplemental Table 7). The total commensal colonization was not significantly affected in the drop-out treatments compared to SynCom-223 (Supplemental Figure 5D, Supplemental Table 6). This suggests that colonization resistance against *Pst* is not mediated by total abundance of strains *in planta* and that Proteobacteria harbour a higher fraction of protective strains.

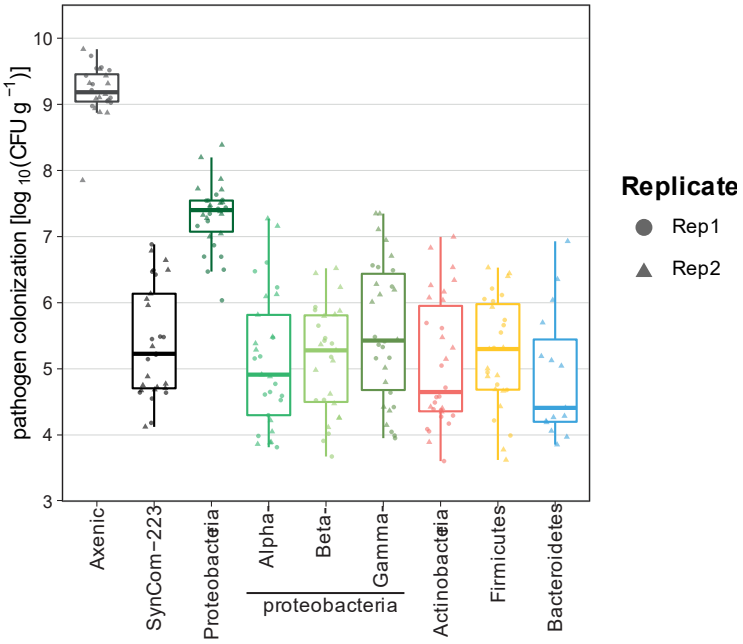


**Figure 3: Impact of dropping out phyla and classes in inoculum on pathogen colonization.** Pathogen colonization at 14 days post infection of different drop-out treatments and the control conditions axenic and SynCom-223. Shapes correspond to replicate experiments and colours are based on phylogeny (n=30, Bacteroidetes n=15).

### Investigation of genera drop-out on plant protection

In a parallel experiment to the phyla and class drop-out, the most numerous genera in terms of strain number or relative abundance of the *At*-LSPHERE were dropped out of the complete synthetic community (SynCom-223) [44,47] (Chapter 2). Namely, the genera *Methylobacterium*, *Sphingomonas*, *Rhizobium*, *Pseudomonas* and *Microbacterium* were dropped out of the inoculum.

Consistent with the results of the phyla and class drop-outs, none of the genera drop-outs were affected in pathogen colonization (Figure 4A). Over the infection time course, the drop-outs of *Methylobacterium*

and *Microbacterium* showed increased pathogen luminescence compared to background (axenic mock-infected) at 12 dpi, and at 6 dpi in the *Microbacterium* drop-out (Supplemental Figure 6A-C, Supplemental Table 8). However, none of the drop-out conditions showed significant differences in pathogen colonization to SynCom-223 (Supplemental Table 6). Commensal colonization was not significantly affected by the drop-out conditions compared to SynCom-223 (Supplemental Figure 6D, Supplemental Table 6).

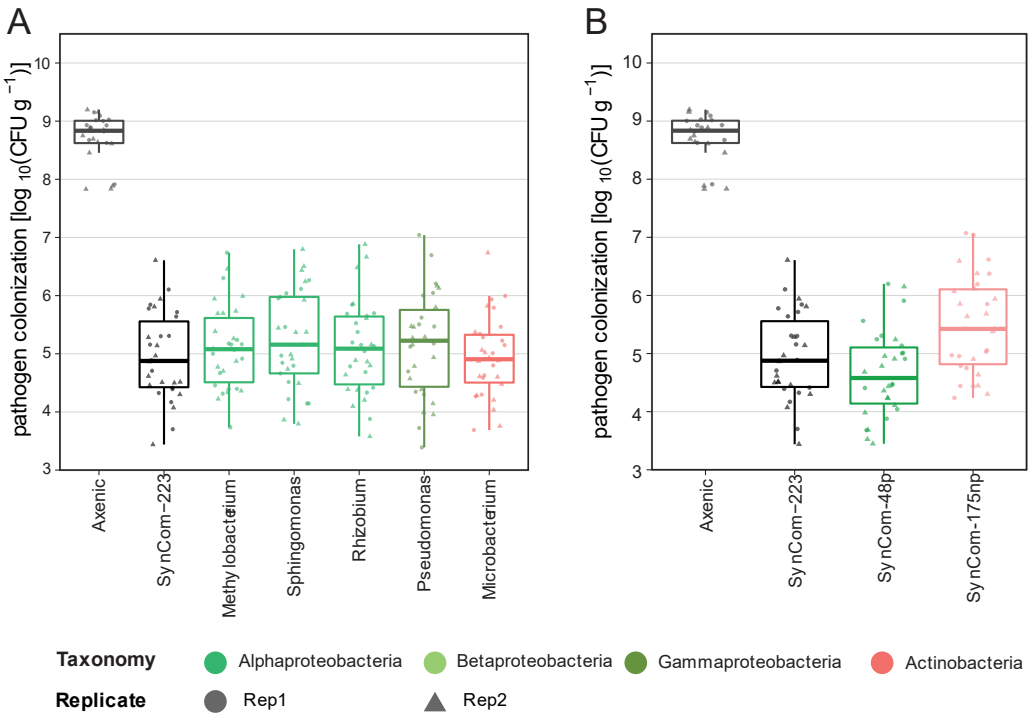


**Figure 4: Impact of dropping out genera and protection-associated strains in inoculum on pathogen colonization.** A. Pathogen colonization at 14 days post infection of different genera drop-out treatments and the control conditions axenic and SynCom-223 (n=30). Shapes correspond to replicate experiments. Boxplot colours are based on treatment or taxonomy. B. Pathogen colonization at 14 days post infection of the different protection-associated groups, protective (SynCom-48p) and non-protective (SynCom-175np) (n=30). Data for control conditions (axenic, SynCom-223) are replicated in panel A and B. Shapes correspond to replicate experiments. Colours correspond to treatment.

### Investigation of protection-associated strain drop-outs on plant protection

In addition to genera drop-outs, strains associated with a specific function can also be eliminated from the inoculum in synthetic community experiments. The strains of the *At*-LSPHERE were previously tested for plant protection in an agar-based gnotobiotic system [36]. To test whether these strains have an impact in a community context in the clay-based system used here, we designed different drop-out experiments. For this, all strains associated with a protection score of above 50 [36] were tested as a synthetic community, corresponding to a total of 48 strains (SynCom-48p, stand of June 2019). A synthetic community was also assembled from the remaining 175 strains with a protection score below 50 (SynCom-175np, stand of June 2019). The latter community can also be thought of as dropping out the strains associated with function of plant protection. The number of strains associated with protection scores of above 50 changed from June

2019 to publication in 2021, with 7 strains being removed (Leaf67, Leaf183, Leaf226, Leaf242, Leaf254, Leaf436, Leaf453) and 2 strains added (Leaf202, Leaf205) to the protective strain group. That meant, two protective strains were still part of SynCom-175np.

As predicted, the SynCom-48p had a low pathogen colonization, non-significant to SynCom-223, while the pathogen colonization of SynCom-175np was significantly higher to both SynCom-223 and SynCom-48Low (Figure 4B, Supplemental Table 6). The pathogen colonization of SynCom-175np was twice as high as that of SynCom-223 and six times higher than that of SynCom-48p (Supplemental Table 5). Over the course of infection, the differences in pathogen colonization and activity (here approximated by luminescence) start to become apparent at 6 dpi, where SynCom-175np started to have higher luminescence signal than background (axenic mock-infected), and higher than that of SynCom-48p at 12 dpi (Supplemental Figure 7A-C, Supplemental Table 9). The pathogen luminescence in SynCom-48p inoculated plants remained low, similar to the background (axenic mock-infected). Commensal colonization in SynCom-48p was higher compared to SynCom-175High, but both communities had commensal colonization comparable to SynCom-223 (Supplemental Figure 7D, Supplemental Table 6).

The combined results showed that plant protection can be compromised by strain drop-outs. However, the differences in plant protection were rather low and the plant protection phenotype was resilient to perturbation.

**Exchanging strains in low-complex synthetic communities have effect on pathogen colonization**

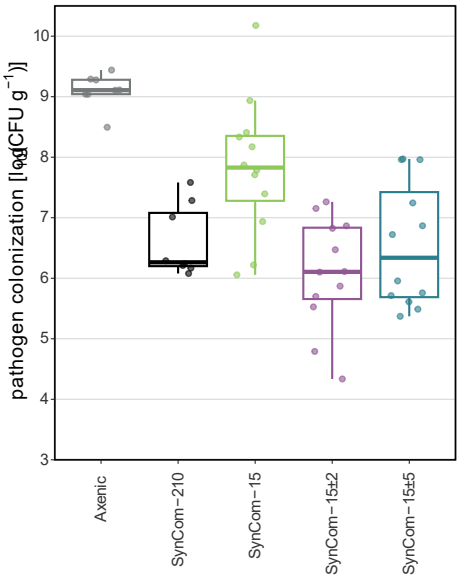


**Figure 5: Impact of exchanging strains in inoculum of SynCom-15 on pathogen colonization.** Pathogen colonization at 14 days post infection of SynCom-15 variations (n=12) in comparison to SynCom-210 and axenic controls (n=5).

Because high complexity communities harbour a high potential for redundant functions, we next tested smaller synthetic communities of 15 strains and changed the community composition based on a previously used focal strain community (SynCom-15) [45]. To potentially improve protection of this communities we exchanged two strains with strains from the same genera that were better at colonizing within a community or had higher protection scores [36] (Supplemental Table 1). Namely, *Rhizobium* Leaf371 and *Pseudomonas* Leaf129 were exchanged for Leaf202 and Leaf83, respectively, to increase colonization potential, forming the community SynCom-15±2 [36,44]. To potentially attenuate protection of the default community, we exchanged five strains mainly on the basis of higher protection scores of the replacement, i.e. *Rhizobium* Leaf371 for Leaf68, *Sphingomonas* Leaf33 for Leaf21, *Duganella*

Leaf61 for Leaf126, *Flavobacterium* Leaf359 for Leaf82 and *Pseudomonas* Leaf129 for Leaf83 to form SynCom-15±5 [36].

The pathogen colonization in both SynCom-15±2 and SynCom-15±5 was non-significantly different from that of SynCom-210, while SynCom-15 has a significantly higher pathogen colonization to the other three communities (Figure 5, Supplemental Table 6). The median pathogen colonization of SynCom-15 was 35 times higher than that of SynCom-210, but 18 times lower than in axenic infected plant, suggesting a reduction in protection compared to SynCom-210 (Supplemental Table 5).

After observing that synthetic communities of the same size but with different strains can affect pathogen colonization, we composed two additional communities. We wanted to test the extent to which the strains with high protection scores would behave differently from strains with low protection score in terms of protection potential and community composition [36]. Under the assumption that strains of the same genus would compete more compared to strains of other genera, e.g. [48], we selected pairs of strains within the same genus that differed in their protection scores from a predefined strain pool [49], ending up with 8 strain pairs (Table 1). The selected strain pairs were grouped into two sets. Set 1 was composed of strains with high protection scores (SynCom-8p, protective, protection score > 50), and set 2 consisted of strains with low(er) protection scores than their counterparts in set 1 (SynCom-8np, non-protective, protection score > 50). While we identified pairs with a mean protection score above and below 50 for all strains, this was not possible for the genus *Pseudomonas*, because the lowest protection score identified was 61 (*Pseudomonas* Leaf48). Nonetheless, we included this genus *Pseudomonas* because it contains a variation in strength of protection [36,50-52]. Apart from *Pseudomonas* Leaf48 that had the lowest protection score of its genus, as mentioned above, we included Leaf15 as a full protective counterpart (protection score 100). In addition to the protection potential and community composition within the SynCom-8p and -8np, we were also interested to see which strains were more competitive during colonization and hypothesized that the strains of the potential more protective community would be more abundant. To test this, we combined the two sets of strains into one community, SynCom-16All.

***Table 1:*** Community composition of SynCom-8p and SynCom-8np to analyse competition of strains with different protection scores. The strains were selected in pairs with one strain having a high protection score, while the other having a lower protection score. The strains are presented in pairs, note that both *Rhizobium* and *Sphingomonas* have two sets of pairs selected.

| Genus [47] | SynCom-8p | Mean protection score of p [36] | SynCom-8np | Mean protection scores of np [36] |
|---|---|---|---|---|
| *Rhizobium* | Leaf68 | 98 | Leaf384 | 38 |
| *Rhizobium* | Leaf311 | 80 | Leaf386 | 35 |
| *Sphingomonas* | Leaf21 | 100 | Leaf28 | 21 |
| *Sphingomonas* | Leaf230 | 58 | Leaf357 | 4 |
| *Pseudomonas* | Leaf15 | 100 | Leaf48 | 61 |
| *Microbacterium* | Leaf203 | 81 | Leaf288 | 13 |
| *Arthrobacter* | Leaf137 | 93 | Leaf69 | 28 |
| *Curtobacterium* | Leaf154 | 96 | Leaf261 | 36 |

First, we compared the pathogen colonization of the three communities compared to each other and the controls conditions, axenic and SynCom-210. The pathogen colonization was significantly higher for SynCom-8np at 14 dpi compared to SynCom-8p, as well as being non-significantly different to axenic infected. This suggested a partial loss of protection in SynCom-8np. No significant differences were between the treatments were observed at 7 dpi, likely due to low sample size (n=5) (Figure 6A, Supplemental Figure 8A,B, Supplemental Table 10). The median pathogen colonization of SynCom-8p was five times lower than that of SynCom-210 (Supplemental Table 5).

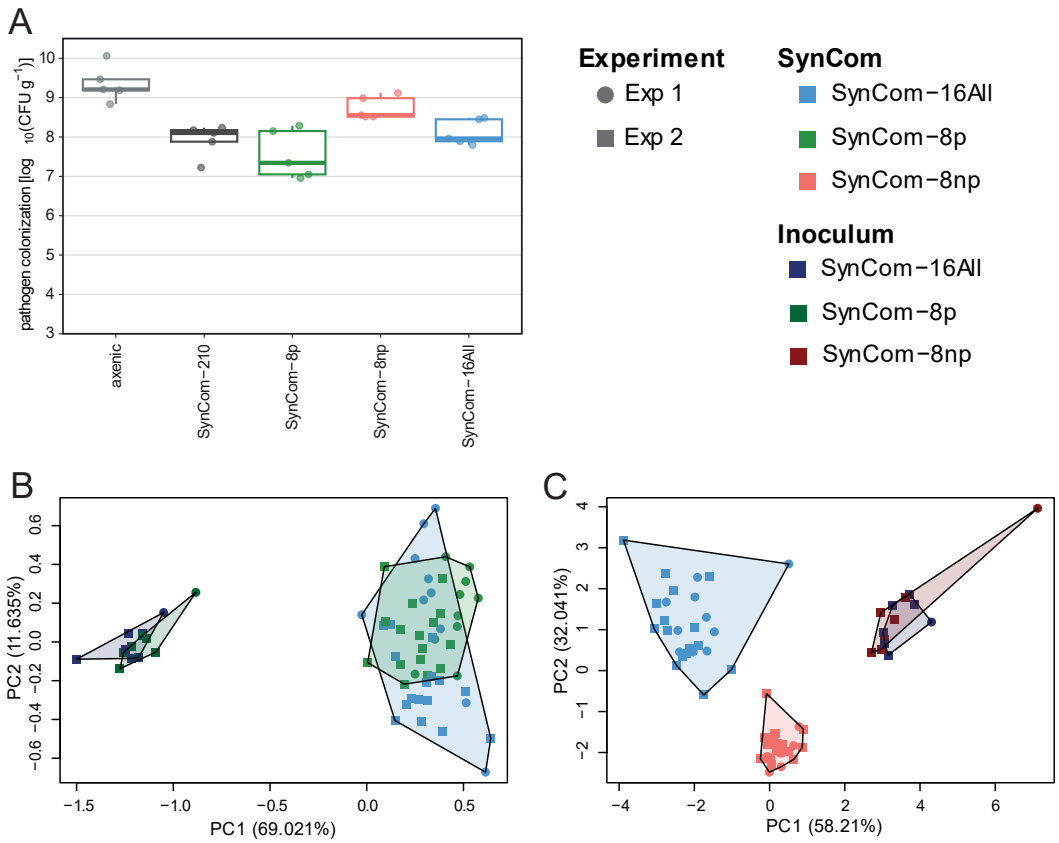


**Figure 6: Impact of creating two opposing sets of strains on community composition and pathogen colonization.** A. Pathogen colonization at 14 days post infection of the different synthetic community variations (n=5). Data of axenic and SynCom-210 are duplicated from Figure 2C. B. PCA of community composition of strains with high protection scores compared between SynCom-16All (blue) and SynCom-8p (green) of mock-infected plants. The inoculum samples are shown in darker shades (n=7), the *in planta* composition in lighter shades (n=26). The samples are distributed across two experiments (symbol shapes), the batch effect between the experiments were accounted for in the analysis. C. PCA of community composition of strains with low protection scores compared between SynCom-16All (blue) and SynCom-8np (red) of mock-infected plants. The inoculum samples are shown in darker shades, the *in planta* composition in lighter shades. The samples are distributed across two experiments (symbol shapes), the batch effect between the experiments were accounted for in the analysis.

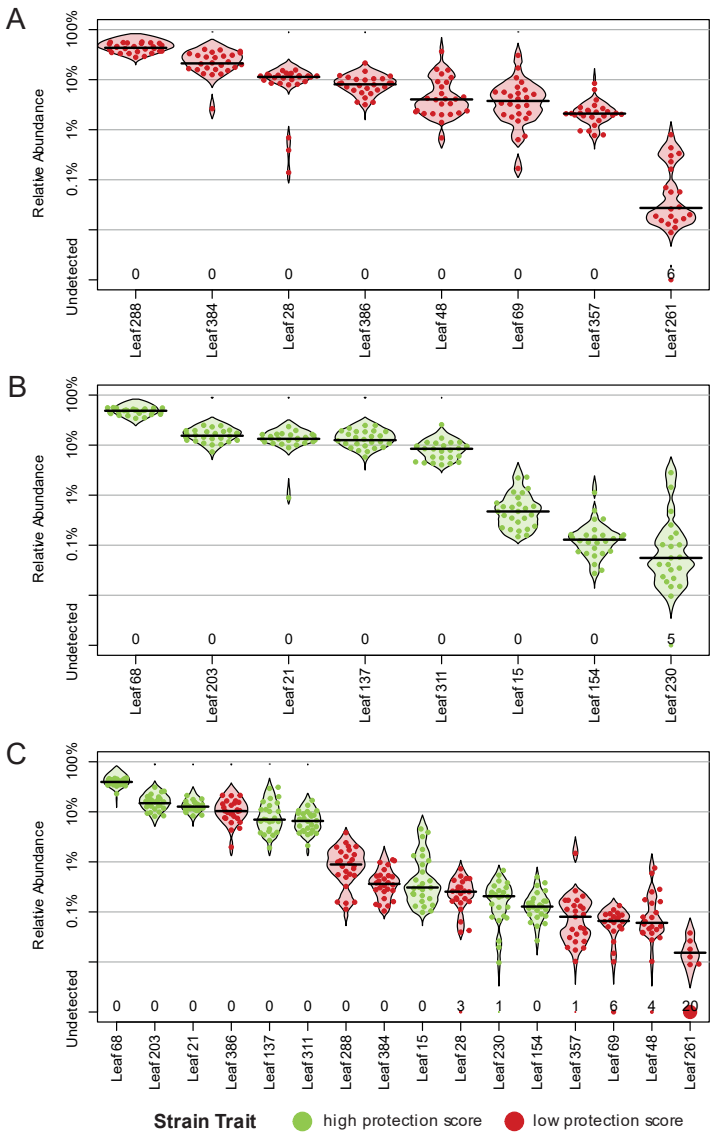


**Figure 7: Community compositions of variations of the SynCom-8s.** Relative strain abundances of in planta established communities of the trait-associated strain competitions in mock-infected plants (n=26). A. Relative strain abundances in SynCom-8np (red). B. Relative strain abundances in SynCom-8p (green). C. Relative strain abundances in SynCom-16All. The strains with high protection scores are shown in green, the strains with low protection scores in red.

Next, we examined how the two sets of strains are impacted in SynCom-16All. For this, we compared the community composition of SynCom-8p and -8np to the respective composition of the respective subset in SynCom-16All. In a PCA analysis, we saw that while the inoculum samples of both SynCom-8p and -8np overlapped with that of SynCom-16All (darker shades in Figure 6BC), the *in planta* samples of SynCom-8np clustered apart from SynCom-16All (Figure 6C), while they overlapped with SynCom-16All for SynCom-8p (Figure 6B). The PERMANOVA comparison of the abundances of the high protection score strains showed a low effect size (4.46%, p-value 0.0198) (Supplemental Figure 9A), but a large size effect for low protection score strains (46.4%, p-value 0.0099) (Supplemental Figure 9B). None of the high

protection score strains were significantly affected in relative abundance when comparing SynCom-8p to SynCom-16All. In contrast, 3 of the 8 low protection score strains decreased in relative abundance in SynCom-16All compared to SynCom-8np, and one strain, *Rhizobium* Leaf386 increased in abundance (Supplemental Figure 9C). Next, we looked at the community composition of all three communities (Figure 7). In SynCom-8np, *Microbacterium* Leaf288 and *Rhizobium* Leaf384 were most abundant in relative abundance, with only *Curtobacterium* Leaf261 being low abundant or even not detected (Figure 7A). SynCom-8p had 5 abundant strains, and three of low abundance (*Pseudomonas* Leaf15, *Curtobacterium* Leaf154, *Sphingomonas* Leaf230) (Figure 7B). In SynCom-16All, the 5 of the 6 most abundant strains were high protection score strains, with the exact rank as in SynCom-8p (Figure 7C). This uneven relative abundance distribution between the strains was not reflected in the inoculum of SynCom-16All, where all strains had a comparable relative abundance (Supplemental Figure 10). This suggested that the low protection score strains of SynCom-8np (in red) were affected not only in abundance, but also in rank in SynCom-16All and were less competitive than the high protection score strains in the community.

**Protection-associated strains have higher colonization levels *in planta***

The community structure of SynCom-16All is in line with our hypothesis that previously identified strains with high protection scores (in green) are more competitive in colonization (Figure 7C). To test whether and how often a strain with a higher protection score will outcompete another strain with a lower protection score, we designed binary competitions *in planta* for each of the strain pairs of the same genus and determined absolute colonization. The strain pairs were grown individually as well as in competition with each other *in planta* (Figure 8). We were interested in two questions (or comparisons): i) Does a beneficial (protective) strain reach a higher total abundance by itself when colonizing the plant compared to a neutral (non-protective) strain? ii) Is the beneficial strain more abundant when competing with the neutral strain? – and vice versa, does the neutral strain experience a decreased in abundance when competing with the beneficial strain?

***Table 2:*** Most strains with high protection score were either better colonizers or reduced strains with lower protection scores in competition. The table summarizes the comparisons of *in planta* colonization: Comparison 1 looked at whether strains with higher protection scores had higher colonization levels by itself than lower protection score strains by themselves. In comparison 2, we looked whether high protection score strains had higher colonization levels in competition compared to low protection score strains. 3 compared colonization levels of low protection strains by itself versus in competition, while 4 compared the high protection score strain by itself versus in competition.

| Comparison | Significant | Non-significant | Exception |
|---|---|---|---|
| 1 | 58.33% | 25% | 16.67% |
| 2 | 91.67% | 8.33% | 0% |
| 3 | 83.33% | 16.67% | 0% |
| 4 | 16.67% | 83.33% | 0% |

All strains were proficient colonizers and had a total abundance *in planta* of over $10^5$ cfu g-1 plant fresh weight, most colonized between $10^7$ to $10^8$ when colonizing in mono association (Figure 7, triangle shape).

The comparisons of binary comparisons and competitions *in planta* are summarized in Table 2. In 7 of 12 of the binary comparisons performed, the strains with higher protection score had higher colonization levels by themselves compared to the strain with lower protection score. The exceptions were *Sphingomonas* Leaf230 and *Arthrobacter* Leaf137 that had lower individual colonization than their counterparts (Leaf357, Leaf69, respectively), but both were unaffected in binary competition (Figure 7H,K). In 11 out of 12 binary competitions, the strain with higher protection score was more abundant than the lower protection score strain when in competition with each other. In 10 out of 12 binary competitions, the strain with the lower protection score had lower colonization levels in competition with a higher protection score strain compared to by itself. Only *Sphingomonas* Leaf21 as a protective strain was affected when competing with another strain in planta (2 of 12 competitions, Figure 7E,F). Despite being affected, *Sphingomonas* Leaf21 was still present at higher abundance compared to the lower protection score strain when in competition with each other. In the two binary competitions, where the higher protection scores strain had a lower colonization level than the lower protection score strain by themselves (*Sphingomonas* Leaf230 vs Leaf357, *Arthrobacter* Leaf137 vs Leaf69, Figure 7H,K), the colonization of the lower protection score strain was reduced when in competition with the higher protection score strain. The two strains with low protection scores that were unaffected in colonization level in binary competitions compared to individual colonisation were *Curtobacterium* Leaf261 and *Microbacterium* Leaf288 (Figure 7I,L). *Curtobacterium* Leaf261 had the lowest colonization of all strains tested by themselves and was not further reduced (Figure 7L). In contrast to that, *Microbacterium* Leaf288 had a lower colonization in competition than by itself, but the reduction was not statistically significant (Figure 7I).

In summary, virtually all the binary competitions showed that the strain with the higher protection potential was more abundant *in planta*, already when inoculated by itself compared to colonization level of lower protection score strain by itself. The difference became even more apparent in competition, where the higher protection score strain reduced the lower protection score strains.

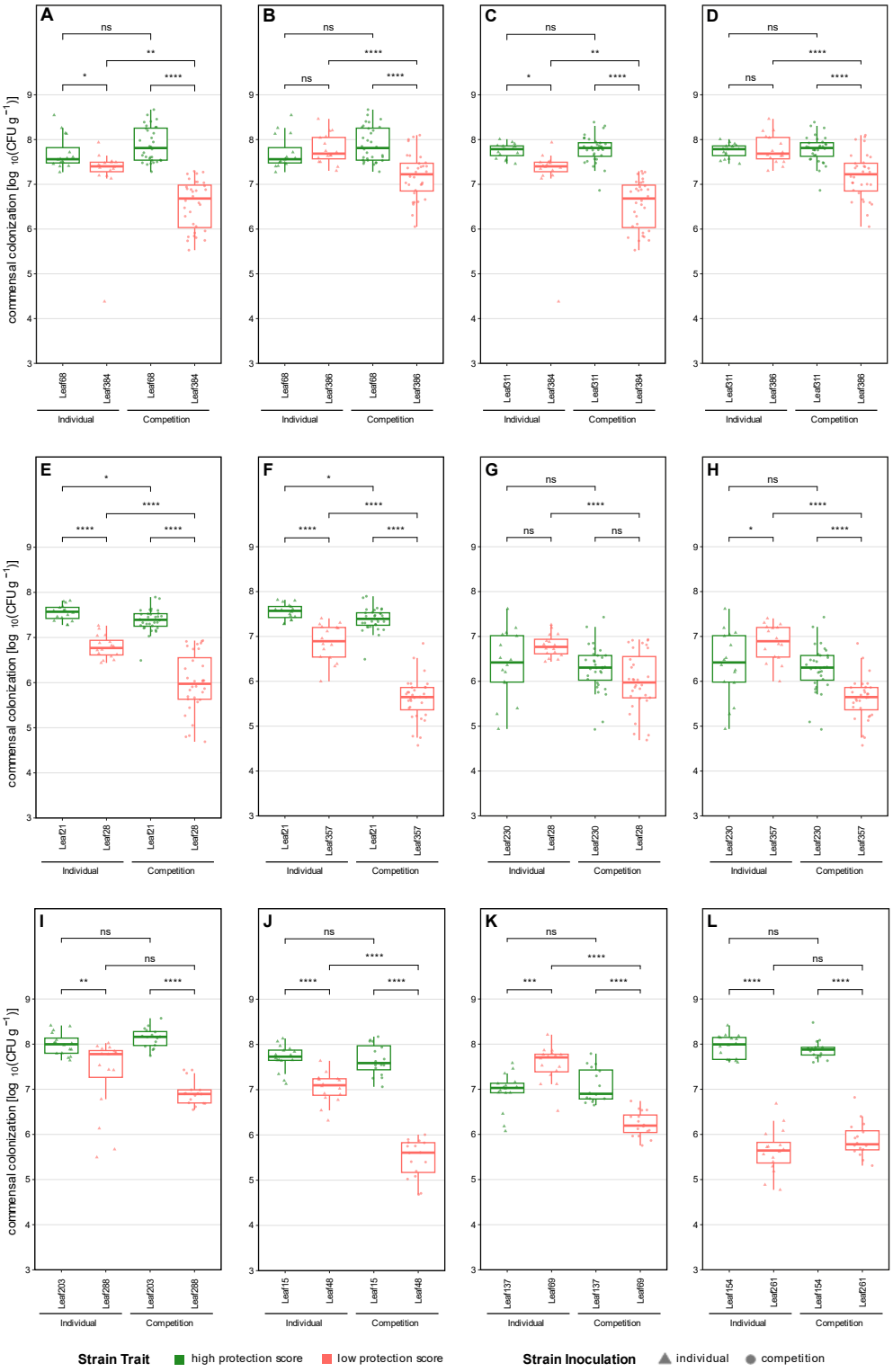


**Figure 8: *In planta* competitions of strains with high protection scores versus strains with low protection scores within the same genus.** Strains grown individually are represented by triangles (n=16), when samples grown in competition with the other strain are represented by circles (n=16). The strain with high protection scores are shown in green, the ones with low protection scores in red. Binary competitions of: A. *Rhizobium* Leaf68 versus Leaf384. B. *Rhizobium* Leaf68 versus Leaf386. C. *Rhizobium* Leaf311 versus Leaf384. D. *Rhizobium* Leaf311 versus Leaf386. E. *Sphingomonas* Leaf21 versus Leaf28. F. *Sphingomonas* Leaf21 versus Leaf357. G. *Sphingomonas* Leaf230 versus Leaf28. H. *Sphingomonas* Leaf230 versus Leaf357. I. *Microbacterium* Leaf203 versus Leaf288. J. *Pseudomonas* Leaf15 versus Leaf48. K. *Arthrobacter* Leaf137 versus Leaf69. L. *Curtobacterium* Leaf154 versus Leaf261. Differences in colonization are assessed through pairwise Welch's t-test with Bonferroni-correction on p-values.

To control if the *in planta* patterns were exclusive to that environment, we performed an *in vitro* competition (Supplemental Figure 11). Here, the strains grew comparable in competition to growing by themselves, except for *Arthrobacter* Leaf69 and *Curtobacterium* Leaf261 (Supplemental Figure 11K,L). In the first case, *Curtobacterium* Leaf261 had a slight lag phase until 24 hours when grown by itself, and that gave it disadvantage when competing with Leaf154, which overgrew it. In contrast to that, *Arthrobacter* Leaf69 grew competitively with its high protection score counterpart Leaf137 when analysed after 8 hours. However, after 24 hours, cell densities of Leaf69 were reduced and only one of four samples had detectable colonies. This might suggest an active killing of Leaf69 by Leaf137 at higher cell densities. However, no direct antibiosis of these two strains was observed in an *in vitro* screen [53].

We can conclude that the strains with high protection score were more competitive than their low protection score counterparts *in planta*, whether that was by reaching higher colonization densities by itself or in presence of other strains or potentially by actively reducing the low protection score counterparts.

**Summary of results**

Here, we showed a link between community composition and pathogen colonization outcomes in the phyllosphere of *Arabidopsis thaliana* through synthetic community experiments. We found that perturbation of strain abundances had no influence on pathogen colonization (Figure 2). Pathogen colonization was only increased when dropping out the biggest phylogenetic group of strains, the Proteobacteria phylum (Figure 3). This suggests that the *At*-LSPHERE strain collection harbours diverse and redundant mechanisms to limit pathogen growth. The synergism of these mechanisms was emphasized in the finding of only a small increase in pathogen colonization when the strains previously associated with high protection scores were removed from the full community (Figure 4B). We saw that exchanging strains for others linked to higher protection scores or higher colonization efficiency improved plant protection in smaller synthetic communities of up to 15 members (Figure 5, 6A). We hypothesised that beneficial strains are better colonizers *in planta*, and showed this to be true in the community composition (Figure 7C), when colonizing the plant individually and in binary competition assays (Figure 8, Table 13).

# Discussion

In this study, we correlated pathogen colonization outcomes as parameter of disease establishment with the community composition to find important patterns of the plant-associated microbiota. To perturb the community composition, we changed the strain abundance and presence at the stage of inoculum in a gnotobiotic system with *A. thaliana* as the host, the representative native bacterial collection *At*-LSPHERE [47] as the source of synthetic communities and challenged the established microbiota with the foliar pathogen *Pseudomonas syringae* pathovar tomato DC3000. We found that the establishment of the strains of the *At*-

LSPHERE *in planta* was resilient to strain abundance changes in the inoculum to most perturbations (Figure 2A, Supplemental Figure 1). Reversing the abundances of the phyla ("reversed phyla") and reducing the genera *Methylophilus* and *Devosia* in the inoculum had significant effects on the community composition (PERMANOVA effect size of 19.3 and 10.3%, respectively), however the most abundant strains remained relatively similar (Supplemental Figure 2A,B,D). The effect size of the PERMANOVA analysis in the significantly changed treatments corresponded to the effect sizes of a prior study where Proteobacteria classes and key strains were dropped out of a 62 member community (range 3 – 13.4%) [44]. Upon infection, all inocula treatments varied in community composition compared to the default at 7 dpi but returned to a non-significant difference at 14 dpi, except for "reversed phyla". This suggests that the microbiota differs in reaction to pathogen challenge initially, but then the converge to a similar state. Despite the perturbed strain abundances in inoculum and differences in community composition, the pathogen colonization was unaffected at both 7 and 14 dpi (Figure 2B,C). This suggests that the strain presence was more important than their abundance in the community, contrary to what was suggested in the rhizosphere microbiota [16].

Next, we investigated the impact on pathogen colonization by removing groups of strains from the inoculum. We found that pathogen colonization was only significantly increased when removing the entire Proteobacteria phylum, by a factor of 150 compared to complete community (SynCom-223) (Figure 3, Supplemental Table 6). Pathogen colonization was not affected when other phyla or Proteobacteria classes were dropped out. Similarly, dropping out the most numerous genera also showed no effect on the pathogen colonization (Figure 4A). Dividing the synthetic community based on previously found protection scores of individual strains [36], revealed a two times higher pathogen colonization in the non-protective strains community, SynCom-175np, compared to SynCom-223 (Figure 4B, Supplemental Table 6). The community composed of all protective strains, SynCom-48p, had a similar pathogen colonization as SynCom-223. This suggests that some functions and mechanisms to reduce pathogen still remain in the community of non-protective strains, while a community reduced to one fourth of the full can still exhibit the full protection potential. In all of the comparisons with the full community (SynCom-210, SynCom-223), we found that the variation in pathogen colonization of the complete synthetic community was spanning two orders of magnitude (Figure 2, 3, 4), making significance testing and interpretation difficult. The results of the drop-out conditions together suggested, that the *At*-LSPHERE harbours diverse and redundant mechanisms of protection against *Ps*t and removal of one part of the microbiota will be compensated by the remaining strains. The phyllosphere is a competitive environment and the interactions between strains are largely negative, suggesting the unoccupied niches of removed strains will be filled by others [44,45].

We also aimed to correlate pathogen outcome with alteration of the community by exchanging individual strains based on prior knowledge of colonization and protection scores [36,44]. When replacing 2 or 5 strains of SynCom-15[45], we found significant reduction in pathogen colonization to both alterations (SynCom-

15±2, SynCom15±5) compared to the original SynCom-15 (Figure 5). Interestingly, the two altered communities had a similar pathogen colonization to the complex community, SynCom-210. This suggests that by exchanging only a few strains, we were able to bring pathogen colonization to the level of a much larger community. This in turn suggests that the presence of beneficial strains is important. It was suggested before that the initial composition is important for plant health outcomes [43]. [16]Our results seem to support this observation.

To further investigate the differences between protective and non-protective strains in terms of colonization levels, we composed two communities of 8 strains. One community was composed of strains associated with a high protection score (SynCom-8p), while the other was composed of strains with a lower protection score (SynCom-8np) (Table 1) [36,49]. While SynCom-8p had a low pathogen colonization, similar to that of SynCom-210, while SynCom-8np had a significantly higher pathogen colonization (Figure 6A, Supplemental Table 10). We hypothesized that the strains of SynCom-8p would be more competitive to the strains in SynCom-8np. To test this, we combined the two sets of strains into SynCom-16All. While the protective strains were unaffected in terms of relative abundance and rank in SynCom-16All compared to SynCom-8p, the non-protective shifted not only in relative abundance, but also ranks compared to SynCom-8np (Figure 6B,C, Figure 7, Supplemental Figure 9B,C).

We further supported the hypothesis that the protective strains were more competitive than non-protective strains of the same genus in binary competition experiments (Figure 8). We found that most protective strains had higher colonization levels by themselves and in competition with non-protective strains, as well as reduced the colonization of non-protective strains in competition compared to their colonization levels when grown alone (Table 2). With the findings of the protective strains being more competitive, and the exchange of strains with lower protection scores for ones with higher protection scores resulting in pathogen colonization similar to complex communities (SynCom-210), we suggest that few key strains highly competitive might drive plant protection. In another setup of analysing and mixing two opposite communities, commensal *Pseudomonas* strains were found to outcompete pathogenic strains *in planta* [51]. Thus, not only presence and absence of strains but also abundance might impact protection outcomes, which was suggested as being an important aspect of beneficial strains [16].

Different mechanisms might contribute to plant protection [36,54]. One of these might be priming of the host plant. In another study, we had investigated plant transcriptome responses to different bacterial strains and correlated plant protection with colonization as well as the overall plant response to colonization and found a weak positive correlation [49]. The strains included in our study of protective versus non-protective strains were also analysed for eliciting a plant response when inoculated individually on the plant [49]. While some strain pairs, like the *Sphingomonas* and *Microbacterium* pairs, showed differences in plant response strength (non-protective eliciting a weak, protective an intermediate strong response), all *Rhizobium* strains elicited a medium plant response, while all *Arthrobacter* and *Pseudomonas* strains elicited a strong response

in the plant. The only pair where the protective strain elicited a weak response, while the non-protective elicited a strong response in the plant, was the one of *Curtobacterium*. Our findings suggest that plant protection and colonization is not only a result of the plant response elicited by the beneficial microbes. However, since all strains of the protective versus non-protective community experiment do elicit a general non-self response in the plant [49], this suggests communication occurs between the plant host and the commensal bacteria. The competitive trait of the strains with higher protection scores might lead to a positive feedback on plant fitness and in turn on these strains, providing an example how ecological interactions can drive evolutionary processes in the long term. Other mechanism of protection may include direct antibiosis of the pathogen. However, an *in vitro* screen found no antibiosis of the involved SynCom-8 strains against the pathogen *Ps*t [53]. It was suggested that phyllosphere strains need a cue for exhibiting antibiosis, which would the lack of antibiosis found [55]. Prior genomic comparison of protective versus non-protective *Rhizobium* strains revealed the presence of type 6 secretion system (T6SS) genes in the protective strains included in the SynCom-8p, SynCom-15±2 and SynCom15±5 (Leaf68, Leaf311, Leaf202), while the non-protective strains in SynCom-8np and SynCom-15 do not encode a T6SS (Leaf371, Leaf384, Leaf386) [36]. The T6SS can lead to killing of other cells, and was experimentally shown to inhibit *Ps*t growth in vitro [36]. Other traits might also be relevant for protection such as biofilm formation, as has been proposed [56,57]. Since the protective strains used in SynCom-8p are at higher frequency and colonization level, biofilm formation could be one mechanism they use. Further investigation into the mechanisms of the protective strains and communities must be made in general.

In summary, we showed that complex synthetic communities cannot be easily perturbed and are resistant to pathogen colonization, suggesting that plant protection is the result of diverse and redundant mechanisms of microbiota members. In lower complex communities, exchanging strains for more competitive and protection-associated ones was shown to be a suitable indicator for pathogen reduction. We showed that the strain conferring protection were abundant and competitive in a community, and individually. Further investigation of the relationship between abundance and protection potential needs to be done. We highlight the importance of community assembly for understanding the protection potential beyond the capacity of individual strains to confer protection.

# Materials and Methods

### Plant growth conditions

For the experiments of this Chapter, except the drop-out experiments, *Arabidopsis thaliana* Col-0 were grown gnotobiotically in 6-well tissue culture plates (TechnoPlasticProducts), as previously described [45]. Briefly, 5 ml calcined clay (Diamond Pro Calcined Clay Drying Agent) was mixed with 2.5 ml 0.5× Murashige and Skoog (½ MS) medium including vitamins, pH 7 (M0222.0050, Duchefa). Surface sterilized seeds were stratified at 4 °C for 4 d and 1 seed was placed in the centre of each well. If a seed did not germinate, a new plant was transplanted at day 10 from surplus plates. Starting at 4 days, each well was watered twice a week with 200 µl 0.5x MS medium, except on the day of inoculation with bacteria.

For the drop-out experiments, the plants were grown as described previously [44,46]. Briefly, 140 ml calcined clay (Diamond Pro Calcined Clay Drying Agent) was mixed with 60 ml ½ MS in gamma-irradiated microboxes (no. O118/80 + OD118 with XXL + (green) filter lid, Saco2). Surface sterilized seeds were stratified at 4 °C for 4 d and 2-3 plants seeded at four spots. On inoculation day (10 d old), the surplus plants were removed, and in case no seed was germinated in a spot, a seedling was transplanted from another spot. Starting from day 7, plants were watered once a week with 500 µl 0.5x MS per plant.

Plates and microboxes were incubated in growth chambers set to 22°C and 54% relative humidity with a 11 h photoperiod. Combined light intensities were set to 180-210 µmol $m^{-2}$ $s^{-1}$ (400-700 nm, PAR) and 4-7 µmol $m^{-2}$ $s^{-1}$ (28-400 nm, UV light). The plants were inoculated with bacterial suspensions at 10d. After 14 days, plants were infected (24 d old) and harvested 14 days post infection (dpi). Where pathogen colonization at 7 dpi was analysed, the plants were infected 21 days after inoculation (31 d old).

### Synthetic community mixing for strain abundance changes in inoculum

Bacterial strains were streaked out on R2A agar (Sigma-Aldrich) supplemented with 0.5% (v/v) methanol (R2A+M) and incubated at 22°C for 6 days. Strains were resuspended individually in 1 ml 10 mM $MgCl_2$ buffer, and vortexed for 10 min. If aggregates were formed in bacterial suspensions, the suspension was left to settle, and supernatant was transferred into a clean, sterile Eppendorf tube.

For the "default" condition, the strains were mixed in a 1:1 ratio into the respective phyla, expect for the Proteobacteria strains. The latter were mixed in a 1:1 ratio into mixes of the genera *Rhizobium, Methylophilus* and *Devosia*, and the remaining strains into the "rest". For the "default", the "Proteobacteria all" mix was created by mixing 1.2 ml *Rhizobium*, 300 µl *Methylophilus* and 150 µl *Devosia*, then filling up to 12 ml with the "rest" mix. The phyla mixes were then combined into the "default" SynCom-210 inoculum by combining 1 ml of Proteobacteria "all" mix, 600 µl of Actinobacteria mix, 120 µl of Bacteroidetes mix and 80 µl of Firmicutes mix. For the "reversed phyla" treatment, 80 µl of "Proteobacteria all" mix, 120 µl of Actinobacteria mix, 600 µl of Bacteroidetes mix and 1 ml of Firmicutes mix were combined. For the

"low *Rhizobium*" treatment, the "*Rhizobium*" mix was reduced tenfold in the Proteobacterium mix, by combining 120 µl *Rhizobium*, 300 µl *Methylophilus* and 150 µl *Devosia*, then filling up to 12 ml with the "rest" mix. For the "low *Methylophilus* low *Devosia*" treatment, 30 µl *Methylophilus* and 15 µl *Devosia* were combined with 1.2 ml *Rhizobium* and then filled up to 12 ml with "rest". In the "with pathogen" treatment, 100 µl of both *Ps*t and *Serratia* Leaf50 were mixed in with 1.2 ml *Rhizobium*, 300 µl *Methylophilus* and 150 µl *Devosia,* then filled up to 12 ml with "rest" of Proteobacteria strains. To create the inocula variations of SynCom-210, the described Proteobacteria mix variations were mixed in with the other phyla as described for "default". 1 ml of each inoculum was spun down in lysis matrix E tubes (FastDNA SPIN Kit for Soil, MP Biomedicals), and stored at −80 °C. The inocula were diluted to $OD_{600}$ of 0.02 in 20 ml 10 mM $MgCl_2$ buffer.

To control the viability and density of strains in the inoculum, tenfold dilution series of all strains were prepared, and 4 µl of each dilution was spotted onto R2A+M agar square plates (Greiner) to determine colony-forming units (cfu).

**Synthetic community mixing for drop-out conditions**

The drop-out experiments were conducted in two parallel experiments with two experimental replicates. First, the phyla and Proteobacteria were dropped out of SynCom-223. In the second experiment, genera were dropped out, and strains were additionally divided into protective and non-protective groups (SynCom-48p, SynCom-175np). For both experiments, bacterial strains were streaked out on R2A agar (Sigma-Aldrich) supplemented with 0.5% (v/v) methanol (R2A+M) and incubated at 22°C for 6 days. With a sterile 1 µl plastic loop, "one loop-full" of strain biomass was resuspended individually in 1 ml 10 mM $MgCl_2$ buffer, and vortexed for 10 min. For the phyla and classes drop-out, strains were mixed in a 1:1 ratio into respective phyla and Proteobacterium classes. For SynCom-223 ("all" condition), 792 µl of Alphaproteobacteria mix, 152 µl of Betaproteobacteria mix, 120 µl of Gammaproteobacteria mix, 520 µl of Actinobacteria mix, 128 µl of Bacteroidetes mix, 64 µl of Firmicutes mix and 8 µl of Deinococcus were mixed. For the genera drop-outs, strains were mixed in a 1:1 ratio into the drop-out genera (*Rhizobium, Sphingomonas, Methylobacterium*, *Pseudomonas, Microbacterium*) and *Rhodococcus* (initially intended as a drop-out condition) and the "rest". For SynCom-223 ("all" condition), 912 µl of the "rest" mix, 120 µl of *Rhizobium*, 296 µl *Sphingomonas*, 256 µl *Methylobacterium* mix, 72 µl *Pseudomonas*, 80 µl *Microbacterium* and 48 µl *Rhodococcus*. For SynCom-48p and SynCom-175np, the strains were mixed in a 1:1 ratio into the respective group. These two conditions were analysed in the same experiment as the genera drop-outs, the SynCom-223 control is the same. For all specified drop-out conditions, the volume of the drop-out group was replaced with 10 mM $MgCl_2$ buffer to have similar strain abundances across each treatment.

To control the viability and density of strains in the inoculum, tenfold dilution series of all strains were prepared, and 4 µl of each dilution was spotted onto R2A+M agar square plates (Greiner) to determine colony-forming units (cfu). 50 µl of dilutions $10^{-4}$ and $10^{-5}$ of a tenfold dilution series of the synthetic communities and binary mixes were plated onto round (9 cm) R2A+M agar plates.

**Synthetic community mixing of exchanging strains**

SynCom-210 and SynCom-15s were prepared from a frozen aliquot (Chapter 2). The aliquots were slowly thawed, spun down at 6000 rcf for 10 min and resuspended in 10 mM MgCl2. Then, $OD_{600}$ was adjusted to 0.02 in 25 ml 10 mM $MgCl_2$ buffer.

The glycerolstocks were for experiments described in Chapter 2. Briefly, bacterial strains were streaked out on R2A agar (Sigma-Aldrich) supplemented with 0.5% (v/v) methanol (R2A+M) and incubated at 22°C for 6 days. With a sterile 1 µl plastic loop, "one loop-full" of biomass of each strain were resuspended individually in 1 ml 10 mM $MgCl_2$ buffer, and vortexed for 10 min. For SynCom-15s, the strains were directly mixed in a 1:1 ratio (SynCom-15±2 was not described but was prepared simultaneously). For SynCom-210, strains were mixed in a 1:1 ration into their respective phyla, which were then mixed into a 50 ml Falcon (25 ml Proteobacteria mix, 14.75 ml Actinobacteria mix, 7.5 ml Bacteroidetes mix, 2.5 ml Firmicutes mix, 0.25 ml *Deinococcus*).

SynCom-8p, SynCom-8np, and SynCom-16All were inoculated two separate experiments. In the first one, pathogen infection was analysed. In the second one, the community composition and binary competition experiments were carried out and remained uninfected. For the first experiment, the communities were composed in the method described for the SynCom-15s using the "loop-full" technique to have strains in a similar cell density. The SynCom-210 control was the same as the "default" treatment from the investigation of changes in abundance in inoculum on community stability and pathogen infection. $OD_{600}$ of pre-inocula was measured and were adjusted to 0.02 in 25 ml 10 mM $MgCl_2$ buffer. In the second experiment, strains were resuspended individually in 1 ml 10 mM $MgCl_2$ buffer, and vortexed for 10 min. Strains were then adjusted to $OD_{600}$ of 0.02 in 25 ml 10 mM $MgCl_2$ buffer (32 ml for *Rhizobium* and *Sphinogomonas* strains) and then mixed in a 1:1 ratio into the respective binary competitions or synthetic communities. This experiment did not include a SynCom-210 control since all plants remained uninfected. For both experiments, 1.5 ml of each inoculum was spun down in lysis matrix E tubes (FastDNA SPIN Kit for Soil, MP Biomedicals), and stored at −80 °C.

To control the viability and density of strains in the inoculum, tenfold dilution series of all strains were prepared, and 4 µl of each dilution was spotted onto R2A+M agar square plates (Greiner) to determine colony-forming units (cfu). 200 µl of dilutions $10^{-4}$ and $10^{-5}$ of a tenfold dilution series of the synthetic communities and binary mixes were plated onto round (9 cm) R2A+M agar plates.

**Plant inoculation**

The term “inoculation” is used to refer to treatment with commensal strains of the *At*-LSPHERE collection, whereas the term “infection” refers to spraying with the pathogen *P. syringae*. On inoculation day, surplus plants were removed from wells or spots in the microboxes. Where necessary, a seedling was transplanted into a well or spot where none had grown. Plants that were grown in 6-well plates were inoculated with 200 µl of inoculum at $OD_{600}$ 0.02 or with buffer (axenic). Plants that were grown in microboxes were inoculated with 0.5 ml of inoculum at $OD_{600}$ 0.02 or with buffer (axenic). Plates and boxes were assigned to their specific treatment and labelled accordingly prior to inoculation.

**Plant infection with pathogen and monitoring**

The infection inoculum of *Pseudomonas syringae* pv. tomato DC3000 *luxCDABE* (*Ps*t) [58] was prepared as described in Innerebner et al. [15]. Briefly, a lawn of *Ps*t was grown on King’s B agar [59] at 28°C overnight, resuspended in 10 ml 10mM $MgCl_2$ and $OD_{600}$ adjusted to 0.1 in the 6-well system or to 0.001 in the microbox system. The plants were sprayed at day 24 (day 31 for 7 dpi) with either buffer (mock-infected controls, NI) or with *Ps*t suspension using a thin-layer chromatography reagent sprayer (Faust Laborbedarf AG). Each plant was sprayed 3-6 times, until it appeared to be thoroughly wet. The pathogen titre corresponded to roughly $10^6$ pathogen cfus per plant. Each microbox was sprayed 6 times, which corresponded to roughly $10^4$ pathogen cfus per plant. Pathogen titre was assessed by CFU determination on King’s B agar.

Luminescence measurements were used as a proxy for pathogen colonization as described previously [36]. In all experiments, the luminescence was measured at 3 and 6 dpi, microboxes were additionally measured at 12 dpi. 6-well plates were placed with a clean lid into the IVIS Spectrum Imaging System (Xenogen), microboxes were placed with an open lid, and luminescence was acquired for 30 s at 500 nm wavelength. If the lids of the 6-well tissue plates showed condensation, they were dried in a laminar flow hood or exchanged with a new lid. In the Living Image Software v.4.2., circular region of interests (ROI) were set around each well (or plant in microboxes, where ROIs were adjusted for size) and the total photon flux per ROI was exported. A photograph of each plate was made at infection timepoint, 7 dpi and 14 dpi were applicable.

***In vitro* competition of bacterial strains**

To investigate the competition between strains of SynCom-8p and SynCom-8np, the strains were inoculated in R2A broth either individually, in binary competition or within communities. For this, bacterial strains were streaked out on R2A agar (Sigma-Aldrich) supplemented with 0.5% (v/v) methanol (R2A+M) and incubated at 22°C for 6 days. With a sterile 1 µl plastic loop, “one loop-full” of biomass of each strain were resuspended individually in 1 ml 10 mM $MgCl_2$ buffer, and vortexed for 10 min. Strains were combined in a 1:1 ratio into either for binary competition, SynCom-8p, SynCom-8np or SynCom-16All. 200 µl of bacterial suspension was added to 20 ml R2A broth with supplemented with 0.5% (v/v) methanol in

shakeflasks with baffles and incubated at 22 °C shaking at 200 rpm. Viability and cell densities of inocula were verified by tenfold dilution series and spotting 4 µl on R2A+M agar square plates (Greiner) and plating 50 µl of dilutions $10^{-5}$ and $10^{-6}$ onto round (9cm) R2A+M plates. After 8, 24 and 48 hours samples were taken for bacterial enumeration. 4 µl of a tenfold dilution series was spotted on R2A+M agar square plates (Greiner) and 50 µl of dilutions $10^{-4}$ and $10^{-5}$ were plated onto round (9cm) R2A+M plates. The agar plates were inoculated at room temperature until colonies could be counted.

**Plant harvest for bacterial enumeration**

Bacterial colonization was measured by washing off the microbiota and spotting dilution series as described before [15]. The plants were removed from the clay substrate with sterile tweezers, and the cotyledons and roots were cut off with a sterile scalpel. The remaining phyllosphere was placed into 2 ml Eppendorf tubes containing 1.3 ml 100 mM phosphate buffer (pH7) supplemented with 0.2% Silwet-L77 (Leu+Gygax). Plant fresh weight was measured on an analytical balance (Mettler-Toledo) with an accuracy of 0.1 mg. The tubes were subsequently washed off by shaking tubes for 15 min at 25 Hz with a TissueLyser II (Qiagen), followed by sonication (ultrasonic bath, Branson) for 5 min. After vortexing the tubes for 10 sec, 100 µl of the solution was transferred to a 96-well plate and a tenfold dilution series was done in 100 mM phosphate buffer (pH7). To assess pathogen colonization, 4 µl of each dilution was spotted on KB agar square plates (Greiner) supplemented with 50 µg/ml kanamycin and 50 µg/ml rifampicin. For total bacterial colonization, 4 µl of each dilution was spotted on R2A+M agar square plates (Greiner) and 50 µl of dilutions 10-3 and 10-4 were plated onto round (9cm) R2A+M agar plates. Plates were incubated at room temperature until cfu could be counted (2-7 days). Strains in binary competition experiments were distinguished based on colony morphology (size, colour, growth speed).

**Plant harvest for community composition, DNA extraction and 16S rDNA amplicon sequencing library preparation and sequencing**

To analyse community composition, plants were removed from clay substrate with sterile tweezers, and the cotyledons and roots were cut off with a sterile scalpel. The remaining phyllosphere was placed into a lysis matrix E tube (FastDNA SPIN Kit for Soil, MP Biomedicals). Plant fresh weight was measured on an analytical balance (Mettler-Toledo) with an accuracy of 0.1 mg. Samples were then frozen in liquid nitrogen and stored at -80 °C.

DNA extraction and 16S rRNA amplicon sequencing was done as described before [44-47]. Briefly, frozen plant and inoculum samples were lyophilised (Christ Alpha 2–4 LD Plus) overnight and subsequently homogenized with a TissueLyser II for 2 min at 25 Hz. The DNA was extracted with the FastDNA SPIN Kit for Soil (MP Biomedicals) according to the manufacturer's instructions. DNA was quantified (Promega QuantiFluor dsDNA, E2670) and normalized to a concentration of 2.5 ng/µl in 50 µl end volume. For samples with lower DNA concentration, the undiluted sample was taken. The 16S amplicon library was

prepared as previously described [44-47]. The V5–V7 region of the 16S rRNA gene was amplified in triplicate with primers 799F [60] and 1193R [61] with the DFS Taq polymerase (Bioron). After pooling triplicate samples, amplification was verified by loading 5 µl of each sample on a 1.5% (w/v) agarose gel. Primers were removed by enzymatic digestion with Antarctic phosphatase (NewEnglandBioLabs) and Exonuclease I (NewEnglandBioLabs). 10 cycles of barcoding-PCR were performed in triplicate with plate-specific forward and well-specific reverse. Triplicates were again pooled and the amplification was verified with a 1.5% (w/v) agarose gel as described for the first PCR. Based on the intensity of the gel band, samples were pooled and to reduce the volume of the library, it was cleaned by bead clean-up (AMPure XP, Beckman Coulter) with a ratio of 0.8:1. Then the library was loaded on a 1.5% agarose gel and the band at approximately 500 bp was cleaned up with the QIAquick gel extraction kit (Qiagen) according to the manufacturer's protocol. The library was cleaned twice by bead clean-up (AMPure XP, Beckman Coulter) with a ratio of 0.8:1.

Library sequencing was performed on the Illumina MiSeq platform using a v3 cycle kit (2 × 300 bp, paired-end) at the Genetic Diversity Center (ETH Zurich). The denatured library was diluted to a final concentration of 10 or 20 pM with addition of 20-30% PhiX. Sequencing was performed with custom sequencing primers as previously described [47]. The 16S rRNA amplicon sequencing samples presented in this study were split over two libraries. The first library contained the experiment of SynCom-210 inocula variation and the first experiment of SynCom-16All, SynCom-8p and SynCom-8np. The second library contained the second experiment of SynCom-16All, SynCom-8p and SynCom-8np and binary competition samples (data of sequenced binary competition is not shown).

**Data analysis**

If not stated otherwise, data was analysed and visualized in the statistical software R v4.2.2 [62]. The packages used for data preparation, analysis and visualization included *tidyverse* v1.3.2 [63], *gridExtra* v2.3 [64], *ggpubr* v0.6.0 [65].

**Pathogen luminescence analysis.** Prior to data analysis, the total flux [p/s] measurements were $\log_{10}$-transformed. Differences in luminescence among different treatments were detected using pairwise Welch's t-tests with Bonferroni-correction on the p-value. In the analysis, measurements of the mock-infected axenic plants served as the background luminescence signal (no bacteria on top of plants).

**Bacterial colonization analysis.** Prior to data analysis, the calculated bacterial cfu per gram fresh weight were $\log_{10}$-transformed. Differences in colonization level among different treatments were detected using pairwise Welch's t-tests with Bonferroni-correction on the p-value. The data was visualized with the lower limit being the calculated detection limit.

**16S rRNA data processing.** The 16S rRNA amplicon sequencing data were analysed as described previously [44-46]. The raw reads of the paired-end sequencing were processed using USEARCH v.11.0.667-i86 linux64 [66]. The reads were merged with the *fastq_mergepairs* command with minimum identity of 90% and minimum overlap of 16 bp. The merged reads were filtered using the command *fastq_filter* with a maximum expected error of 1 and a minimum length of 200 bp. A 16S rDNA reference database was composed based on the amplicon sequencing variants (ASV) of the V5-V7 region 16S rRNA gene sequences of the At-LPSHERE strains [45]. The command *otutab* was used to classify and count reads with 100% identity to the 16S rDNA reference database and assign them to individual samples, to generate an ASV table. The sequences with a barcode corresponding to a sample but no match to the reference database were added up and included as an additional line for sequencing depth estimation, but not further investigated. Control samples of axenic plants, and water controls of the extraction and processing controls were used to detect possible systematic contaminations but excluded for further analysis. The 16S rRNA amplicon sequencing samples presented in this study were split over two libraries. ASV tables of the two libraries were combined and analysed together.

**Comparing and visualising community composition based on 16s rRNA amplicon sequencing.** Prior to data analysis, the pathogen abundance was omitted, and for low complex communities (SynCom-15s) ASV tables were reduced to ASVs present in the communities. The community composition comparisons and ASV changes between treatments were analysed as described before [44-46] with the R package *phylloR* version 1.0.1 available on GitHub (https://github.com/MicrobiologyETHZ/phylloR/). Briefly, after filtering for the comparisons of interest, the ASV table was log-normalized for sequence depth and variance-stabilized by DESeq2 v1.38.3[67]. For visualization of the overall comparison of two treatments, the *plotPCA* function in the package *phylloR* was used. The function applies a principal component analysis (PCA) to the transformed OTU table using the *prcomp* command and calculated the effect size, which is the variance explained by the compared factor, and the p-value of the comparisons were calculated by PERMANOVA using the *adonis* function of the package *vegan* v2.6-4 [68] with Euclidean distance. In the *phylloR* package, PERMANOVA was modified to account for the batch effect between replicate experiments with the strata argument. Changes in ASV abundances between two groups was analysed through the function *plotCommunityChanges* in the *phylloR* package. The output of *DESeq2* provided $\log_2$-fold change values and p-values (Wald tests, Benjamini-Hochberg adjusted). The community composition was visualized through the function *plotCommunity*, where the relative abundance values were calculated by proportional normalization of each sample by its sequencing depth.

Shannon's diversity scores, Pielou's evenness and species richness were calculated using the package *vegan* v2.6-4 [68]. In a first step, ASV tables were transposed, and samples of interest were filtered for. Next, the samples were rarefied to 2500, leaving 4 samples non-rarefied (LibUid "Lib330", "Lib4348", "Lib333",

"Lib4374"). The species number (richness) was calculated using the function *specnumber*. The diversity scores were calculated with the function *diversity* with index set to "shannon". Pielou's evenness was calculated by dividing the diversity by the log-transformed species richness. Differences in diversity scores among different treatments were detected using pairwise Welch's t-tests with Bonferroni-correction on the p-value.

**Data and Code availability**

Data files and R script used for preparation of data, visualization and analysis are stored on a gitlab repository (https://gitlab.ethz.ch/thesisbe/june2022.git). Raw sequencing data are stored on TAPES (\\LTS22\biol_lts_cifs\biol-micro\gr_vorholt\OMICS\barbmuel).

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# Supplemental Tables

**Supplemental Table 1:** Representation of At-LSPHERE strains in synthetic communities described in chapter III.

| Strain | ASV representative | ASV size (n strains) | Phylum [1] | Class [1] | Genus [1] | SynCom-210 | SynCom-223 | SynCom-48p | SynCom-175np | SynCom-15 | SynCom-15±2 | SynCom-15±5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Leaf129 | Leaf129 | 1 | Proteobacteria | Gammaproteobacteria | *Pseudomonas* | 1 | 1 | 1 | 0 | 1 | 0 | 0 |
| Leaf88 | Leaf88 | 9 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| Leaf33 | Leaf33 | 1 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| Leaf405 | Leaf405 | 1 | Bacteroidetes | Flavobacteriia | *Chryseobacterium* | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| Leaf371 | Leaf371 | 4 | Proteobacteria | Alphaproteobacteria | *Rhizobium* | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| Leaf299 | Leaf299 | 3 | Actinobacteria | Actinobacteria | *Rathayibacter* | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| Leaf220 | Leaf220 | 1 | Proteobacteria | Betaproteobacteria | *Variovorax* | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| Leaf374 | Leaf374 | 1 | Actinobacteria | Actinobacteria | *Nocardioides* | 0 | 1 | 0 | 1 | 1 | 1 | 1 |
| Leaf61 | Leaf61 | 1 | Proteobacteria | Betaproteobacteria | *Duganella* | 1 | 1 | 1 | 0 | 1 | 1 | 0 |
| Leaf187 | Leaf187 | 2 | Firmicutes | Bacilli | *Exiguobacterium* | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| Leaf41 | Leaf41 | 1 | Bacteroidetes | Sphingobacteriia | *Pedobacter* | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| Leaf67 | Leaf67 | 1 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| Leaf359 | Leaf359 | 1 | Bacteroidetes | Flavobacteriia | *Flavobacterium* | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| Leaf427 | Leaf427 | 2 | Proteobacteria | Alphaproteobacteria | *Aurantimonas* | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| Leaf272 | Leaf272 | 1 | Actinobacteria | Actinobacteria | *Aeromicrobium* | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| Leaf82 | Leaf82 | 1 | Bacteroidetes | Flavobacteriia | *Flavobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 1 |
| Leaf126 | Leaf126 | 1 | Proteobacteria | Betaproteobacteria | *Duganella* | 1 | 1 | 1 | 0 | 0 | 0 | 1 |
| Leaf202 | Leaf155 | 4 | Proteobacteria | Alphaproteobacteria | *Rhizobium* | 1 | 1 | 0 | 1 | 0 | 1 | 0 |
| Leaf21 | Leaf21 | 1 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 1 | 0 | 0 | 0 | 1 |
| Leaf83 | Leaf83 | 1 | Proteobacteria | Gammaproteobacteria | *Pseudomonas* | 1 | 1 | 0 | 1 | 0 | 1 | 1 |
| Leaf68 | Leaf155 | 4 | Proteobacteria | Alphaproteobacteria | *Rhizobium* | 1 | 1 | 1 | 0 | 0 | 0 | 1 |
| Leaf7 | Leaf247 | 4 | Actinobacteria | Actinobacteria | *Rhodococcus* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf15 | Leaf15 | 2 | Proteobacteria | Gammaproteobacteria | *Pseudomonas* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf44 | Leaf186 | 5 | Actinobacteria | Actinobacteria | *Frigoribacterium* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf48 | Leaf48 | 1 | Proteobacteria | Gammaproteobacteria | *Pseudomonas* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf49 | Leaf49 | 1 | Firmicutes | Bacilli | *Bacillus* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf51 | Leaf51 | 1 | Proteobacteria | Gammaproteobacteria | *Serratia* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf53 | Leaf53 | 1 | Proteobacteria | Gammaproteobacteria | *Erwinia* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf58 | Leaf58 | 1 | Proteobacteria | Gammaproteobacteria | *Pseudomonas* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf59 | Leaf59 | 1 | Proteobacteria | Gammaproteobacteria | *Pseudomonas* | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf98 | Leaf15 | 2 | Proteobacteria | Gammaproteobacteria | *Pseudomonas* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf127 | Leaf127 | 1 | Proteobacteria | Gammaproteobacteria | *Pseudomonas* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf130 | Leaf130 | 1 | Proteobacteria | Gammaproteobacteria | *Acinetobacter* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf131 | Leaf131 | 2 | Proteobacteria | Gammaproteobacteria | *Xanthomonas* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf137 | Leaf137 | 1 | Actinobacteria | Actinobacteria | *Arthrobacter* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf141 | Leaf141 | 1 | Actinobacteria | Actinobacteria | *Arthrobacter* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf145 | Leaf145 | 1 | Actinobacteria | Actinobacteria | *Arthrobacter* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf148 | Leaf131 | 2 | Proteobacteria | Gammaproteobacteria | *Xanthomonas* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf151 | Leaf151 | 1 | Actinobacteria | Actinobacteria | *Microbacterium* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf154 | Leaf154 | 2 | Actinobacteria | Actinobacteria | *Curtobacterium* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf155 | Leaf155 | 4 | Proteobacteria | Alphaproteobacteria | *Rhizobium* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf160 | Leaf160 | 1 | Proteobacteria | Betaproteobacteria | *Acidovorax* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf167 | Leaf155 | 4 | Proteobacteria | Alphaproteobacteria | *Rhizobium* | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf177 | Leaf177 | 1 | Proteobacteria | Betaproteobacteria | *Burkholderia* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf179 | Leaf179 | 3 | Actinobacteria | Actinobacteria | *Microbacterium* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf183 | Leaf154 | 2 | Actinobacteria | Actinobacteria | *Curtobacterium* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf198 | Leaf198 | 5 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf203 | Leaf179 | 3 | Actinobacteria | Actinobacteria | *Microbacterium* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf226 | Leaf226 | 1 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf230 | Leaf198 | 5 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf233 | Leaf233 | 2 | Actinobacteria | Actinobacteria | *Rhodococcus* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf242 | Leaf198 | 5 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf254 | Leaf186 | 5 | Actinobacteria | Actinobacteria | *Frigoribacterium* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf257 | Leaf257 | 1 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf262 | Leaf262 | 1 | Proteobacteria | Alphaproteobacteria | *Rhizobium* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf311 | Leaf311 | 1 | Proteobacteria | Alphaproteobacteria | *Rhizobium* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf335 | Leaf210 | 4 | Actinobacteria | Actinobacteria | *Agreia* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf337 | Leaf337 | 1 | Actinobacteria | Actinobacteria | *Arthrobacter* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf347 | Leaf347 | 2 | Actinobacteria | Actinobacteria | *Microbacterium* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf351 | Leaf347 | 2 | Actinobacteria | Actinobacteria | *Microbacterium* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf434 | Leaf434 | 1 | Proteobacteria | Gammaproteobacteria | *Pseudomonas* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf436 | Leaf179 | 3 | Actinobacteria | Actinobacteria | *Microbacterium* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf453 | Leaf386 | 3 | Proteobacteria | Alphaproteobacteria | *Rhizobium* | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Leaf1 | Leaf171 | 3 | Actinobacteria | Actinobacteria | *Plantibacter* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf2 | Leaf2 | 1 | Proteobacteria | Alphaproteobacteria | *Novosphingobium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf3 | Leaf3 | 1 | Actinobacteria | Actinobacteria | *Sanguibacter* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf5 | Leaf24 | 2 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf8 | Leaf186 | 5 | Actinobacteria | Actinobacteria | *Frigoribacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf9 | Leaf11 | 6 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf10 | Leaf10 | 1 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf11 | Leaf11 | 6 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf13 | Leaf13 | 1 | Firmicutes | Bacilli | *Bacillus* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf16 | Leaf16 | 3 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf17 | Leaf17 | 1 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf20 | Leaf198 | 5 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf22 | Leaf22 | 2 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf23 | Leaf11 | 6 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf24 | Leaf24 | 2 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf25 | Leaf11 | 6 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf26 | Leaf26 | 1 | Proteobacteria | Alphaproteobacteria | *Sphingobium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf28 | Leaf28 | 1 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf29 | Leaf16 | 3 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf30 | Leaf30 | 1 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf32 | Leaf16 | 3 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf34 | Leaf34 | 2 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf38 | Leaf34 | 2 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf42 | Leaf11 | 6 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf62 | Leaf22 | 2 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf64 | Leaf64 | 1 | Proteobacteria | Alphaproteobacteria | *Devosia* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf69 | Leaf69 | 1 | Actinobacteria | Actinobacteria | *Arthrobacter* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |

Supplemental Table 1 continued

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Leaf70 | Leaf70 | 1 | Proteobacteria | Gammaproteobacteria | *Stenotrophomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf72 | Leaf72 | 1 | Firmicutes | Bacilli | *Paenibacillus* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf75 | Leaf75 | 1 | Firmicutes | Bacilli | *Bacillus* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf76 | Leaf191 | 3 | Proteobacteria | Betaproteobacteria | *Acidovorax* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf78 | Leaf78 | 1 | Proteobacteria | Betaproteobacteria | *Acidovorax* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf84 | Leaf191 | 3 | Proteobacteria | Betaproteobacteria | *Acidovorax* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf85 | Leaf85 | 1 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf86 | Leaf86 | 1 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf87 | Leaf100 | 5 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf89 | Leaf88 | 9 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf90 | Leaf119 | 4 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf91 | Leaf91 | 1 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf92 | Leaf122 | 2 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf93 | Leaf106 | 2 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf94 | Leaf88 | 9 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf99 | Leaf99 | 1 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf100 | Leaf100 | 5 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf102 | Leaf100 | 5 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf104 | Leaf88 | 9 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf106 | Leaf106 | 2 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf108 | Leaf108 | 3 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf111 | Leaf88 | 9 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf112 | Leaf100 | 5 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf113 | Leaf88 | 9 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf117 | Leaf88 | 9 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf119 | Leaf119 | 4 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf121 | Leaf119 | 4 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf122 | Leaf122 | 2 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf123 | Leaf119 | 4 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf125 | Leaf88 | 9 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf139 | Leaf139 | 1 | Proteobacteria | Betaproteobacteria | *Massilia* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf159 | Leaf159 | 3 | Actinobacteria | Actinobacteria | *Microbacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf161 | Leaf159 | 3 | Actinobacteria | Actinobacteria | *Microbacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf164 | Leaf164 | 1 | Actinobacteria | Actinobacteria | *Rathayibacter* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf168 | Leaf168 | 1 | Proteobacteria | Alphaproteobacteria | *Brevundimonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf171 | Leaf171 | 3 | Actinobacteria | Actinobacteria | *Plantibacter* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf172 | Leaf172 | 2 | Actinobacteria | Actinobacteria | *Clavibacter* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf176 | Leaf176 | 1 | Bacteroidetes | Sphingobacteriia | *Pedobacter* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf180 | Leaf180 | 1 | Bacteroidetes | Flavobacteriia | *Chryseobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf182 | Leaf182 | 1 | Firmicutes | Bacilli | *Brevibacillus* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf185 | Leaf185 | 2 | Actinobacteria | Actinobacteria | *Rathayibacter* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf186 | Leaf186 | 5 | Actinobacteria | Actinobacteria | *Frigoribacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf189 | Leaf189 | 1 | Bacteroidetes | Cytophagia | *Dyadobacter* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf191 | Leaf191 | 3 | Proteobacteria | Betaproteobacteria | *Acidovorax* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf194 | Leaf194 | 3 | Bacteroidetes | Sphingobacteriia | *Pedobacter* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf196 | Leaf187 | 2 | Firmicutes | Bacilli | *Exiguobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf201 | Leaf201 | 1 | Bacteroidetes | Flavobacteriia | *Chryseobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf205 | Leaf198 | 5 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf208 | Leaf208 | 1 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf210 | Leaf210 | 4 | Actinobacteria | Actinobacteria | *Agreia* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf216 | Leaf216 | 1 | Bacteroidetes | Sphingobacteriia | *Pedobacter* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf222 | Leaf222 | 1 | Actinobacteria | Actinobacteria | *Agromyces* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf225 | Leaf247 | 4 | Actinobacteria | Actinobacteria | *Rhodococcus* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf231 | Leaf231 | 1 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf234 | Leaf234 | 1 | Actinobacteria | Actinobacteria | *Arthrobacter* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf244 | Leaf210 | 4 | Actinobacteria | Actinobacteria | *Agreia* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf245 | Leaf245 | 1 | Actinobacteria | Actinobacteria | *Aeromicrobium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf247 | Leaf247 | 4 | Actinobacteria | Actinobacteria | *Rhodococcus* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf258 | Leaf247 | 4 | Actinobacteria | Actinobacteria | *Rhodococcus* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf261 | Leaf261 | 1 | Actinobacteria | Actinobacteria | *Curtobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf263 | Leaf172 | 2 | Actinobacteria | Actinobacteria | *Clavibacter* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf264 | Leaf264 | 2 | Actinobacteria | Actinobacteria | *Leifsonia* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf265 | Leaf265 | 1 | Proteobacteria | Betaproteobacteria | *Pseudorhodoferax* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf267 | Leaf267 | 1 | Proteobacteria | Betaproteobacteria | *Variovorax* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf274 | Leaf274 | 1 | Proteobacteria | Betaproteobacteria | *Pseudorhodoferax* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf278 | Leaf233 | 2 | Actinobacteria | Actinobacteria | *Rhodococcus* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf280 | Leaf280 | 1 | Proteobacteria | Alphaproteobacteria | *Brevundimonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf283 | Leaf210 | 4 | Actinobacteria | Actinobacteria | *Agreia* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf285 | Leaf285 | 2 | Actinobacteria | Actinobacteria | *Nocardioides* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf288 | Leaf288 | 1 | Actinobacteria | Actinobacteria | *Microbacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf289 | Leaf289 | 2 | Actinobacteria | Actinobacteria | *Aeromicrobium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf291 | Leaf289 | 2 | Actinobacteria | Actinobacteria | *Aeromicrobium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf294 | Leaf185 | 2 | Actinobacteria | Actinobacteria | *Rathayibacter* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf296 | Leaf299 | 3 | Actinobacteria | Actinobacteria | *Rathayibacter* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf304 | Leaf304 | 1 | Actinobacteria | Actinobacteria | *Frondihabitans* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf306 | Leaf306 | 2 | Proteobacteria | Alphaproteobacteria | *Rhizobium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf307 | Leaf285 | 2 | Actinobacteria | Actinobacteria | *Nocardioides* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf314 | Leaf171 | 3 | Actinobacteria | Actinobacteria | *Plantibacter* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf320 | Leaf159 | 3 | Actinobacteria | Actinobacteria | *Microbacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf321 | Leaf306 | 2 | Proteobacteria | Alphaproteobacteria | *Rhizobium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf324 | Leaf324 | 1 | Proteobacteria | Alphaproteobacteria | *Aureimonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf325 | Leaf264 | 2 | Actinobacteria | Actinobacteria | *Leifsonia* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf326 | Leaf326 | 1 | Deinococcus-Thermus | Deinococci | *Deinococcus* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf334 | Leaf334 | 2 | Actinobacteria | Actinobacteria | *Cellulomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf336 | Leaf336 | 1 | Actinobacteria | Actinobacteria | *Leifsonia* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf339 | Leaf339 | 2 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf341 | Leaf371 | 4 | Proteobacteria | Alphaproteobacteria | *Rhizobium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf343 | Leaf343 | 1 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf344 | Leaf344 | 1 | Proteobacteria | Alphaproteobacteria | *Bosea* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf354 | Leaf354 | 1 | Actinobacteria | Actinobacteria | *Williamsia* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf357 | Leaf357 | 1 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf361 | Leaf361 | 1 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf363 | Leaf363 | 1 | Proteobacteria | Alphaproteobacteria | *Brevundimonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf369 | Leaf369 | 1 | Actinobacteria | Actinobacteria | *Geodermatophilus* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf380 | Leaf380 | 1 | Actinobacteria | Actinobacteria | *Blastococcus* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |

Supplemental Table 1 continued

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Leaf383 | Leaf371 | 4 | Proteobacteria | Alphaproteobacteria | *Rhizobium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf384 | Leaf371 | 4 | Proteobacteria | Alphaproteobacteria | *Rhizobium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf386 | Leaf386 | 3 | Proteobacteria | Alphaproteobacteria | *Rhizobium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf391 | Leaf386 | 3 | Proteobacteria | Alphaproteobacteria | *Rhizobium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf394 | Leaf394 | 1 | Bacteroidetes | Flavobacteriia | *Chryseobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf395 | Leaf334 | 2 | Actinobacteria | Actinobacteria | *Cellulomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf396 | Leaf396 | 2 | Proteobacteria | Alphaproteobacteria | *Bradyrhizobium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf399 | Leaf108 | 3 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf400 | Leaf400 | 1 | Proteobacteria | Betaproteobacteria | *Acidovorax* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf404 | Leaf404 | 2 | Bacteroidetes | Flavobacteriia | *Chryseobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf406 | Leaf406 | 1 | Firmicutes | Bacilli | *Bacillus* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf407 | Leaf11 | 6 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf408 | Leaf414 | 3 | Proteobacteria | Betaproteobacteria | *Methylophilus* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf412 | Leaf412 | 1 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf414 | Leaf414 | 3 | Proteobacteria | Betaproteobacteria | *Methylophilus* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf415 | Leaf186 | 5 | Actinobacteria | Actinobacteria | *Frigoribacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf416 | Leaf414 | 3 | Proteobacteria | Betaproteobacteria | *Methylophilus* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf420 | Leaf420 | 1 | Proteobacteria | Alphaproteobacteria | *Devosia* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf443 | Leaf443 | 1 | Proteobacteria | Alphaproteobacteria | *Aurantimonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf446 | Leaf446 | 1 | Actinobacteria | Actinobacteria | *Marmoricola* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf454 | Leaf454 | 1 | Proteobacteria | Alphaproteobacteria | *Aurantimonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf456 | Leaf456 | 1 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf459 | Leaf459 | 1 | Proteobacteria | Betaproteobacteria | *Methylophilus* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf460 | Leaf427 | 2 | Proteobacteria | Alphaproteobacteria | *Aurantimonas* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf465 | Leaf88 | 9 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf466 | Leaf108 | 3 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf469 | Leaf100 | 5 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf4 | Leaf4 | 1 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf37 | Leaf339 | 2 | Proteobacteria | Alphaproteobacteria | *Sphingomonas* | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf73 | Leaf73 | 1 | Proteobacteria | Betaproteobacteria | *Acidovorax* | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf118 | Leaf118 | 1 | Proteobacteria | Alphaproteobacteria | *Methylobacterium* | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf132 | Leaf194 | 3 | Bacteroidetes | Sphingobacteriia | *Pedobacter* | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf170 | Leaf194 | 3 | Bacteroidetes | Sphingobacteriia | *Pedobacter* | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf248 | Leaf299 | 3 | Actinobacteria | Actinobacteria | *Rathayibacter* | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf250 | Leaf250 | 1 | Bacteroidetes | Sphingobacteriia | *Pedobacter* | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf313 | Leaf404 | 2 | Bacteroidetes | Flavobacteriia | *Chryseobacterium* | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf350 | Leaf350 | 1 | Actinobacteria | Actinobacteria | *Aeromicrobium* | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf401 | Leaf396 | 2 | Proteobacteria | Alphaproteobacteria | *Bradyrhizobium* | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| Leaf50 | Leaf50 | 1 | Proteobacteria | Gammaproteobacteria | *Serratia* | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

[1] Y. Bai et al., Functional overlap of the Arabidopsis leaf and root microbiota. Nature 528, 364-369 (2015).

**Supplemental Table 2:** Table to summarize relative abundance changes of ASVs in inocula treatments versus default SynCom-210 composition in mock-infected plants.

| Community | Treatment | Group 1 | Group 2 | ASV Representative | Phyla | Genus | fold change | p-value | significance[1] | Comment |
|---|---|---|---|---|---|---|---|---|---|---|
| SynCom-210 | mock-infected | "default" inoculum | reversed phyla | Leaf210 | Actinobacteria | Agreia | 10.1 | 0.01 | ** | |
| SynCom-210 | mock-infected | "default" inoculum | reversed phyla | Leaf414 | Proteobacteria | Methylophilus | 0.00126 | 0.000000619 | **** | nd 8/10 in group 2 |
| SynCom-210 | mock-infected | "default" inoculum | reversed phyla | Leaf131 | Proteobacteria | Xanthomonas | 0.0662 | 0.0343 | * | nd 8/10 in group 2 |
| SynCom-210 | mock-infected | "default" inoculum | reversed phyla | Leaf344 | Proteobacteria | Bosea | 0.0147 | 0.00604 | ** | nd 10/10 in group 2 |
| SynCom-210 | mock-infected | "default" inoculum | reversed phyla | Leaf64 | Proteobacteria | Devosia | 0.0131 | 0.000288 | *** | nd 9/10 in group 2 |
| SynCom-210 | mock-infected | "default" inoculum | reversed phyla | Leaf26 | Proteobacteria | Sphingobium | 0.0679 | 0.000797 | *** | nd 6/10 in group 2 |
| SynCom-210 | mock-infected | "default" inoculum | reversed phyla | Leaf108 | Proteobacteria | Methylobacterium | 0.0407 | 0.00282 | ** | nd 5/10 in group 2 |
| SynCom-210 | mock-infected | "default" inoculum | reversed phyla | Leaf306 | Proteobacteria | Rhizobium | 0.0241 | 0.0104 | * | nd 10/10 in group 2 |
| SynCom-210 | mock-infected | "default" inoculum | reversed phyla | Leaf98 | Proteobacteria | Pseudomonas | 0.0255 | 0.000576 | *** | nd 7/10 in group 2 |
| SynCom-210 | mock-infected | "default" inoculum | reversed phyla | Leaf420 | Proteobacteria | Devosia | 0.0126 | 0.000288 | *** | nd 8/10 in group 2 |
| SynCom-210 | mock-infected | "default" inoculum | reversed phyla | Leaf159 | Actinobacteria | Microbacterium | 14 | 0.01 | ** | |
| SynCom-210 | mock-infected | "default" inoculum | reversed phyla | Leaf53 | Proteobacteria | Erwinia | 0.0164 | 0.0000835 | **** | nd 10/10 in group 2 |
| SynCom-210 | mock-infected | "default" inoculum | low *Rhizobium* | Leaf311 | Proteobacteria | Rhizobium | 0.0067 | 0.0000774 | **** | nd 6/10 in group 2 |
| SynCom-210 | mock-infected | "default" inoculum | low *Rhizobium* | Leaf306 | Proteobacteria | Rhizobium | 0.0136 | 0.00105 | ** | nd 8/10 in group 2 |
| SynCom-210 | mock-infected | "default" inoculum | low *Methylophilus* , low *Devosia* | Leaf414 | Proteobacteria | Methylophilus | 0.000952 | 2.29E-09 | **** | nd 6/10 in group 2 |
| SynCom-210 | mock-infected | "default" inoculum | low *Methylophilus* , low *Devosia* | Leaf64 | Proteobacteria | Devosia | 0.00565 | 2.77E-08 | **** | nd 7/10 in group 2 |
| SynCom-210 | mock-infected | "default" inoculum | low *Methylophilus* , low *Devosia* | Leaf337 | Actinobacteria | Arthrobacter | 0.0499 | 0.0109 | * | nd 6/10 in group 2 |
| SynCom-210 | mock-infected | "default" inoculum | low *Methylophilus* , low *Devosia* | Leaf420 | Proteobacteria | Devosia | 0.00145 | 4.1E-10 | **** | nd 9/10 in group 2 |
| SynCom-210 | mock-infected | "default" inoculum | with pathogen | Leaf50 | Proteobacteria | Serratia | 90.4 | 0.0000358 | **** | added in group 2, ignore |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 3:** Bonferroni-corrected p-values of pairwise Welch's t-tests of Shannon's diversity, Pielou's evenness and species richness of different SynCom-210 inocula compared to default SynCom-210, and of different infection treatments within inocula treatments.

| Score | Treatment | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|---|
| Diversity | mock-infected | SynCom-210 | reversed Phyla | 0 | **** |
| Diversity | mock-infected | SynCom-210 | low *Rhizobium* | 1 | |
| Diversity | mock-infected | SynCom-210 | low *Methylophilus* , low *Devosia* | 1 | |
| Diversity | mock-infected | SynCom-210 | with pathogen | 1 | |
| Diversity | SynCom-210 | mock-infected | 7 dpi | 0.18648 | |
| Diversity | SynCom-210 | mock-infected | 14 dpi | 1 | |
| Diversity | reversed Phyla | mock-infected | 7 dpi | 1 | |
| Diversity | reversed Phyla | mock-infected | 14 dpi | 1 | |
| Diversity | low *Rhizobium* | mock-infected | 7 dpi | 1 | |
| Diversity | low *Rhizobium* | mock-infected | 14 dpi | 1 | |
| Diversity | low *Methylophilus* , low *Devosia* | mock-infected | 7 dpi | 1 | |
| Diversity | low *Methylophilus* , low *Devosia* | mock-infected | 14 dpi | 1 | |
| Diversity | with pathogen | mock-infected | 7 dpi | 1 | |
| Diversity | with pathogen | mock-infected | 14 dpi | 1 | |
| Richness | mock-infected | SynCom-210 | reversed Phyla | 0 | **** |
| Richness | mock-infected | SynCom-210 | low *Rhizobium* | 1 | |
| Richness | mock-infected | SynCom-210 | low *Methylophilus* , low *Devosia* | 0.06208 | |
| Richness | mock-infected | SynCom-210 | with pathogen | 1 | |
| Richness | SynCom-210 | mock-infected | 7 dpi | 1 | |
| Richness | SynCom-210 | mock-infected | 14 dpi | 1 | |
| Richness | reversed Phyla | mock-infected | 7 dpi | 1 | |
| Richness | reversed Phyla | mock-infected | 14 dpi | 1 | |
| Richness | low *Rhizobium* | mock-infected | 7 dpi | 1 | |
| Richness | low *Rhizobium* | mock-infected | 14 dpi | 1 | |
| Richness | low *Methylophilus* , low *Devosia* | mock-infected | 7 dpi | 1 | |
| Richness | low *Methylophilus* , low *Devosia* | mock-infected | 14 dpi | 0.04642 | * |
| Richness | with pathogen | mock-infected | 7 dpi | 1 | |
| Richness | with pathogen | mock-infected | 14 dpi | 1 | |
| Evenness | mock-infected | SynCom-210 | reversed Phyla | 0.05844 | |
| Evenness | mock-infected | SynCom-210 | low *Rhizobium* | 1 | |
| Evenness | mock-infected | SynCom-210 | low *Methylophilus* , low *Devosia* | 1 | |
| Evenness | mock-infected | SynCom-210 | with pathogen | 1 | |
| Evenness | SynCom-210 | mock-infected | 7 dpi | 1 | |
| Evenness | SynCom-210 | mock-infected | 14 dpi | 1 | |
| Evenness | reversed Phyla | mock-infected | 7 dpi | 1 | |
| Evenness | reversed Phyla | mock-infected | 14 dpi | 1 | |
| Evenness | low *Rhizobium* | mock-infected | 7 dpi | 1 | |
| Evenness | low *Rhizobium* | mock-infected | 14 dpi | 1 | |
| Evenness | low *Methylophilus* , low *Devosia* | mock-infected | 7 dpi | 1 | |
| Evenness | low *Methylophilus* , low *Devosia* | mock-infected | 14 dpi | 1 | |
| Evenness | with pathogen | mock-infected | 7 dpi | 1 | |
| Evenness | with pathogen | mock-infected | 14 dpi | 1 | |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 4:** Bonferroni-corrected p-values of pairwise Welch's t-tests of pathogen colonization at 7 or 14 dpi of different SynCom-210 inocula compared to default SynCom-210 and axenic infected controls, and comparing different infection timepoints within inocula treatments.

| Timepoint | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|
| 7 dpi | axenic | "default" SynCom-210 | 1 | |
| 7 dpi | axenic | reversed Phyla | 1 | |
| 7 dpi | axenic | low *Rhizobium* | 0.11122 | |
| 7 dpi | axenic | low *Methylophilus* low *Devosia* | 1 | |
| 7 dpi | axenic | with pathogen | 0.48509 | |
| 7 dpi | "default" SynCom-210 | reversed Phyla | 1 | |
| 7 dpi | "default" SynCom-210 | low *Rhizobium* | 1 | |
| 7 dpi | "default" SynCom-210 | low *Methylophilus* low *Devosia* | 1 | |
| 7 dpi | "default" SynCom-210 | with pathogen | 1 | |
| axenic | 7 dpi | 14 dpi | 1 | |
| "default" SynCom-210 | 7 dpi | 14 dpi | 1 | |
| reversed Phyla | 7 dpi | 14 dpi | 1 | |
| low *Rhizobium* | 7 dpi | 14 dpi | 1 | |
| low *Methylophilus* low *Devosia* | 7 dpi | 14 dpi | 1 | |
| with pathogen | 7 dpi | 14 dpi | 1 | |
| 14 dpi | axenic | "default" SynCom-210 | 0.00257 | ** |
| 14 dpi | axenic | reversed Phyla | 0.00087 | *** |
| 14 dpi | axenic | low *Rhizobium* | 0.00006 | **** |
| 14 dpi | axenic | low *Methylophilus* low *Devosia* | 0.00194 | ** |
| 14 dpi | axenic | with pathogen | 0.00072 | *** |
| 14 dpi | "default" SynCom-210 | reversed Phyla | 1 | |
| 14 dpi | "default" SynCom-210 | low *Rhizobium* | 1 | |
| 14 dpi | "default" SynCom-210 | low *Methylophilus* low *Devosia* | 1 | |
| 14 dpi | "default" SynCom-210 | with pathogen | 1 | |
| 14 dpi | "default" SynCom-210 | with pathogen, non-infected | 0 | **** |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 5:** Table to summarize the median pathogen colonization at specified timepoints, and comparing the untransformed values of the treatment to the "all" control (SynCom-210 or SynCom-223) (factor=(treatment-control)/control).

| Experiment | Inoculum/Drop-Out Condition | Timepoint | median pathogen colonization $log_{10}$([cfu $g^{-1}$ fresh weight]) | factor changes compared to SynCom-210 or 223 |
|---|---|---|---|---|
| SynCom-210 Inoculum Variation | Axenic | 7 dpi | 9.238682459 | 2.518382353 |
| SynCom-210 Inoculum Variation | SynCom-210 | 7 dpi | 8.692339425 | 0 |
| SynCom-210 Inoculum Variation | Reversed Phyla | 7 dpi | 8.762287571 | 0.174757282 |
| SynCom-210 Inoculum Variation | Low Rhizobium | 7 dpi | 8.178004454 | -0.694039735 |
| SynCom-210 Inoculum Variation | Low Methylophilus, Low Devosia | 7 dpi | 8.56494009 | -0.254237288 |
| SynCom-210 Inoculum Variation | with pathogen | 7 dpi | 8.61425627 | -0.164556962 |
| SynCom-210 Inoculum Variation | Axenic | 14 dpi | 9.207525422 | 2.27480916 |
| SynCom-210 Inoculum Variation | SynCom-210 | 14 dpi | 8.102080246 | -0.743113772 |
| SynCom-210 Inoculum Variation | Reversed Phyla | 14 dpi | 7.869418841 | -0.849658314 |
| SynCom-210 Inoculum Variation | Low Rhizobium | 14 dpi | 7.412498729 | -0.9475 |
| SynCom-210 Inoculum Variation | Low Methylophilus, Low Devosia | 14 dpi | 8.36031524 | -0.534439834 |
| SynCom-210 Inoculum Variation | with pathogen | 14 dpi | 7.891747306 | -0.841726619 |
| SynCom-210 Inoculum Variation | with pathogen | non-infected | 3.609336582 | -0.99999174 |
| Phyla Drop-Out | Axenic | 14 dpi | 9.183140307 | 9176.211983 |
| Phyla Drop-Out | SynCom-223 | 14 dpi | 5.220429544 | 0 |
| Phyla Drop-Out | Proteobacteria | 14 dpi | 7.400561182 | 150.4020091 |
| Phyla Drop-Out | Alphaproteobacteria | 14 dpi | 4.898184919 | -0.523837296 |
| Phyla Drop-Out | Betaproteobacteria | 14 dpi | 5.151112267 | -0.147522897 |
| Phyla Drop-Out | Gammaproteobacteria | 14 dpi | 5.427972581 | 0.61266083 |
| Phyla Drop-Out | Actinobacteria | 14 dpi | 4.649037465 | -0.731707877 |
| Phyla Drop-Out | Bacteroidetes | 14 dpi | 4.409792835 | -0.84534524 |
| Phyla Drop-Out | Firmicutes | 14 dpi | 4.974779181 | -0.431998298 |
| Genera Drop-Out | Axenic | 14 dpi | 8.83462783 | 9074.868444 |
| Genera Drop-Out | SynCom-223 | 14 dpi | 4.876739638 | 0 |
| Genera Drop-Out | Methylobacterium | 14 dpi | 5.078474943 | 0.591238599 |
| Genera Drop-Out | Microbacterium | 14 dpi | 4.905938223 | 0.069543825 |
| Genera Drop-Out | Pseudomonas | 14 dpi | 5.224203653 | 1.225686617 |
| Genera Drop-Out | Rhizobium | 14 dpi | 5.087801739 | 0.625781214 |
| Genera Drop-Out | Sphingomonas | 14 dpi | 5.156404474 | 0.903990758 |
| Protective Strains Drop-Out | Axenic | 14 dpi | 8.83462783 | 9074.868444 |
| Protective Strains Drop-Out | SynCom-223 | 14 dpi | 4.876739638 | 0 |
| Protective Strains Drop-Out | NonprotectiveStrains | 14 dpi | 5.423979911 | 2.525658734 |
| Protective Strains Drop-Out | ProtectiveStrains | 14 dpi | 4.578840164 | -0.496382833 |
| SynComs-15 | Axenic | 14 dpi | 9.107913 | 697.9832997 |
| SynComs-15 | SynCom-210 | 14 dpi | 6.263446201 | 0 |
| SynComs-15 | SynCom-15High | 14 dpi | 7.829256397 | 35.79681224 |
| SynComs-15 | SynCom-15Mid | 14 dpi | 6.105520688 | -0.304856467 |
| SynComs-15 | SynCom-15Low | 14 dpi | 6.338706874 | 0.189215806 |
| Trait competition | Axenic | 7 dpi | 9.238682459 | 2.518382353 |
| Trait competition | SynCom-210 | 7 dpi | 8.692339425 | 0 |
| Trait competition | SynCom-16All | 7 dpi | 8.584170899 | -0.220472441 |
| Trait competition | SynCom-8Low | 7 dpi | 8.79518459 | 0.2672 |
| Trait competition | SynCom-8High | 7 dpi | 8.980404444 | 0.941176471 |
| Trait competition | Axenic | 14 dpi | 9.207525422 | 11.7480916 |
| Trait competition | SynCom-210 | 14 dpi | 8.102080246 | 0 |
| Trait competition | SynCom-16All | 14 dpi | 7.962211439 | -0.275345168 |
| Trait competition | SynCom-8Low | 14 dpi | 7.343091341 | -0.825814863 |
| Trait competition | SynCom-8High | 14 dpi | 8.552841969 | 1.823330516 |

**Supplemental Table 6:** Bonferroni-corrected p-values of pairwise Welch's t-tests of total bacterial colonization comparing drop-out conditions with controls (SynCom-223, axenic). Controls for genera and functional group drop-outs are duplicated. For comparisons of commensals, only p-values different from 1 are shown.

| Experiment | Colonization by | Inoculation/Drop-out | Drop-out | p-value | significance [1] |
|---|---|---|---|---|---|
| Phyla and Class Drop-out | *Ps* t | axenic | Proteobacteria | 0 | **** |
| Phyla and Class Drop-out | *Ps* t | axenic | Alphaproteobacteria | 0 | **** |
| Phyla and Class Drop-out | *Ps* t | axenic | Betaproteobacteria | 0 | **** |
| Phyla and Class Drop-out | *Ps* t | axenic | Gammaproteobacteria | 0 | **** |
| Phyla and Class Drop-out | *Ps* t | axenic | Actinobacteria | 0 | **** |
| Phyla and Class Drop-out | *Ps* t | axenic | Bacteroidetes | 0 | **** |
| Phyla and Class Drop-out | *Ps* t | axenic | Firmicutes | 0 | **** |
| Phyla and Class Drop-out | *Ps* t | SynCom-223 | Proteobacteria | 0 | **** |
| Phyla and Class Drop-out | *Ps* t | SynCom-223 | Alphaproteobacteria | 1 | |
| Phyla and Class Drop-out | *Ps* t | SynCom-223 | Betaproteobacteria | 1 | |
| Phyla and Class Drop-out | *Ps* t | SynCom-223 | Gammaproteobacteria | 1 | |
| Phyla and Class Drop-out | *Ps* t | SynCom-223 | Actinobacteria | 1 | |
| Phyla and Class Drop-out | *Ps* t | SynCom-223 | Bacteroidetes | 1 | |
| Phyla and Class Drop-out | *Ps* t | SynCom-223 | Firmicutes | 1 | |
| Phyla and Class Drop-out | Commensals | SynCom-223 | Proteobacteria | 0.6644 | |
| Phyla and Class Drop-out | Commensals | Proteobacteria | Alphaproteobacteria | 0.02745 | * |
| Phyla and Class Drop-out | Commensals | Proteobacteria | Betaproteobacteria | 0.00364 | ** |
| Phyla and Class Drop-out | Commensals | Proteobacteria | Bacteroidetes | 0.0092 | ** |
| Phyla and Class Drop-out | Commensals | Alphaproteobacteria | Gammaproteobacteria | 0.07362 | |
| Phyla and Class Drop-out | Commensals | Betaproteobacteria | Gammaproteobacteria | 0.01079 | * |
| Phyla and Class Drop-out | Commensals | Betaproteobacteria | Actinobacteria | 0.40682 | |
| Phyla and Class Drop-out | Commensals | Betaproteobacteria | Firmicutes | 0.46308 | |
| Phyla and Class Drop-out | Commensals | Bacteroidetes | Gammaproteobacteria | 0.02196 | * |
| Phyla and Class Drop-out | Commensals | Bacteroidetes | Actinobacteria | 0.4247 | |
| Phyla and Class Drop-out | Commensals | Bacteroidetes | Firmicutes | 0.47326 | |
| Genera drop-out | *Ps* t | axenic | Methylobacterium | 0 | **** |
| Genera drop-out | *Ps* t | axenic | Microbacterium | 0 | **** |
| Genera drop-out | *Ps* t | axenic | Pseudomonas | 0 | **** |
| Genera drop-out | *Ps* t | axenic | Rhizobium | 0 | **** |
| Genera drop-out | *Ps* t | axenic | Sphingomonas | 0 | **** |
| Genera drop-out | *Ps* t | SynCom-223 | Methylobacterium | 1 | |
| Genera drop-out | *Ps* t | SynCom-223 | Microbacterium | 1 | |
| Genera drop-out | *Ps* t | SynCom-223 | Pseudomonas | 1 | |
| Genera drop-out | *Ps* t | SynCom-223 | Rhizobium | 1 | |
| Genera drop-out | *Ps* t | SynCom-223 | Sphingomonas | 1 | |
| Genera drop-out | Commensals | SynCom-223 | Pseudomonas | 0.60657 | |
| Genera drop-out | Commensals | SynCom-223 | Rhizobium | 1 | |
| Genera drop-out | Commensals | SynCom-223 | Sphingomonas | 1 | |
| Genera drop-out | Commensals | SynCom-223 | Methylobacterium | 1 | |
| Genera drop-out | Commensals | SynCom-223 | Microbacterium | 1 | |
| functional group drop-out | *Ps* t | axenic | SynCom-48p | 0 | **** |
| functional group drop-out | *Ps* t | axenic | SynCom-175np | 0 | **** |
| functional group drop-out | *Ps* t | SynCom-223 | SynCom-48p | 0.77149 | |
| functional group drop-out | *Ps* t | SynCom-223 | SynCom-175np | 0.02637 | * |
| functional group drop-out | *Ps* t | SynCom-48p | SynCom-175np | 0.00013 | *** |
| functional group drop-out | Commensals | SynCom-223 | SynCom-48p | 0.1113 | |
| functional group drop-out | Commensals | SynCom-223 | SynCom-175np | 0.32403 | |
| functional group drop-out | Commensals | SynCom-48p | SynCom-175np | 0.00104 | ** |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 7:** Bonferroni-corrected p-values of pairwise Welch's t-tests of pathogen luminescence at 3, 6, 12 dpi of phyla drop-out conditions compared to controls (SynCom-223, axenic mock-infected, axenic infected).

| Timepoint | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|
| 3dpi | axenic mock-infected | axenic infected | 0 | **** |
| 3dpi | axenic mock-infected | SynCom-223 | 1 | |
| 3dpi | axenic mock-infected | Proteobacteria | 0.00003 | **** |
| 3dpi | axenic mock-infected | Alphaproteobacteria | 1 | |
| 3dpi | axenic mock-infected | Betaproteobacteria | 1 | |
| 3dpi | axenic mock-infected | Gammaproteobacteria | 1 | |
| 3dpi | axenic mock-infected | Actinobacteria | 1 | |
| 3dpi | axenic mock-infected | Bacteroidetes | 0.61701 | |
| 3dpi | axenic mock-infected | Firmicutes | 1 | |
| 3dpi | axenic infected | SynCom-223 | 0 | **** |
| 3dpi | axenic infected | Proteobacteria | 0 | **** |
| 3dpi | axenic infected | Alphaproteobacteria | 0 | **** |
| 3dpi | axenic infected | Betaproteobacteria | 0 | **** |
| 3dpi | axenic infected | Gammaproteobacteria | 0 | **** |
| 3dpi | axenic infected | Actinobacteria | 0 | **** |
| 3dpi | axenic infected | Bacteroidetes | 0 | **** |
| 3dpi | axenic infected | Firmicutes | 0 | **** |
| 3dpi | SynCom-223 | Proteobacteria | 0.00001 | **** |
| 3dpi | SynCom-223 | Alphaproteobacteria | 1 | |
| 3dpi | SynCom-223 | Betaproteobacteria | 1 | |
| 3dpi | SynCom-223 | Gammaproteobacteria | 1 | |
| 3dpi | SynCom-223 | Actinobacteria | 1 | |
| 3dpi | SynCom-223 | Bacteroidetes | 1 | |
| 3dpi | SynCom-223 | Firmicutes | 1 | |
| 6dpi | axenic mock-infected | axenic infected | 0 | **** |
| 6dpi | axenic mock-infected | SynCom-223 | 1 | |
| 6dpi | axenic mock-infected | Proteobacteria | 0 | **** |
| 6dpi | axenic mock-infected | Alphaproteobacteria | 1 | |
| 6dpi | axenic mock-infected | Betaproteobacteria | 1 | |
| 6dpi | axenic mock-infected | Gammaproteobacteria | 1 | |
| 6dpi | axenic mock-infected | Actinobacteria | 1 | |
| 6dpi | axenic mock-infected | Bacteroidetes | 1 | |
| 6dpi | axenic mock-infected | Firmicutes | 1 | |
| 6dpi | axenic infected | SynCom-223 | 0 | **** |
| 6dpi | axenic infected | Proteobacteria | 0 | **** |
| 6dpi | axenic infected | Alphaproteobacteria | 0 | **** |
| 6dpi | axenic infected | Betaproteobacteria | 0 | **** |
| 6dpi | axenic infected | Gammaproteobacteria | 0 | **** |
| 6dpi | axenic infected | Actinobacteria | 0 | **** |
| 6dpi | axenic infected | Bacteroidetes | 0 | **** |
| 6dpi | axenic infected | Firmicutes | 0 | **** |
| 6dpi | SynCom-223 | Proteobacteria | 0 | **** |
| 6dpi | SynCom-223 | Alphaproteobacteria | 1 | |
| 6dpi | SynCom-223 | Betaproteobacteria | 1 | |
| 6dpi | SynCom-223 | Gammaproteobacteria | 1 | |
| 6dpi | SynCom-223 | Actinobacteria | 1 | |
| 6dpi | SynCom-223 | Bacteroidetes | 1 | |
| 6dpi | SynCom-223 | Firmicutes | 1 | |
| 12dpi | axenic mock-infected | axenic infected | 0 | **** |
| 12dpi | axenic mock-infected | SynCom-223 | 1 | |
| 12dpi | axenic mock-infected | Proteobacteria | 0 | **** |
| 12dpi | axenic mock-infected | Alphaproteobacteria | 1 | |
| 12dpi | axenic mock-infected | Betaproteobacteria | 1 | |
| 12dpi | axenic mock-infected | Gammaproteobacteria | 1 | |

Supplemental Table 7 continued

| | | | | |
|---|---|---|---|---|
| 12dpi | axenic mock-infected | Actinobacteria | 1 | |
| 12dpi | axenic mock-infected | Bacteroidetes | 1 | |
| 12dpi | axenic mock-infected | Firmicutes | 1 | |
| 12dpi | axenic infected | SynCom-223 | 0 | **** |
| 12dpi | axenic infected | Proteobacteria | 0 | **** |
| 12dpi | axenic infected | Alphaproteobacteria | 0 | **** |
| 12dpi | axenic infected | Betaproteobacteria | 0 | **** |
| 12dpi | axenic infected | Gammaproteobacteria | 0 | **** |
| 12dpi | axenic infected | Actinobacteria | 0 | **** |
| 12dpi | axenic infected | Bacteroidetes | 0 | **** |
| 12dpi | axenic infected | Firmicutes | 0 | **** |
| 12dpi | SynCom-223 | Proteobacteria | 0 | **** |
| 12dpi | SynCom-223 | Alphaproteobacteria | 1 | |
| 12dpi | SynCom-223 | Betaproteobacteria | 1 | |
| 12dpi | SynCom-223 | Gammaproteobacteria | 1 | |
| 12dpi | SynCom-223 | Actinobacteria | 1 | |
| 12dpi | SynCom-223 | Bacteroidetes | 1 | |
| 12dpi | SynCom-223 | Firmicutes | 1 | |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 8:** Bonferroni-corrected p-values of pairwise Welch's t-tests of pathogen luminescence at 3, 6, 12 dpi of genera drop-out conditions compared to controls (SynCom-223, axenic mock-infected, axenic infected).

| Timepoint | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|
| 3dpi | axenic mock-infected | axenic infected | 0 | **** |
| 3dpi | axenic mock-infected | SynCom-223 | 1 | |
| 3dpi | axenic mock-infected | Methylobacterium | 1 | |
| 3dpi | axenic mock-infected | Microbacterium | 0.10252 | |
| 3dpi | axenic mock-infected | Pseudomonas | 1 | |
| 3dpi | axenic mock-infected | Rhizobium | 1 | |
| 3dpi | axenic mock-infected | Sphingomonas | 1 | |
| 3dpi | axenic infected | SynCom-223 | 0 | **** |
| 3dpi | axenic infected | Methylobacterium | 0 | **** |
| 3dpi | axenic infected | Microbacterium | 0 | **** |
| 3dpi | axenic infected | Pseudomonas | 0 | **** |
| 3dpi | axenic infected | Rhizobium | 0 | **** |
| 3dpi | axenic infected | Sphingomonas | 0 | **** |
| 3dpi | SynCom-223 | Methylobacterium | 1 | |
| 3dpi | SynCom-223 | Microbacterium | 1 | |
| 3dpi | SynCom-223 | Pseudomonas | 1 | |
| 3dpi | SynCom-223 | Rhizobium | 1 | |
| 3dpi | SynCom-223 | Sphingomonas | 1 | |
| 6dpi | axenic mock-infected | axenic infected | 0 | **** |
| 6dpi | axenic mock-infected | SynCom-223 | 0.59732 | |
| 6dpi | axenic mock-infected | Methylobacterium | 1 | |
| 6dpi | axenic mock-infected | Microbacterium | 0.02956 | * |
| 6dpi | axenic mock-infected | Pseudomonas | 0.05201 | |
| 6dpi | axenic mock-infected | Rhizobium | 0.48173 | |
| 6dpi | axenic mock-infected | Sphingomonas | 1 | |
| 6dpi | axenic infected | SynCom-223 | 0 | **** |
| 6dpi | axenic infected | Methylobacterium | 0 | **** |
| 6dpi | axenic infected | Microbacterium | 0 | **** |
| 6dpi | axenic infected | Pseudomonas | 0 | **** |
| 6dpi | axenic infected | Rhizobium | 0 | **** |
| 6dpi | axenic infected | Sphingomonas | 0 | **** |
| 6dpi | SynCom-223 | Methylobacterium | 1 | |
| 6dpi | SynCom-223 | Microbacterium | 1 | |
| 6dpi | SynCom-223 | Pseudomonas | 1 | |
| 6dpi | SynCom-223 | Rhizobium | 1 | |
| 6dpi | SynCom-223 | Sphingomonas | 1 | |
| 12dpi | axenic mock-infected | axenic infected | 1 | |
| 12dpi | axenic mock-infected | SynCom-223 | 0.29051 | |
| 12dpi | axenic mock-infected | Methylobacterium | 0.04055 | * |
| 12dpi | axenic mock-infected | Microbacterium | 0.00053 | *** |
| 12dpi | axenic mock-infected | Pseudomonas | 0.06087 | |
| 12dpi | axenic mock-infected | Rhizobium | 1 | |
| 12dpi | axenic mock-infected | Sphingomonas | 0.49452 | |
| 12dpi | axenic infected | SynCom-223 | 0 | **** |
| 12dpi | axenic infected | Methylobacterium | 0 | **** |
| 12dpi | axenic infected | Microbacterium | 0 | **** |
| 12dpi | axenic infected | Pseudomonas | 0 | **** |
| 12dpi | axenic infected | Rhizobium | 0 | **** |
| 12dpi | axenic infected | Sphingomonas | 0 | **** |
| 12dpi | SynCom-223 | Methylobacterium | 1 | |
| 12dpi | SynCom-223 | Microbacterium | 1 | |
| 12dpi | SynCom-223 | Pseudomonas | 1 | |
| 12dpi | SynCom-223 | Rhizobium | 1 | |
| 12dpi | SynCom-223 | Sphingomonas | 1 | |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 9:** Bonferroni-corrected p-values of pairwise Welch's t-tests of pathogen luminescence at 3, 6, 12 dpi of protection-associated strains drop-out conditions compared to controls (SynCom-223, axenic mock-infected, axenic infected). Control conditions are duplicated from Supplemental Table 8.

| Timepoint | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|
| 3dpi | axenic mock-infected | axenic infected | 0 | **** |
| 3dpi | axenic mock-infected | SynCom-223 | 1 | |
| 3dpi | axenic mock-infected | SynCom-48p | 1 | |
| 3dpi | axenic mock-infected | SynCom-175np | 0.30044 | |
| 3dpi | axenic infected | SynCom-223 | 0 | **** |
| 3dpi | axenic infected | SynCom-48p | 0 | **** |
| 3dpi | axenic infected | SynCom-175np | 0 | **** |
| 3dpi | SynCom-223 | SynCom-48p | 1 | |
| 3dpi | SynCom-223 | SynCom-175np | 1 | |
| 3dpi | SynCom-48p | SynCom-175np | 1 | |
| 6dpi | axenic mock-infected | axenic infected | 0 | **** |
| 6dpi | axenic mock-infected | SynCom-223 | 0.78114 | |
| 6dpi | axenic mock-infected | SynCom-48p | 0.69599 | |
| 6dpi | axenic mock-infected | SynCom-175np | 0.00683 | ** |
| 6dpi | axenic infected | SynCom-223 | 0 | **** |
| 6dpi | axenic infected | SynCom-48p | 0 | **** |
| 6dpi | axenic infected | SynCom-175np | 0 | **** |
| 6dpi | SynCom-223 | SynCom-48p | 1 | |
| 6dpi | SynCom-223 | SynCom-175np | 1 | |
| 6dpi | SynCom-48p | SynCom-175np | 1 | |
| 12dpi | axenic mock-infected | axenic infected | 0 | **** |
| 12dpi | axenic mock-infected | SynCom-223 | 0.15929 | |
| 12dpi | axenic mock-infected | SynCom-48p | 1 | |
| 12dpi | axenic mock-infected | SynCom-175np | 0.00006 | **** |
| 12dpi | axenic infected | SynCom-223 | 0 | **** |
| 12dpi | axenic infected | SynCom-48p | 0 | **** |
| 12dpi | axenic infected | SynCom-175np | 0 | **** |
| 12dpi | SynCom-223 | SynCom-48p | 1 | |
| 12dpi | SynCom-223 | SynCom-175np | 0.53017 | |
| 12dpi | SynCom-48p | SynCom-175np | 0.01205 | * |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 10:** Bonferroni-corrected p-values of Welch's t-test comparing pathogen colonization at of low complex communities with exchanged strains.

| Infection Time | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|
| 14 dpi | axenic | SynCom-15 | 0.03381 | * |
| 14 dpi | axenic | SynCom-15±2 | 0 | **** |
| 14 dpi | axenic | SynCom-15±5 | 0 | **** |
| 14 dpi | SynCom-210 | SynCom-15 | 0.04444 | * |
| 14 dpi | SynCom-210 | SynCom-15±2 | 1 | |
| 14 dpi | SynCom-210 | SynCom-15±5 | 1 | |
| 14 dpi | SynCom-15 | SynCom-15±2 | 0.00018 | *** |
| 14 dpi | SynCom-15 | SynCom-15±5 | 0.01048 | * |
| 14 dpi | SynCom-15±2 | SynCom-15±5 | 1 | |
| 7 dpi | axenic | SynCom-16All | 0.28506 | |
| 7 dpi | axenic | SynCom-8np | 1 | |
| 7 dpi | axenic | SynCom-8p | 0.68992 | |
| 7 dpi | SynCom-210 | SynCom-16All | 1 | |
| 7 dpi | SynCom-210 | SynCom-8np | 1 | |
| 7 dpi | SynCom-210 | SynCom-8p | 1 | |
| 7 dpi | SynCom-16All | SynCom-8np | 1 | |
| 7 dpi | SynCom-16All | SynCom-8p | 1 | |
| 7 dpi | SynCom-8np | SynCom-8p | 1 | |
| 14 dpi | axenic | SynCom-16All | 0.00786 | ** |
| 14 dpi | axenic | SynCom-8np | 1 | |
| 14 dpi | axenic | SynCom-8p | 0.00002 | **** |
| 14 dpi | SynCom-210 | SynCom-16All | 1 | |
| 14 dpi | SynCom-210 | SynCom-8np | 0.41664 | |
| 14 dpi | SynCom-210 | SynCom-8p | 1 | |
| 14 dpi | SynCom-16All | SynCom-8np | 1 | |
| 14 dpi | SynCom-16All | SynCom-8p | 1 | |
| 14 dpi | SynCom-8np | SynCom-8p | 0.01332 | * |

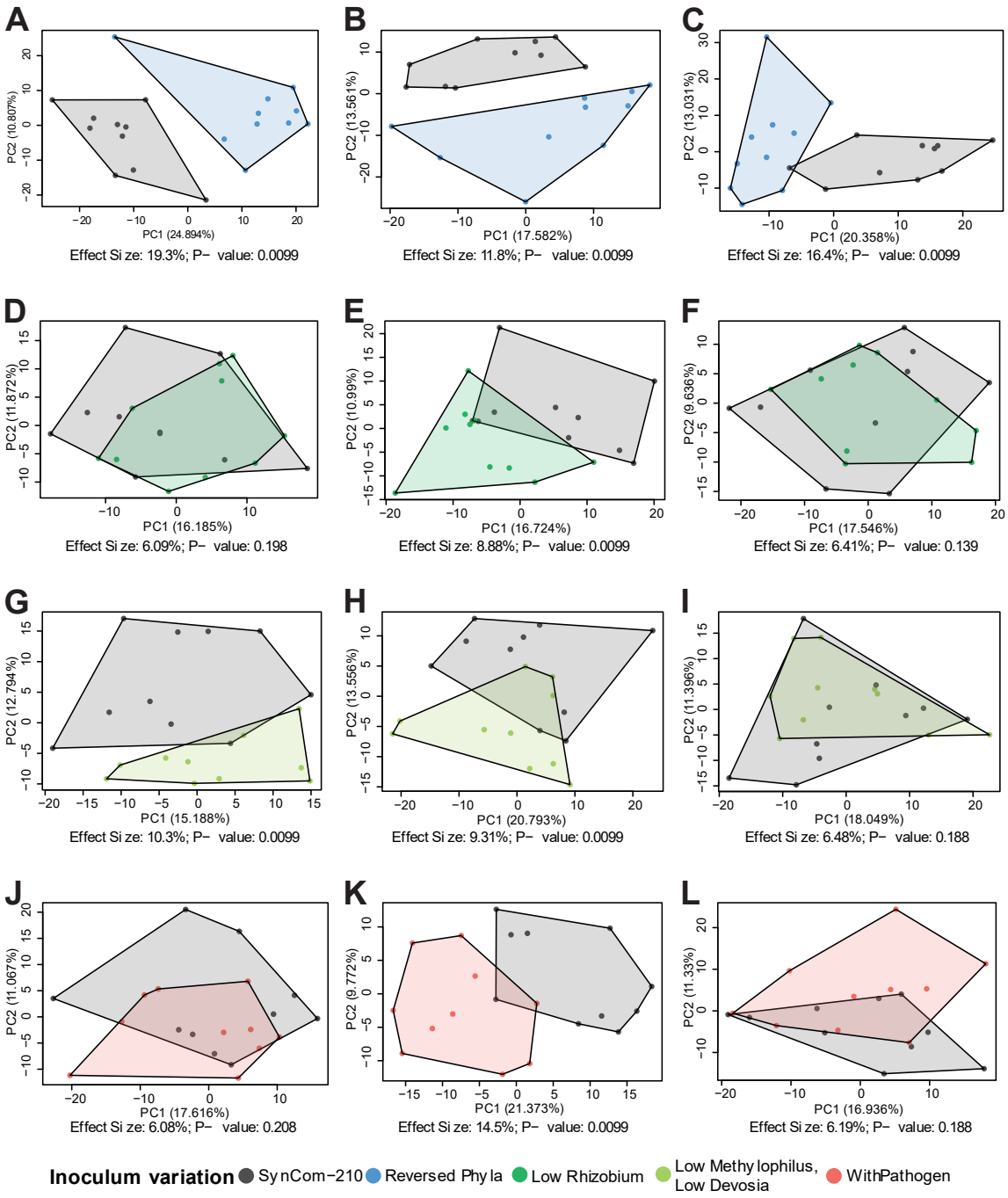
[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 11:** Bonferroni-corrected p-values of pairwise Welch's t-tests of Shannon's diversity, Pielou's evenness and species richness of protection-associated strains competition communities.

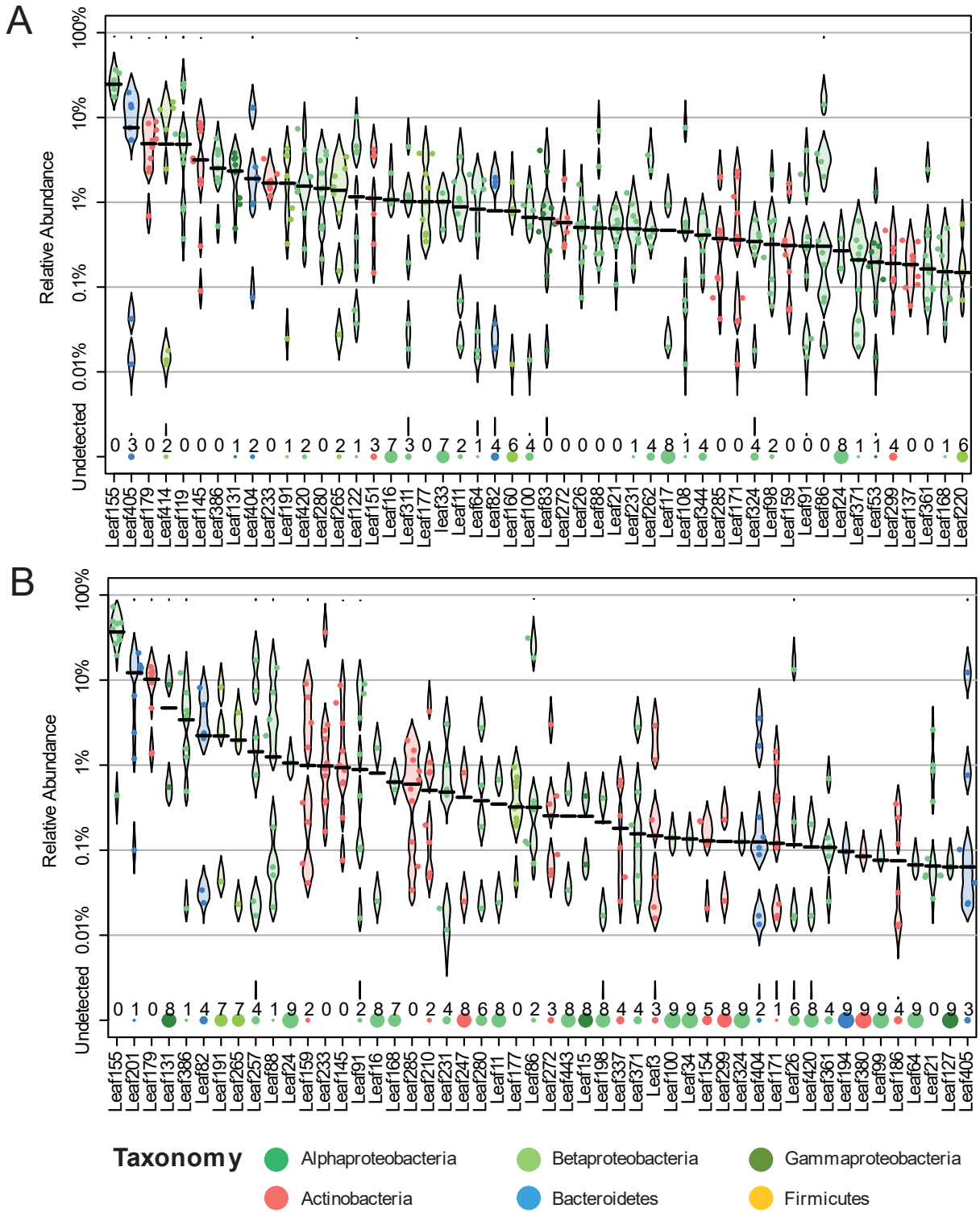
| Score | Treatment | Group 1 | Group 2 | p-value | significance [1] |
|---|---|---|---|---|---|
| Diversity | mock-infected | SynCom-210 | SynCom-16All | 0 | **** |
| Diversity | mock-infected | SynCom-210 | SynCom-8np | 0 | **** |
| Diversity | mock-infected | SynCom-210 | SynCom-8p | 0 | **** |
| Diversity | mock-infected | SynCom-16All | SynCom-8np | 0.04493 | * |
| Diversity | mock-infected | SynCom-16All | SynCom-8p | 1 | |
| Diversity | mock-infected | SynCom-8np | SynCom-8p | 1 | |
| Diversity | SynCom-16All | mock-infected | 7 dpi | 1 | |
| Diversity | SynCom-16All | mock-infected | 14 dpi | 1 | |
| Diversity | SynCom-8np | mock-infected | 7 dpi | 1 | |
| Diversity | SynCom-8np | mock-infected | 14 dpi | 1 | |
| Diversity | SynCom-8p | mock-infected | 7 dpi | 0.91848 | |
| Diversity | SynCom-8p | mock-infected | 14 dpi | 1 | |
| Evenness | mock-infected | SynCom-210 | SynCom-16All | 1 | |
| Evenness | mock-infected | SynCom-210 | SynCom-8np | 1 | |
| Evenness | mock-infected | SynCom-210 | SynCom-8p | 1 | |
| Evenness | mock-infected | SynCom-16All | SynCom-8np | 1 | |
| Evenness | mock-infected | SynCom-16All | SynCom-8p | 1 | |
| Evenness | mock-infected | SynCom-8np | SynCom-8p | 1 | |
| Evenness | SynCom-16All | mock-infected | 7 dpi | 0.01086 | * |
| Evenness | SynCom-16All | mock-infected | 14 dpi | 1 | |
| Evenness | SynCom-8np | mock-infected | 7 dpi | 1 | |
| Evenness | SynCom-8np | mock-infected | 14 dpi | 1 | |
| Evenness | SynCom-8p | mock-infected | 7 dpi | 1 | |
| Evenness | SynCom-8p | mock-infected | 14 dpi | 1 | |
| Richness | mock-infected | SynCom-210 | SynCom-16All | 0 | **** |
| Richness | mock-infected | SynCom-210 | SynCom-8np | 0 | **** |
| Richness | mock-infected | SynCom-210 | SynCom-8p | 0 | **** |
| Richness | mock-infected | SynCom-16All | SynCom-8np | 1 | |
| Richness | mock-infected | SynCom-16All | SynCom-8p | 1 | |
| Richness | mock-infected | SynCom-8np | SynCom-8p | 1 | |
| Richness | SynCom-16All | mock-infected | 7 dpi | 1 | |
| Richness | SynCom-16All | mock-infected | 14 dpi | 0.03871 | * |
| Richness | SynCom-8np | mock-infected | 7 dpi | 1 | |
| Richness | SynCom-8np | mock-infected | 14 dpi | 1 | |
| Richness | SynCom-8p | mock-infected | 7 dpi | 0.56151 | |
| Richness | SynCom-8p | mock-infected | 14 dpi | 1 | |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

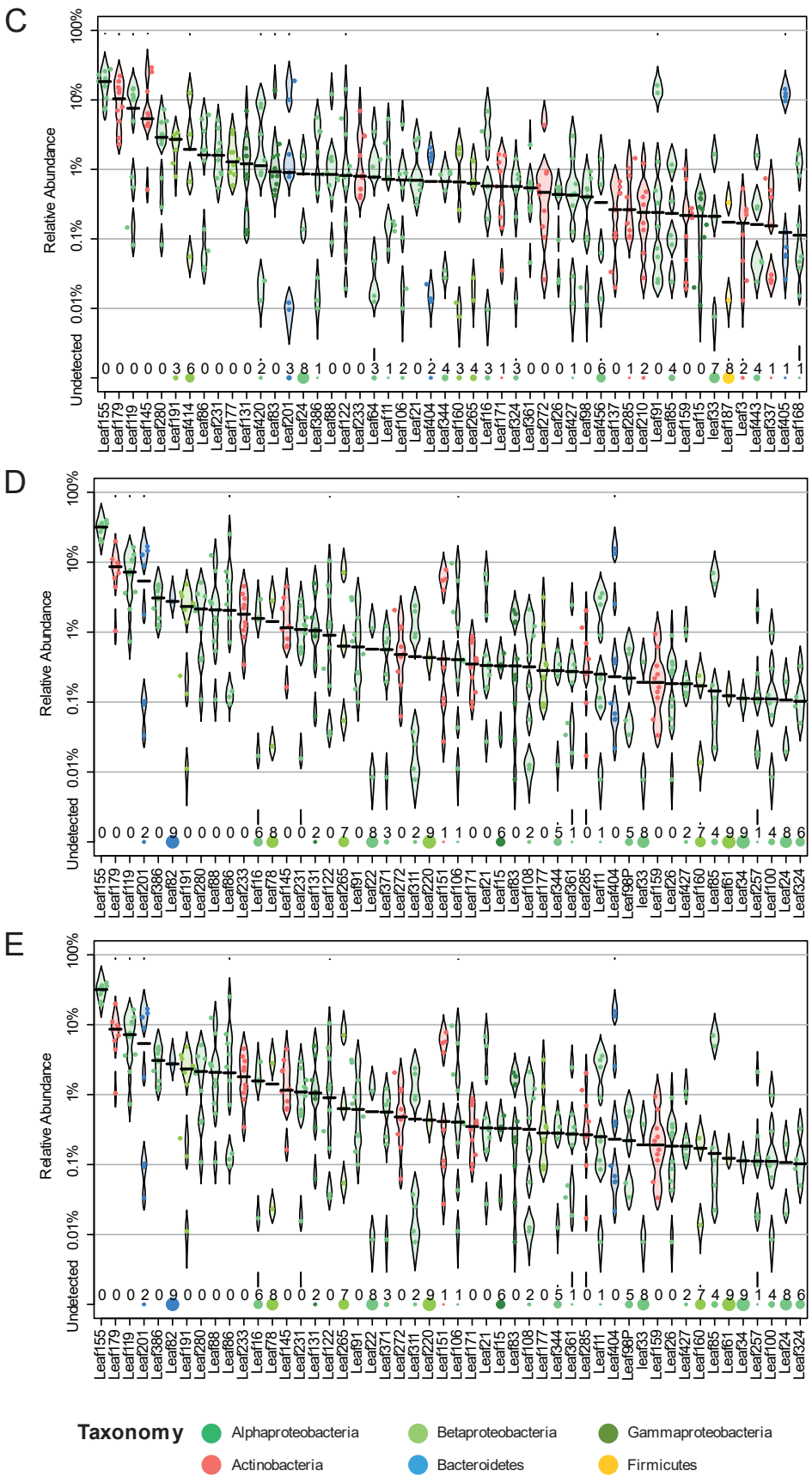
# Supplemental Figures



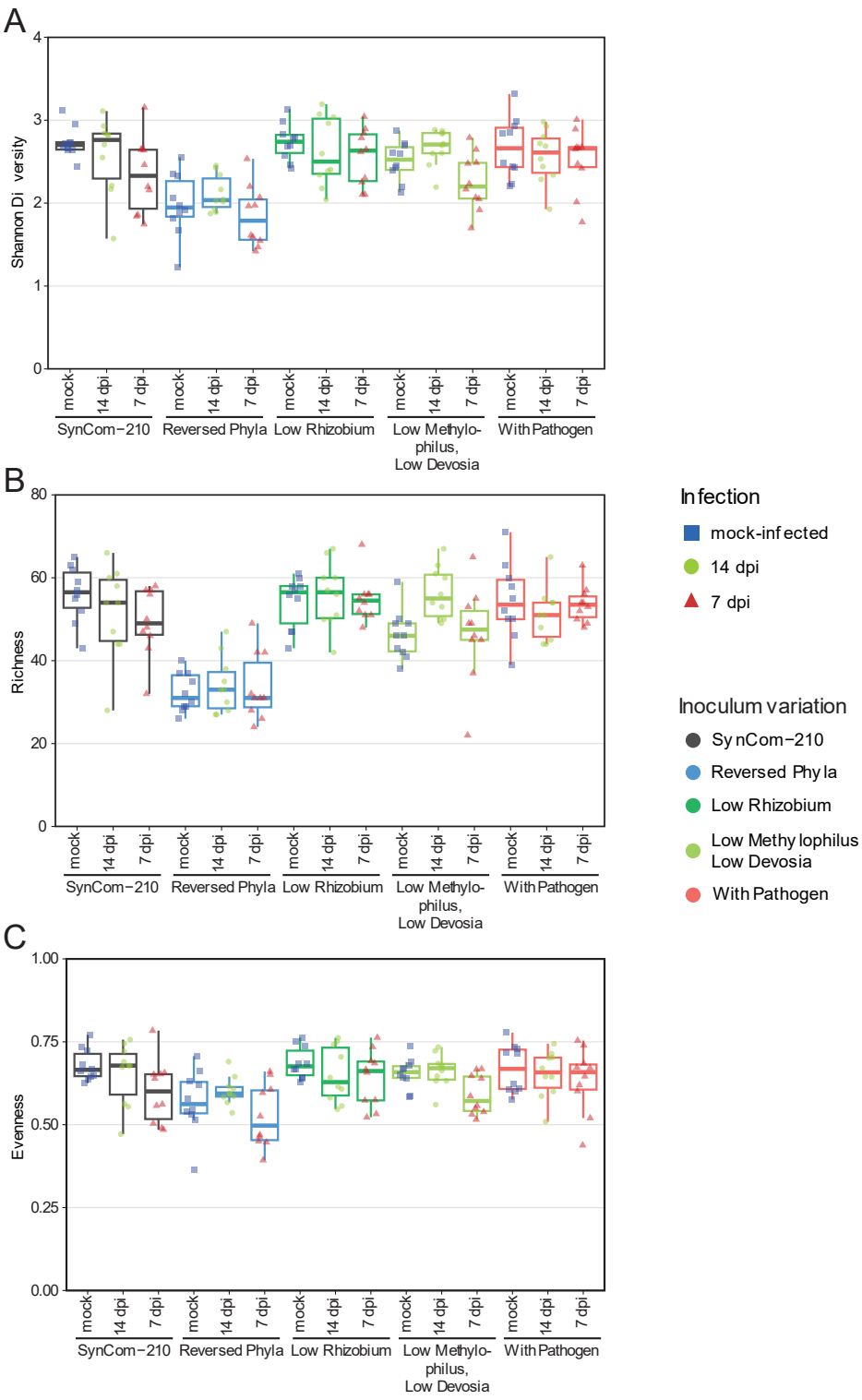

**Supplemental Figure 1: Impact of SynCom-210 inocula variations on community composition in different infection treatments.** PCA and PERMANOVA analysis of community composition of the default treatment (black) versus inocula variations (in colours) (n=10). Mock-infected conditions are compared in the left column (A,D,G,J), infected samples at 7 days post infection are compared in the middle column (B,E,H,K), and in the right column infected samples at 14 days post infection are compared (C,F,I,L). A,B,C. Comparison of default versus "reversed phyla" (blue) treatment. D,E,F. Comparison of default versus "Low *Rhizobium*" (green) treatment. G,H,I. Comparison of default versus "Low *Methylophilus*, Low *Devosia*" (light green) treatment. J,K,L. Comparison of default versus "with pathogen" (red) treatment.



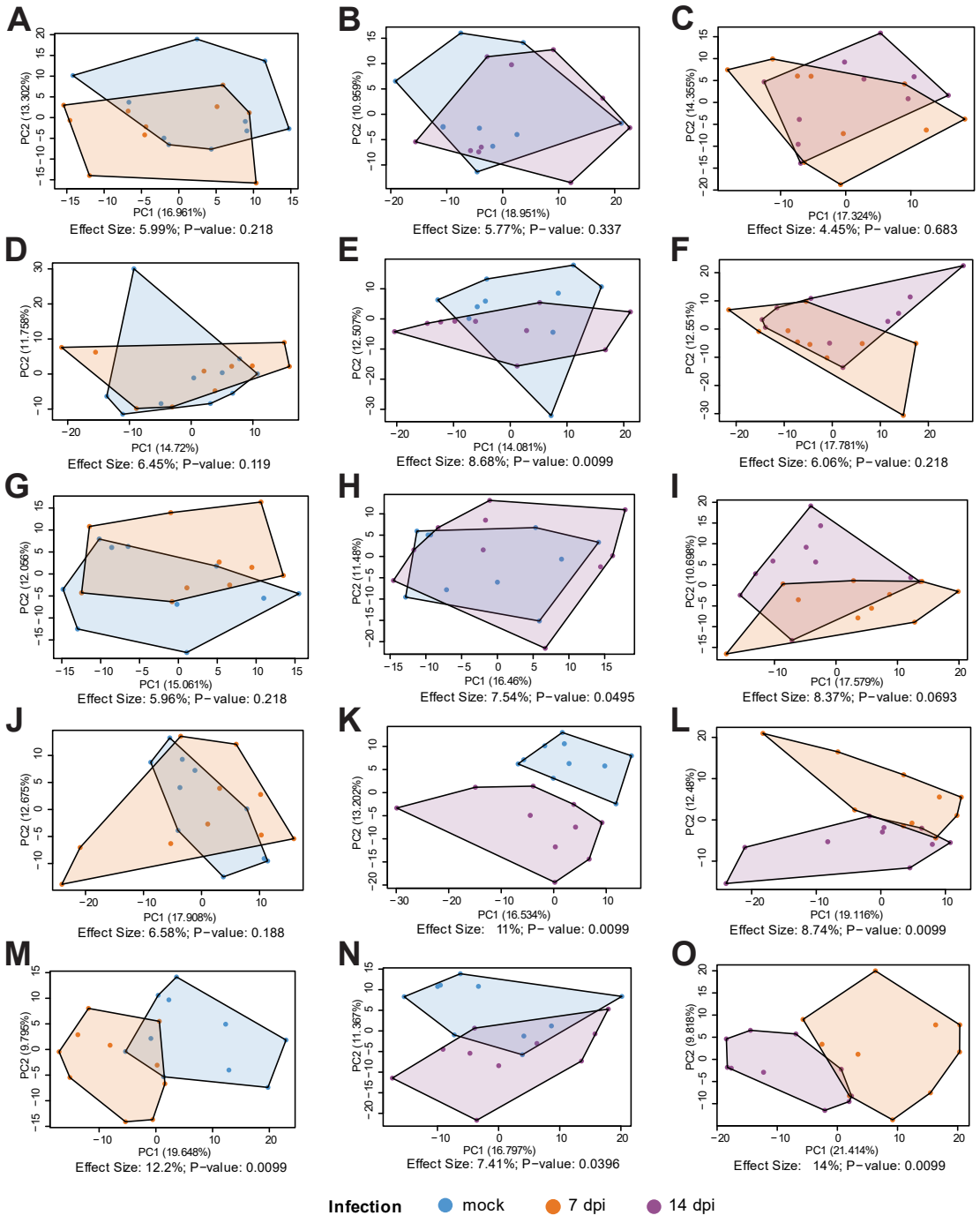

**Supplemental Figure 2: Community composition of SynCom-210 inocula variations.** Relative abundances of strains in community composition of mock-infected strains (n=10). Colours represent taxonomy of the strains. A. Community composition of the default SynCom-210 treatment. B. Community composition of the "reversed phyla" treatment. Continued on next page: C. Community composition of the "Low *Rhizobium*" treatment. D. Community composition of the "Low *Methylophilus*, Low *Devosia*" treatment. E. Community composition of the "with pathogen" treatment.



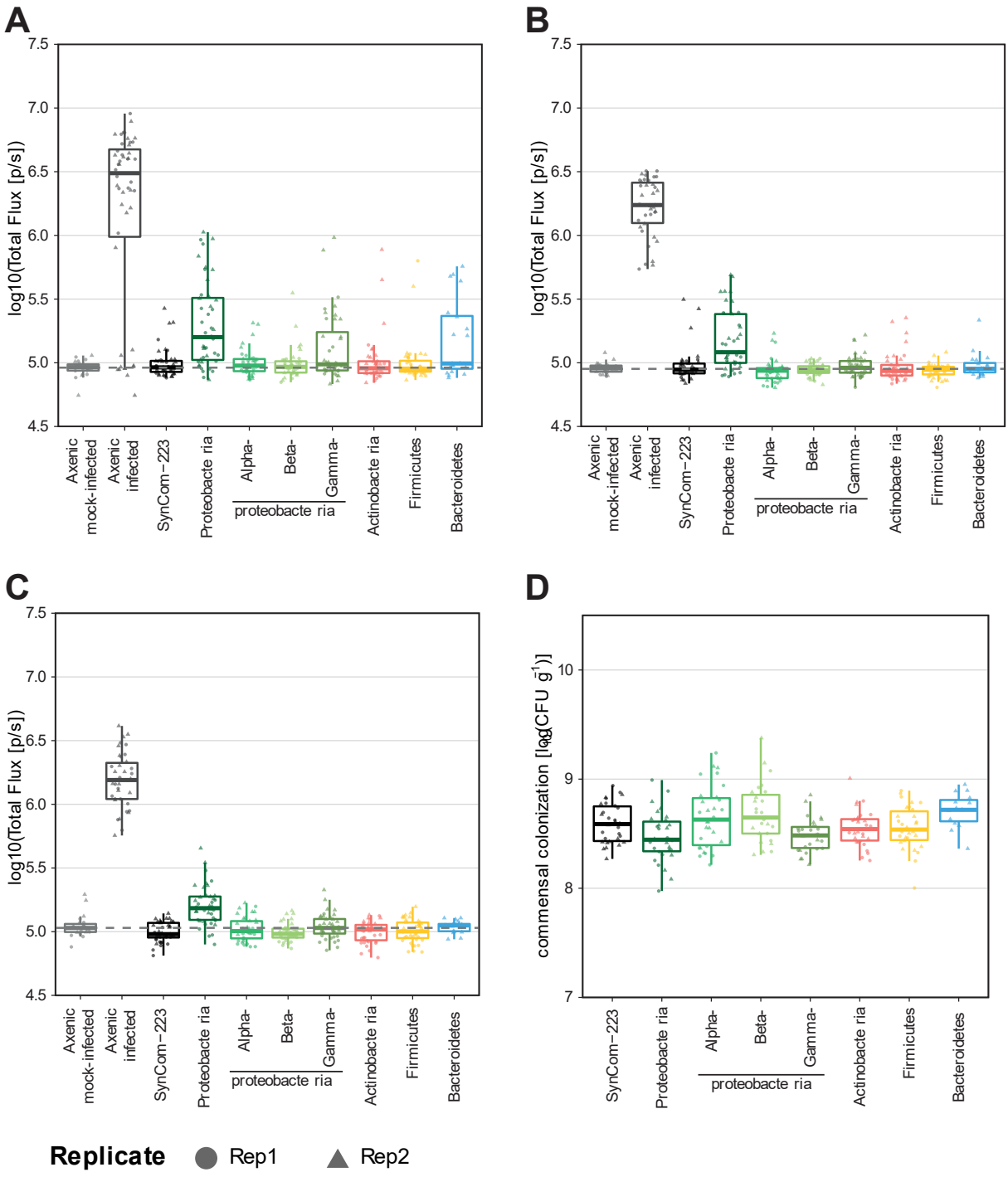

**Supplemental Figure 2: continued.**



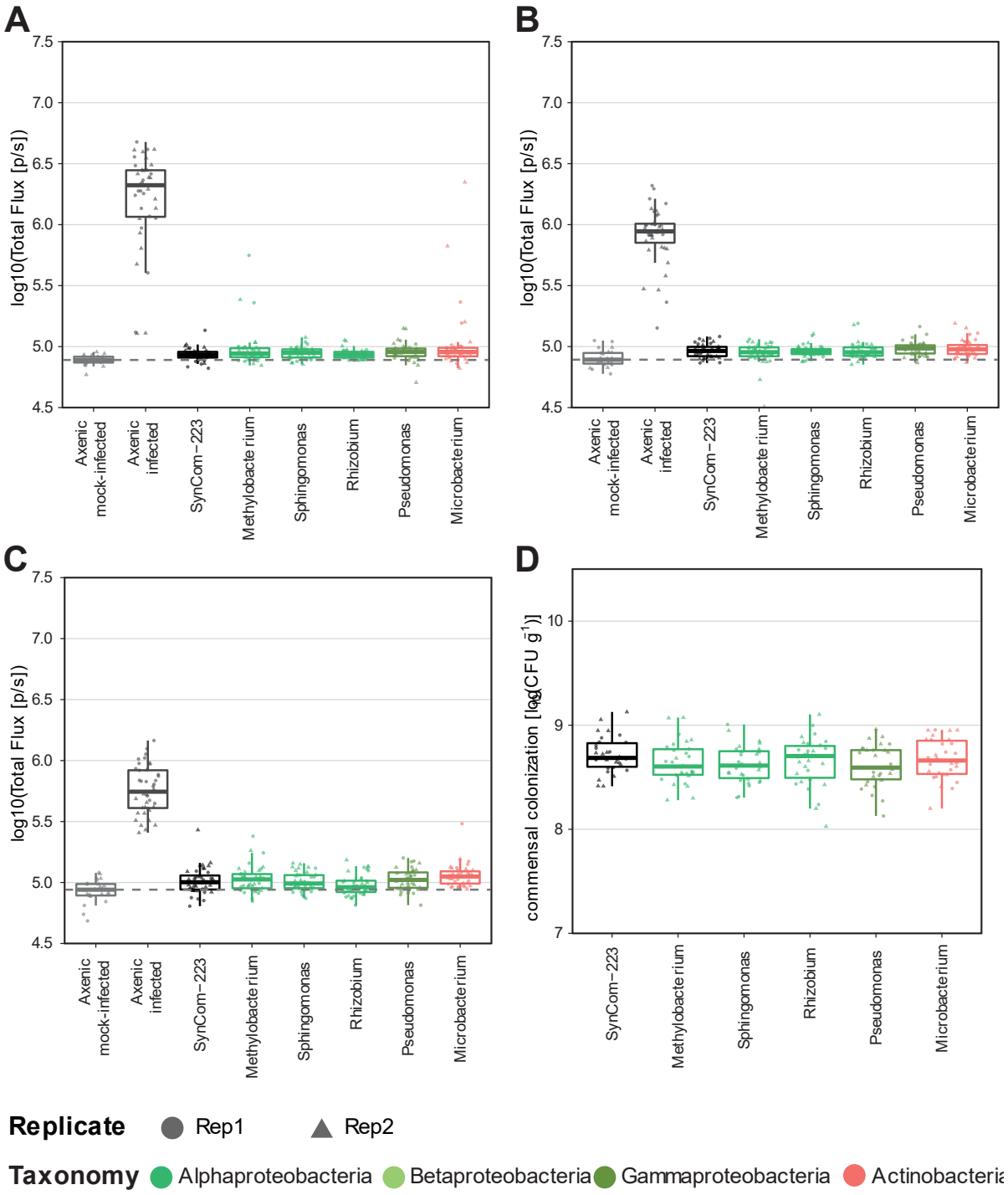

**Supplemental Figure 3: Diversity scores in SynCom-210 inocula variations.** Shannon's diversity, species richness and Pielou's evenness were calculated from rarefied data (see methods) (n=10). Boxplot colours refer to inoculum variation. Infection is indicated by both symbol colour and shape. Mock-infected samples are shown in blue squares, samples at 7 days post infection are shown in red triangles, and samples at 14 days post infection are shown in green circles. A. Shannon's diversity. B. Species richness. C. Pielou's evenness.



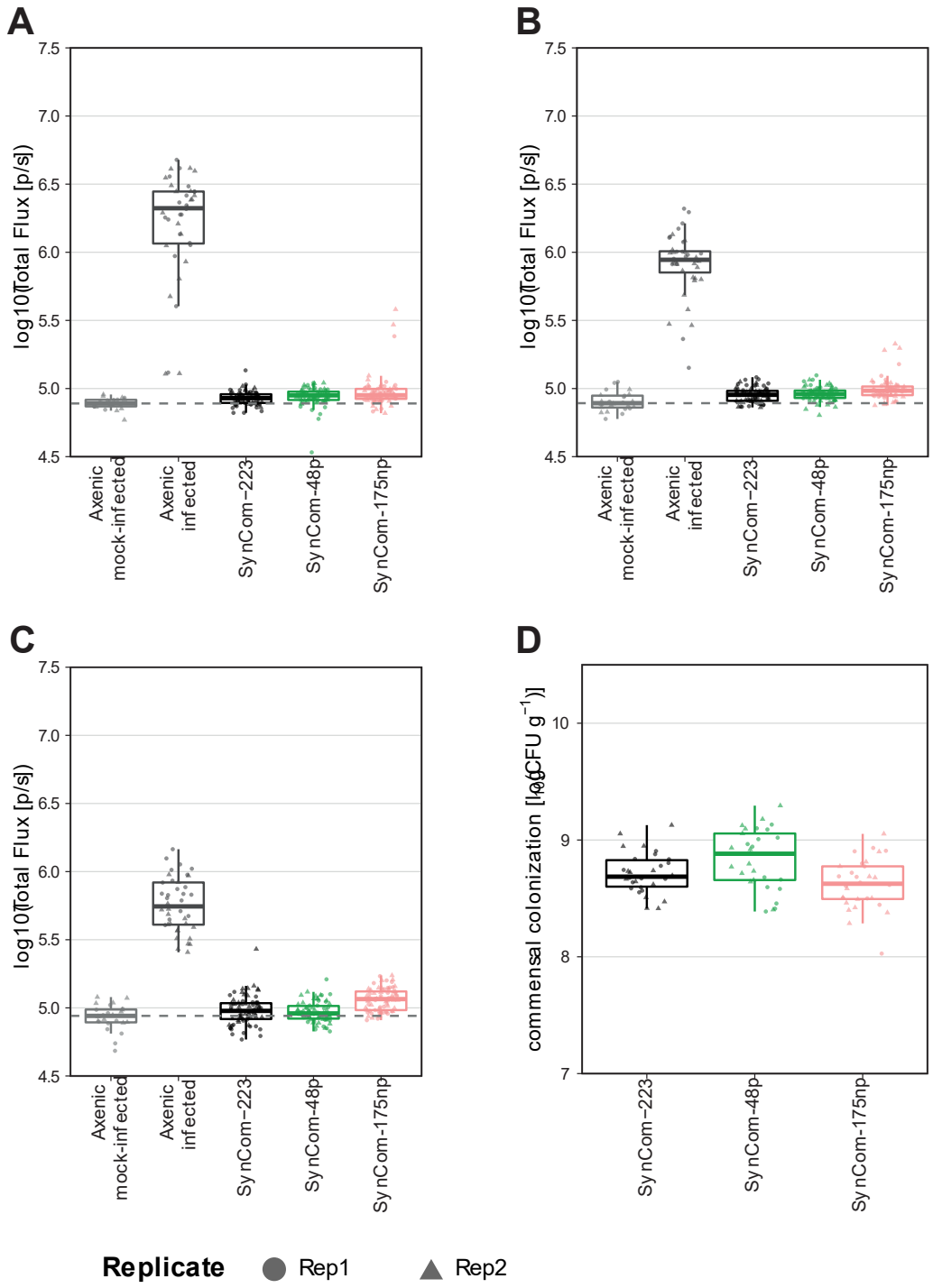

**Supplemental Figure 4: Impact of infection on community compositions of SynCom-210 inocula variations.** PCA and PERMANOVA analysis of community composition of the different infection states within each inoculum variations (n=10). In the left column mock-infected samples (blue) are compared to infected samples at 7 days post infection (orange) (A,D,G,J). In the middle column, mock-infected samples (blue) are compared to those at 14 days post infection (purple) (B,E,H,K). In the right column infected samples at 7 days post infection (orange) are compared to those at 14 days post infection (purple) (C,F,I,L). A,B,C. Comparison of infection states within the default SynCom-210 treatments. D,E,F. Comparison of infection states within the "reversed phyla" treatment. G,H,I. Comparison of infection states within the "Low *Rhizobium*" treatment. J,K,L. Comparison of infection states within "Low *Methylophilus*, Low *Devosia*" treatment. M,N,O. Comparison of infection states within "with pathogen" treatment.



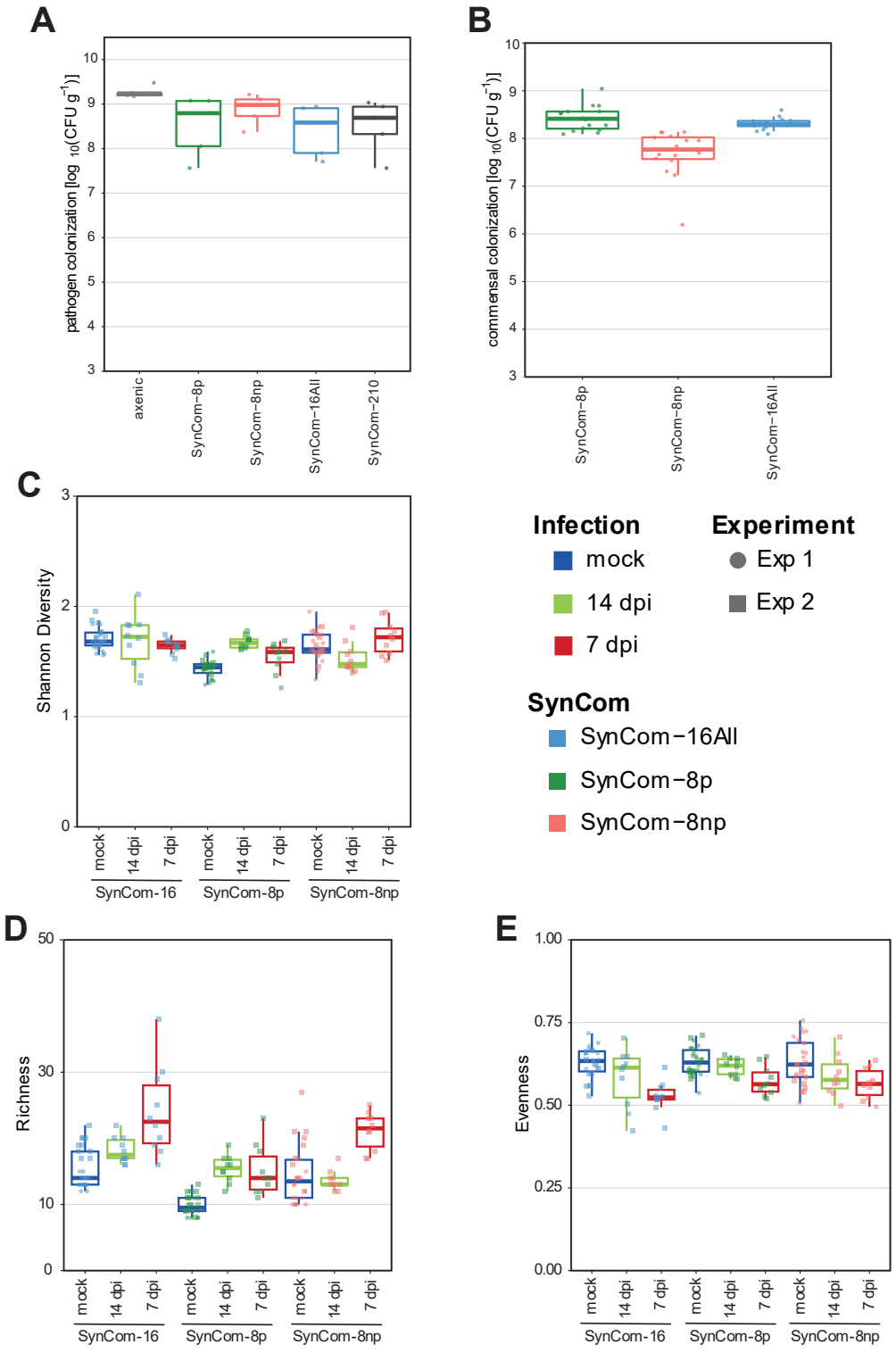

**Supplemental Figure 5: Impact of phyla and classes drop-out on pathogen luminescence and total commensal colonization.** Measured pathogen luminescence of the phyla and classes drop-out conditions and control conditions (axenic mock-infected, axenic infected, SynCom-223) (n=40, Bacteroidetes=20). Axenic mock-infected samples (n=24) are used as a measure of the background luminescence, the median is depicted as a grey dashed line. The two replicate experiments are shown with different symbol shapes. A. Pathogen luminescence at 3 days post infection. B. Pathogen luminescence at 6 days post infection. C. Pathogen luminescence at 12 days post infection. D. Total bacterial colonization of different phyla and class drop-outs compared to SynCom-223 (n=30). The two replicate experiments are shown with different symbol shapes.



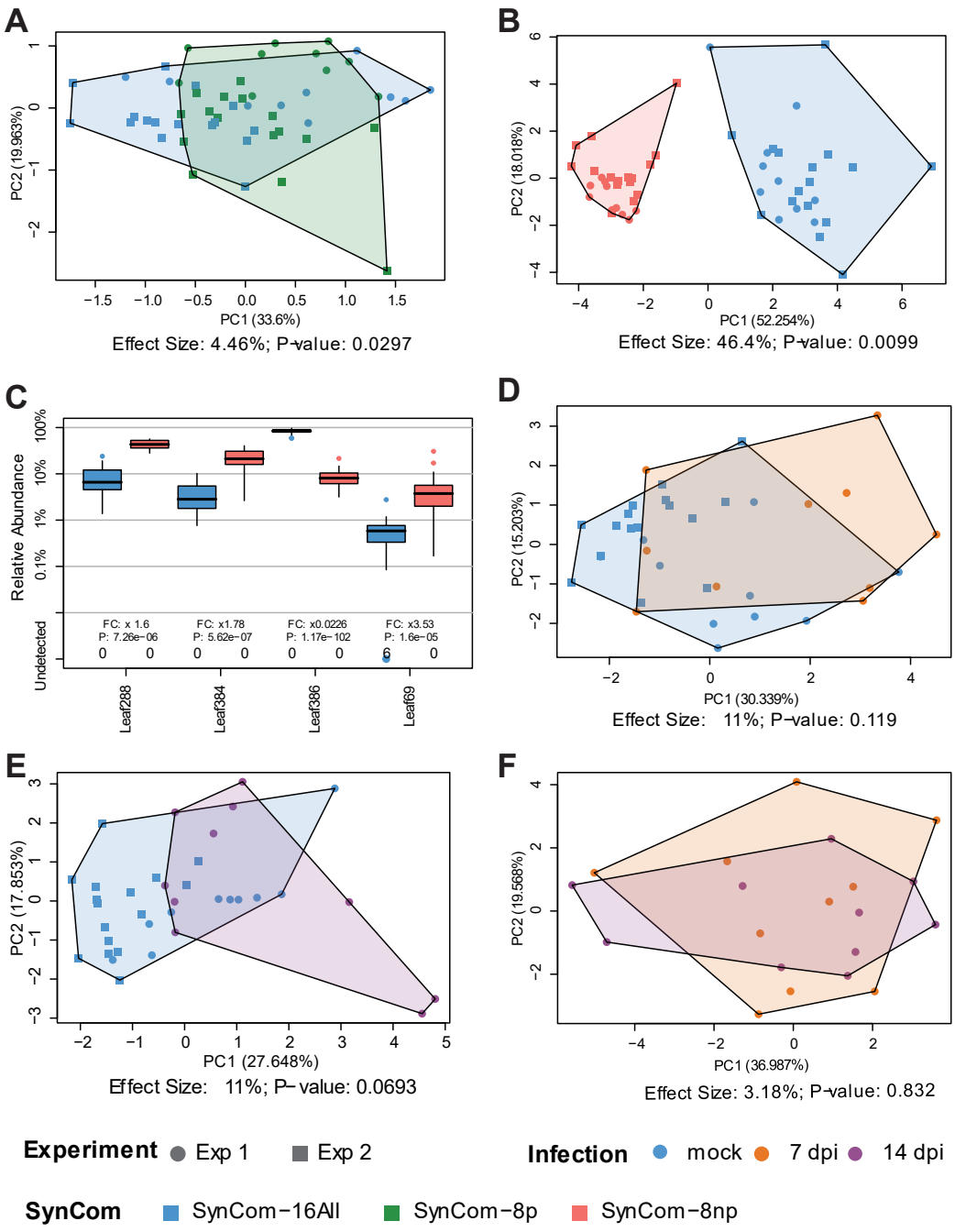

**Supplemental Figure 6: Impact of genera drop-out on pathogen luminescence and total commensal colonization.** Measured pathogen luminescence of the genera drop-out conditions and control conditions (axenic mock-infected, axenic infected, SynCom-223) (n=40). Axenic mock-infected samples (n=24) are used as a measure of the background luminescence, the median is depicted as a grey dashed line. The two replicate experiments are shown with different symbol shapes. Boxplot colours are based on control condition or taxonomy of drop-out. A. Pathogen luminescence at 3 days post infection. B. Pathogen luminescence at 6 days post infection. C. Pathogen luminescence at 12 days post infection. D. Total bacterial colonization of different genera drop-outs compared to SynCom-223 (n=30). The two replicate experiments are shown with different symbol shapes. Boxplot colours are based on control condition or taxonomy of drop-out.



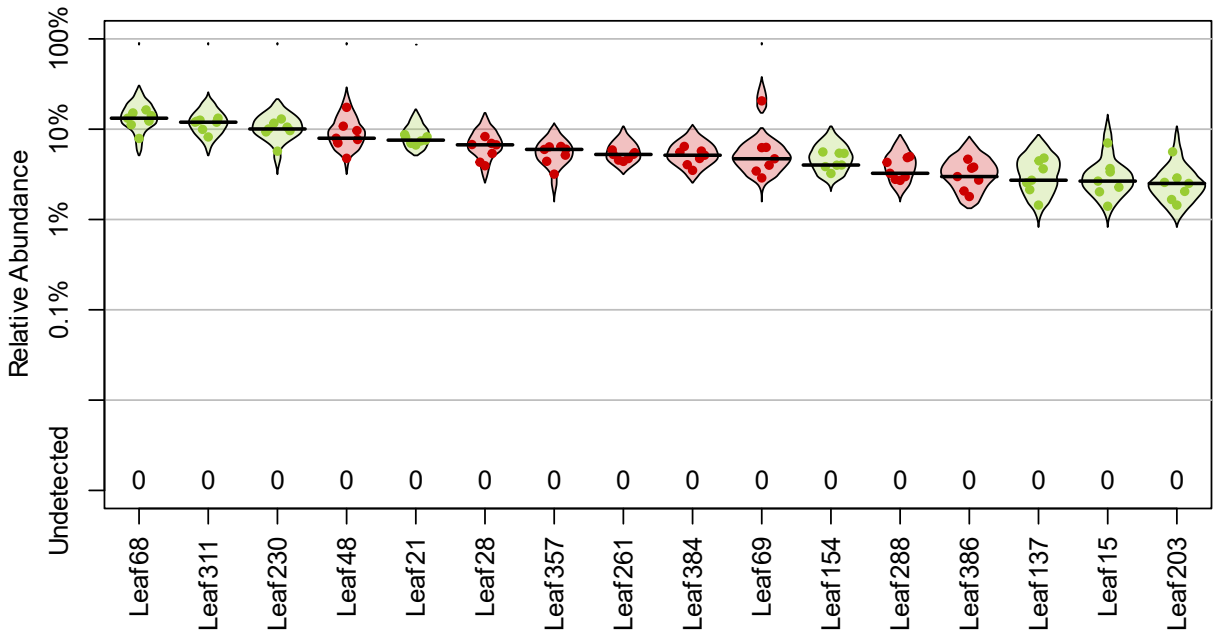

**Supplemental Figure 7: Impact of grouping strains based on protectiveness on pathogen luminescence and total commensal colonization.** Measured pathogen luminescence of the two communities containing either all protective strains (SynCom-48p, green) or all non-protective strains (SynCom-175np, red) and control conditions (axenic mock-infected, axenic infected, SynCom-223) (n=40). Axenic mock-infected samples (n=24) are used as a measure of the background luminescence, the median is depicted as a grey dashed line. The two replicate experiments are shown with different symbol shapes. A. Pathogen luminescence at 3 days post infection. B. Pathogen luminescence at 6 days post infection. C. Pathogen luminescence at 12 days post infection. D. Total bacterial colonization of different genera drop-outs compared to SynCom-223 (n=30). The two replicate experiments are shown with different symbol shapes. Data for axenic and SynCom-223 conditions are repeated from Figure 6.



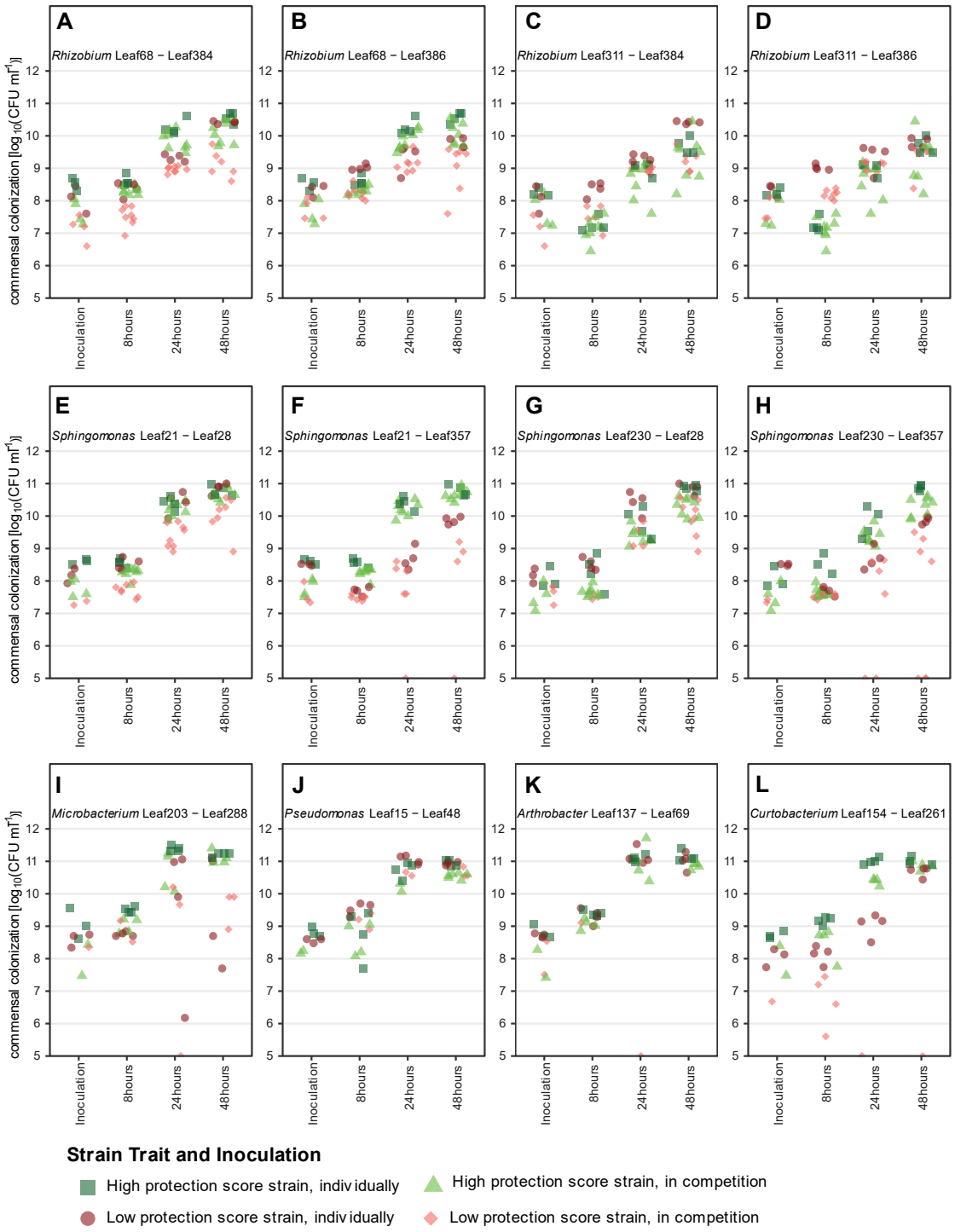

**Supplemental Figure 8: Impact of protection-associated strains on bacterial colonization and community diversity scores.** A. Pathogen colonization at 7 days post infection (n=5). Data of axenic and SynCom-210 are duplicated from Figure 2B. B. Total commensal colonization of the three different communities (n=16). The diversity scores were calculated on rarefied data (see methods). Boxplot colours represent infection state, while symbol colour represents synthetic community analysed (mock-infected n=26, 7 and 14 dpi n=10). C. Shannon's diversity. D. Species richness. E. Pielou's evenness.




**Supplemental Figure 9: Effect of protection-associated strains on community composition.** A. PCA and PERMANOVA analysis comparing strains with high protection scores between SynCom-16All (blue) versus SynCom-8p (green) in mock-infected samples (n=26). Shapes correspond to the two experiments, and batch effect was accounted for in analysis. B. PCA and PERMANOVA analysis comparing strains with high protection scores between SynCom-16All (blue) versus SynCom-8np (red) in mock-infected samples (n=26). Shapes correspond to the two experiments, and batch effect was accounted for in analysis. C. Strain abundance changes comparing SynCom-16All (blue) versus SynCom-8np (red) in mock-infected samples (n=26). D. PCA and PERMANOVA analysis comparing mock-infected samples (blue, n=26) versus samples at 7 days post infection (orange, n=10) in SynCom-16All. E. PCA and PERMANOVA analysis comparing mock-infected samples (blue, n=26) versus samples at 14 days post infection (purple, n=10) in SynCom-16All. F. PCA and PERMANOVA analysis comparing samples at 7 days post infection (orange, n=10) versus samples at 14 days post infection (purple, n=10) in SynCom-16All.




**Supplemental Figure 10: Strain abundances in SynCom-16All inoculum.** Relative strain abundances in inoculum of SynCom-16All (n=7). Strains with high protection scores are shown in green, strains with low protection scores are shown in red.




**Supplemental Figure 11: *In vitro* competition between strains with high and low protection scores within the same genus.** Strains with high protection scores grown individually are represented by light green triangles (n=8), when grown in competition are represented by dark green squares (n=4). Strains with low protection scores grown individually are represented by light red diamonds (n=8), when grown in competition are represented by dark red circles (n=4). Binary competitions of: A. *Rhizobium* Leaf68 versus Leaf384. B. *Rhizobium* Leaf68 versus Leaf386. C. *Rhizobium* Leaf311 versus Leaf384. D. *Rhizobium* Leaf311 versus Leaf386. E. *Sphingomonas* Leaf21 versus Leaf28. F. *Sphingomonas* Leaf21 versus Leaf357. G. *Sphingomonas* Leaf230 versus Leaf28. H. *Sphingomonas* Leaf230 versus Leaf357. I. *Microbacterium* Leaf203 versus Leaf288. J. *Pseudomonas* Leaf15 versus Leaf48. K. Arthrobacter Leaf137 versus Leaf69. L. *Curtobacterium* Leaf154 versus Leaf261.

# Chapter IV

# Identifying microbial patterns important for plant protection using machine learning in synthetic community experiments

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Part of this chapter was submitted for publication

**Author contributions**

B.E., J.M., C.M.V. and J.A.V. designed the study. B.E. performed experimental laboratory work. C.M.V., M.B.M. and B.A.M. contributed to the plant experiments. M.B.M. performed part of the pathogen luminescence measurements. B.E., J.M. and C.M.V. analysed data. B.A.M. contributed to machine learning analysis. B.E., J.M., C.M.V. and J.A.V. wrote the manuscript.

# Abstract

Plant-associated microbiomes contribute to host phenotypes such as resistance to biotic and abiotic stresses. While beneficial candidate strains contributing to such host phenotypes can be tested individually, strain performance may vary in different biotic contexts. Therefore, the identification of beneficial strains within a microbiome can provide valuable insight into their potential. In this study, we present an experimental and analytical approach to explore properties relevant for a microbiota-conferred host phenotype, here plant protection as a microbiota-mediated function, in a synthetic community context. We screened randomly assembled synthetic communities (SynComs), followed by classification and regression analyses as well as empirical validation of the results. We illustrate our approach by investigating plant protection with 35 bacterial strains isolated from *Arabidopsis thaliana,* combined in 136 SynComs of five strains each. Plants were inoculated with SynComs and subsequently infected with the foliar pathogen *Pseudomonas syringae* DC3000. We identified strain identity as the most important predictor of pathogen reduction. We assessed the prediction of microbiota-mediated protection based on 70 synthetic test communities. The microbiota-based classifiers correctly identified 94-100% of protective communities (recall) in comparison to random classifiers with a recall of 32%Additional validation experiments confirmed three strains as the main drivers of pathogen reduction and two additional strains that conferred protection in combination. Beyond the specific application presented in our study, we provide a framework that can be adapted to identifying features relevant for microbiota function in other biological systems.


# Introduction

Complex multicellular organisms such as plants, vertebrates, and invertebrates are colonized by diverse microbes, collectively called the microbiota [1,2]. Research has uncovered that these microbial communities are important for host development and health. The gut microbiota of healthy individuals, for example, confers some level of protection against pathogens [3-6], also referred to as colonization resistance [7], aids in the training of the immune system [8-10], and is crucial for the digestion of food [11,12]. Similarly, plant-associated microbiota impact the state of their hosts by increasing nutrient availability [13,14], priming the plant immune system [15,16], and alleviating biotic and abiotic stresses [17-19] - or impacting flowering time [20,21]. The realization that host-associated microbes strongly affect host phenotypes led to the prospect that manipulation or engineering of microbiomes could be an effective and sustainable approach to address various challenges in medicine and agriculture [22-27]. The identification of beneficial microbes and relevant microbiota properties for host traits is therefore a primary objective in microbiome research.

Microbiota-associated host phenotypes are often postulated based on observational studies that correlate the composition of microbial communities and host phenotypes. However, despite the need in capturing complex biotic interactions, causality cannot be deduced from correlation analyses alone. Instead, experiments in which interacting partners can be manipulated are required to establish causal

relationships [1,28,29]. As a first step in investigating the causality of host-microbiota interactions, it is common practice to add individual microbial strains to the host to assess the effects on the phenotype of interest, such as resistance to pathogen colonization. This has led to the identification of a suite of microbes that are able to protect hosts against specific pathogens. Evidently, examining microbes individually falls short in providing information about the effect of a microbe in the presence of varying microbiota members [30-35]. Synthetic microbial community (SynCom) experiments can be used to bridge this gap between single strain inoculation and observational studies with natural microbiota.

The phyllosphere (above-ground) microbiota of *Arabidopsis thaliana* is a well-suited model system to examine properties of microbial communities that are relevant for microbiota-associated host phenotypes [36,37]. Representative collections of plant-associated bacteria allow the assembly of SynComs of varying composition and complexity [33,38-40]. Furthermore, a previously established gnotobiotic model system enables the design of experiments in which the effects of members of the microbiota on the foliar model pathogenic bacterium *Pseudomonas syringae* DC3000 can be determined under controlled conditions [15,41]. Such experimental systems coupled with machine learning approaches hold great promise to identify microbiota features linked with host phenotypes conserved across different biotic contexts [33,42,43].

Here, we present an experimental and analytical approach to investigate microbiota properties that are relevant for plant protection using a collection of randomly assembled SynComs of environmentally representative strains isolated from healthy Arabidopsis plants. We also provide a detailed rationale and calculations for the general design used in this study to facilitate its implementation in other host microbiota systems.

# Results

### Experimental Design to screen random synthetic communities for plant protection

To test whether properties of microbial communities relevant for a microbiota-conferred host phenotype can be identified by using random synthetic communities and machine-learning analyses, we set up a screen for plant protection in a gnotobiotic model system (Figure 1). The plant protection assays consisted in growing axenic *A. thaliana* Col-0 in a clay substrate in microboxes [41]. After ten days, plants were inoculated with randomly assembled synthetic communities (SynComs) of *At*-LSPHERE strains[38], and at 14 days post inoculation plants were sprayed with the pathogen *Pseudomonas syringae* DC3000 (*Pst*). The infection was monitored 14 days post infection (dpi; Supplemental Figure 1A,B).

A prerequisite for the statistical analyses was the observation of differences in pathogen colonization (our readout for plant protection) across SynComs treatments. We opted to keep the size of the SynCom inoculum (i.e. the number of strains in a SynCom) constant to remove variation in SynCom complexity as a variable. It has previously been shown that variation in plant protection is observed with SynComs of 10 strains using an agar-based assay [32]; therefore, we considered a size of less than 10 strains feasible. In addition, we wanted to quantify the absolute abundances of the different SynCom members and the pathogen, for which simple colony-forming-unit determinations are readily applicable. We estimated

that a limit of five community members would increase the chance of distinguishing all strains in each random SynCom based on colony morphologies.

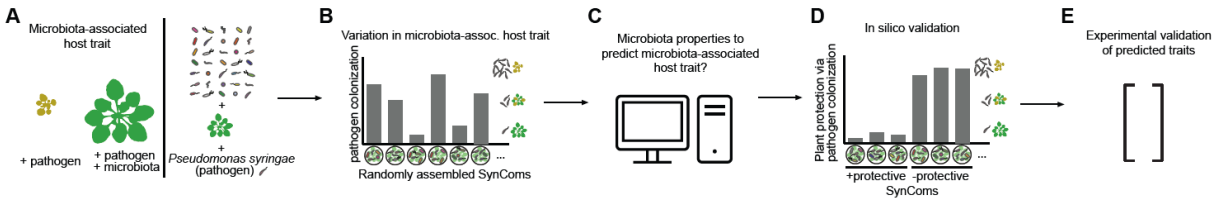


**Figure 1: Overview of screening strategy.** Overview of the screening approach which consists in A. starting from preliminary knowledge about microbiota-associated host trait; B. using a collection of bacteria to randomly constitute synthetic communities inoculated to the host and measurements of the community and host traits of interest; C. statistical models to identify properties of communities which correlate with variation in the trait of interest (e.g., machine learning); D. validation of models with an independent dataset; E. empirical validation of the communities' properties identified as being of potential influence on host trait.

A prerequisite for the statistical analyses was the observation of differences in pathogen colonization (our readout for plant protection) across SynComs treatments. We opted to keep the size of the SynCom inoculum (i.e. the number of strains in a SynCom) constant to remove variation in SynCom complexity as a variable. It has previously been shown that variation in plant protection is observed with SynComs of 10 strains using an agar-based assay [32]; therefore, we considered a size of less than 10 strains feasible. In addition, we wanted to quantify the absolute abundances of the different SynCom members and the pathogen, for which simple colony-forming-unit determinations are readily applicable. We estimated that a limit of five community members would increase the chance of distinguishing all strains in each random SynCom based on colony morphologies.

In a pilot experiment, we tested whether different SynComs with five strains (Mini5SynComs) lead to variation in pathogen colonization patterns. We randomly assembled 17 communities from a pool of 137 strains (SynCom-137; [44]), inoculated plants with these communities and subsequently infected them with *Pst* or buffer (see "Synthetic community assembly and controls" in Methods). At 3-, 6- and 12-days post infection, we measured pathogen luminescence as a proxy for pathogen colonization, which was enabled by using a *luxCDABE*-tagged derivative of the pathogen (see "Data analysis" in Methods for details) [45]. Indeed, we detected variation in luminescence signals between the different Mini5SynComs (Welch's ANOVA at 12 dpi, *p-value* = 6.2 x $10^{-7}$) indicating that Mini5SynComs are suitable for our approach. We also noted that 7 of the 17 Mini5SynComs did not show significantly elevated luminescence signals relative to the background signal of uninfected plants (one-tailed Welch's *t*-tests, unadjusted *p-value* > 0.05, Table S1, Supplemental Figure S2). We therefore decided to use CFU enumeration rather than indirect luminescence signal measurements to assess pathogen colonization in the following experiments.

Another important aspect of the experimental design was the replication scheme for each Mini5SynCom, which was also evaluated in the pilot experiment (see "Data analyses" in Methods). In the pilot each Mini5SynCom was inoculated onto 16 plants distributed among four microboxes to

control for potential differences between microboxes. The box-to-box variation of pathogen luminescence was not significant in 14 of the 19 treatments at 12 dpi, even without multiple testing p-value correction (*p-value* > 0.05) (Supplemental Figure 3). We therefore decided to use only one microbox per Mini5SynCom in the main experimental setup to increase the total number of alternative Mini5SynComs screened.

The size of the pool of strains to assemble in random communities was another important component of our experimental design. Due to space partitioning we decided to split the screen into two separate main experiments, each with control communities from the pilot experiment (M6 and M12, renamed SynCom-High and SynCom-Low, respectively) and axenic references (SI 1). Altogether, we planned to use 160 microboxes of which 136 were dedicated to screening random Mini5SynCom communities. Based on this number of communities screened, we calculated the expected prevalence of a strain across the Mini5SynComs with different sizes of the pool from which the communities were randomly built (see "Strain pool size for community screen" in Methods for calculation; Supplemental Figure 2A). We aimed to achieve a representation of a specific strain in about 20 independent Mini5SynComs and calculated that this representation is reached for five-member SynComs at a strain pool size of 35 or lower (Supplemental Figure 2A). Regarding the source pool of strains, we used the 10 strains from the control communities associated with low and high pathogen colonization from the pilot experiment (SynCom-Low and SynCom-High, respectively), and selected additional strains from the SynCom-137, bringing the pool size to 35 strains in total to reflect phylogenetic diversity, include diverse levels of individual protection [32] and to generate overlap of strains used in prior studies (Figure 2B; Supplemental Table 2; Supplemental Figure 1C) [41,46,47].

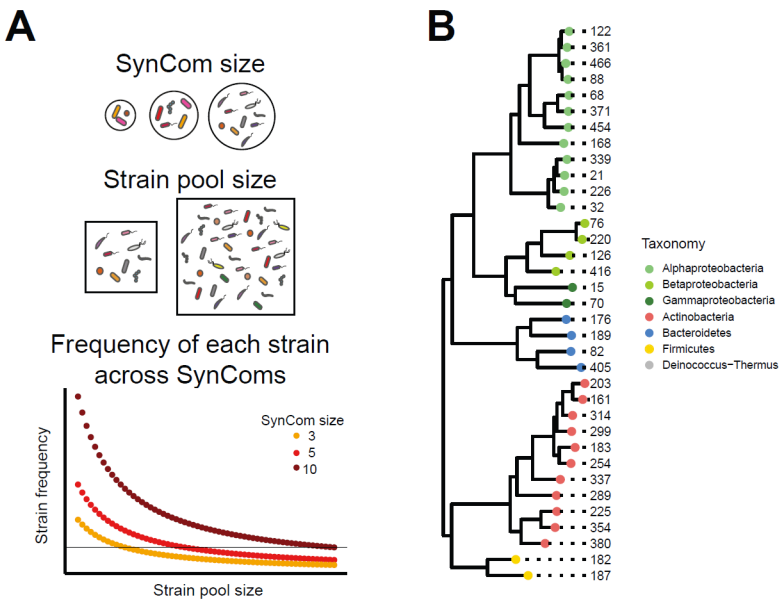


**Figure 2: Experimental constraints and strain selection.** A. SynCom and strain-pool sizes are the two parameters which will influence the expected prevalence of strains across synthetic communities for a known number of experimental units. B. Phylogenetic diversity of the collection of strains (SynCom-35) used for the screening experiment.

**Screening of random Mini5SynComs for plant protection**

We partitioned our screen of 136 randomly assembled Mini5SynComs in two independent experiments (experiments 1 and 2). For each plant, fresh weight and bacterial colonization of both pathogen and commensals (*i.e.*, Mini5SynCom strains) were determined at 14 dpi (Figure 3). In each experiment we included as controls axenic uninfected plants (axenic NI), axenic infected plants (axenic), a SynCom of the entire pool of 35 strains (SynCom-35), as well as SynCom-Low and SynCom-High of the pilot experiment (see "Synthetic community assembly and controls" in Methods for details; SI 1).

Median pathogen colonization (median of plant measurements per microboxes) ranged from four to nine orders of magnitude in cfu $g^{-1}$ plant fresh weight across all SynComs tested (Figure 3A). The controls SynCom-Low and SynCom-35 strongly reduced pathogen colonization, being in the first quartile of communities ranked according to the median pathogen colonization, while axenic infected controls had the highest median pathogen colonization of their respective experiments. In contrast to pathogen colonization, overall commensal colonization was more similar between different communities (Figure 3B). Median commensal colonization ranged from seven to nine orders of magnitude in cfu $g^{-1}$ plant fresh weight and the median plant weight was between 12.5 and 68.1 mg (Figure 3C; SI 1). Axenic-infected plants showed the lowest median plant weight (20.6 and 12.4 mg for experiment 1 and 2, respectively).

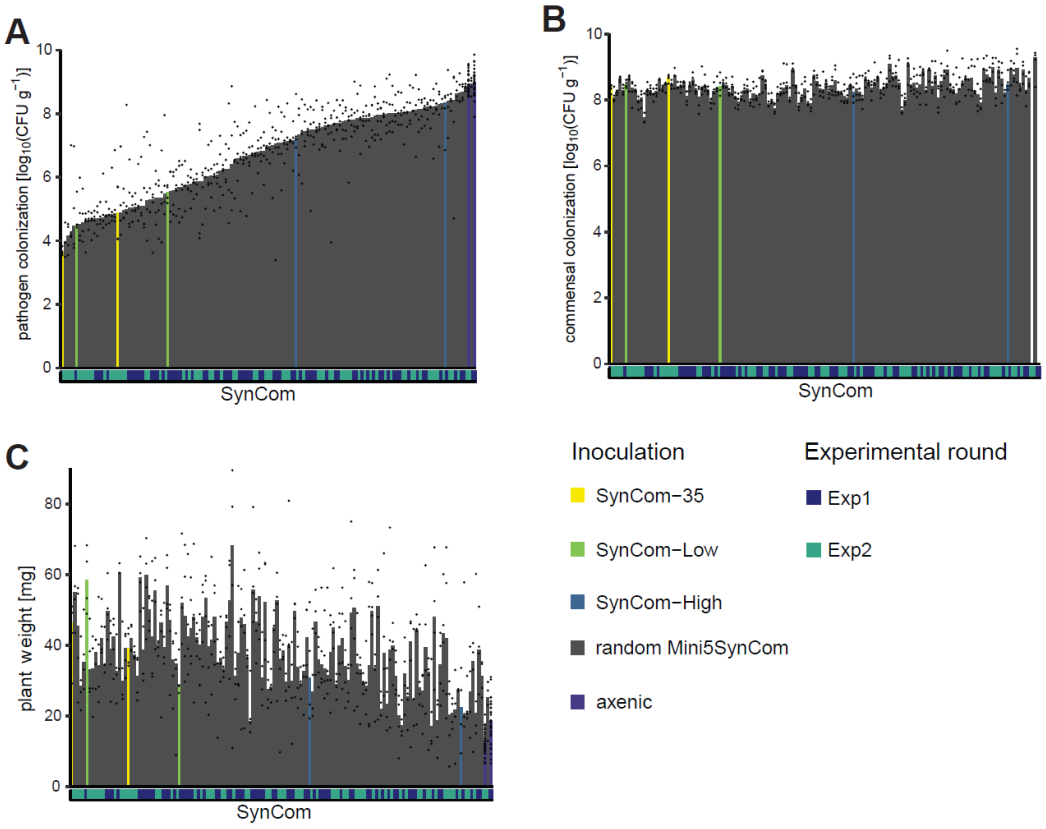


**Figure 3: Results of screen of 136 randomly assembled SynComs.** A. Pathogen colonization, B. commensal colonization, C. plant weight. Bars are medians with individual plant measurements superimposed as a scatter plot; x-axes represent individual treatments coloured according to experimental rounds. The order of treatments is fixed across all panels and ordered in ascending pathogen colonization. Abbreviations: Exp, experiment.

**Correlation among pathogen colonization, overall commensal colonization, and Mini5SynCom evenness**

We first associated properties of overall Mini5SynComs, specifically commensal colonization and community evenness, with pathogen colonization, respectively. We fit linear mixed models with the $log_{10}$-transformed pathogen colonization as dependent variable, and either the $log_{10}$-transformed commensal colonization (centered), or evenness as fixed effects (see "Data analysis" in Methods for details). For overall commensal colonization, the four best models (delta AIC < 4) supported that an increase in one order of magnitude in commensal colonization was associated with an equivalent increase in pathogen colonization considering all Mini5SynComs (Figure 4A; Supplemental Table 3). The standard errors of the corresponding coefficients were consistently below one order of magnitude, and the standard deviations of residuals were one order of magnitude (Supplemental Table 3, Supplemental Figure 4A). We can therefor conclude a positive relationship between pathogen and commensal colonization, which is consistent over different community compositions (in the best model: commensal effect = 1.4, standard deviation of commensal effect = 0.6; Supplemental Table 3).

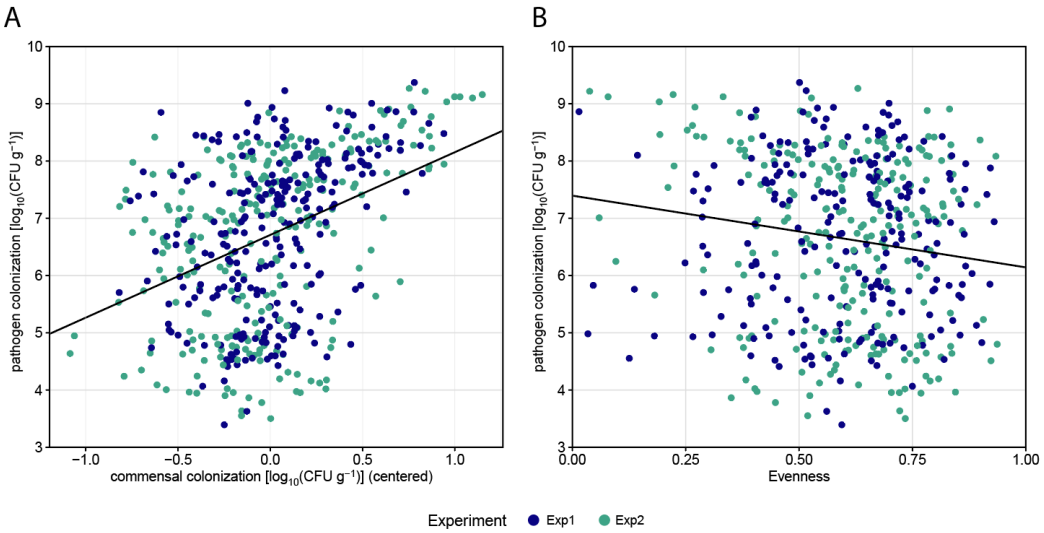


**Figure 4: Correlation among pathogen colonization, overall commensal colonization, and evenness.** Regression lines were obtained from best mixed effect models with similar random structure including random intercepts and random slopes for the box effect, and different fixed effects. A. Pathogen against overall Mini5SynCom commensal colonization, the latter used as fixed effect in the model. B. Pathogen against Mini5SynCom evenness, the latter used as fixed effect in the model. Abbreviations: Exp, experiment.

Variation in pathogen colonization was also associated with changes in evenness of Mini5SynComs. However, the three best models (delta AIC < 4) supported that the entire range of the evenness function (zero to one) corresponded within one order of magnitude to the decrease in pathogen colonization, which is a small value compared to the six orders of magnitudes spanned by pathogen colonization across Mini5SynComs (Supplemental Table 3; Figure 4B). For all models, the standard errors of the corresponding coefficients were consistently less than one order of magnitude, and the standard deviations of residuals were one order of magnitude (Figure Table 3, Supplemental Figure 4B). The relationship between evenness and pathogen colonization was less consistent across different

community compositions with a relatively high estimate of the standard deviation of this fixed effect (in the best model: evenness effect = -1.3, standard deviation of the evenness effect = 2.2; Supplemental Table 3). Altogether, both commensal colonization and community evenness show correlation with pathogen colonization. However, due to the high standard deviations seen, we thought the two parameters unfit for accurate prediction of pathogen colonization outcomes in our system.

**High predictiveness and recall of pathogen reduction by synthetic microbiota composition using machine learning**

Apart from the general community parameters, we were interested to test whether community composition (i.e. strain identity) allows prediction of pathogen colonization. We applied supervised machine-learning algorithms, i.e. random forest (RF) and elastic-net regularized generalized linear models (GLMNet), to train models to predict the outcome of pathogen infection based on Mini5SynCom composition (see "Data analysis" in Methods for details). We implemented those methods rather than simpler classification and regression analyses to limit overfitting.

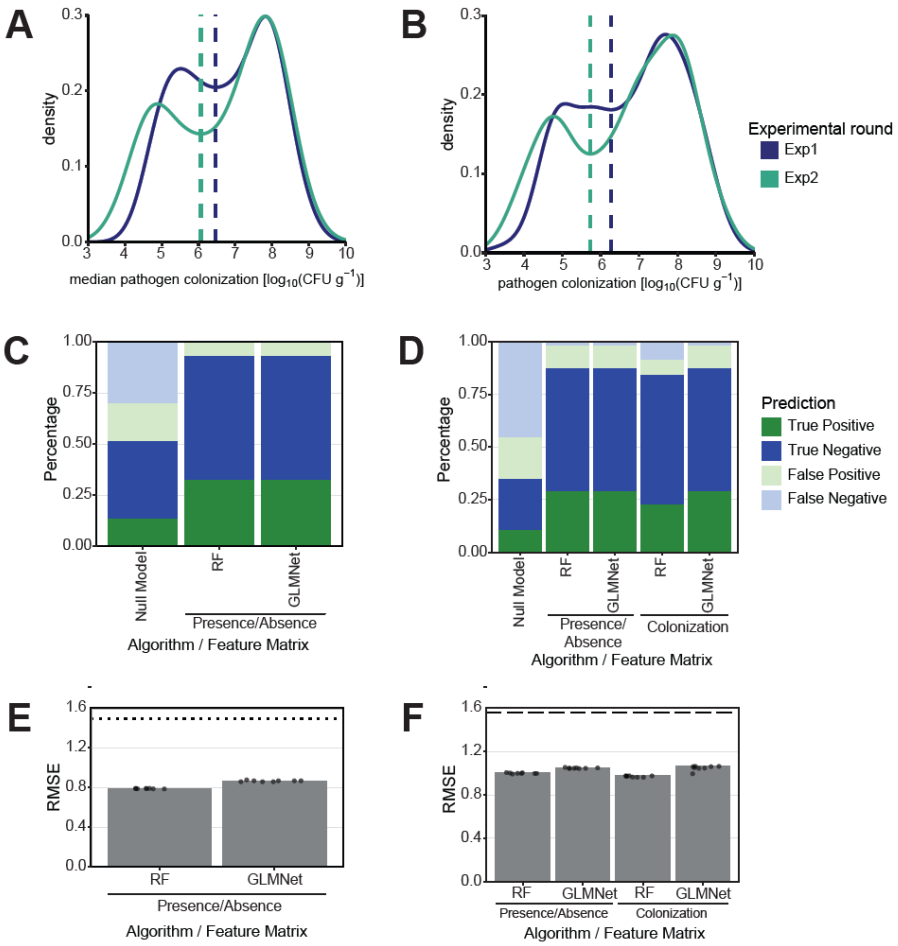


**Figure 5: Predictive outcome of machine learning.** A, B. Density curves of pathogen colonization with global minima used for splitting into "protected" and "not-protected" classes presented as vertical dashed lines. C, D. Performances of classification algorithms compared to a random classification (i.e., no model). E, F. Root mean square errors (RMSE) of the regression algorithms with dashed corresponding to a null model. A,C,E. Results derived from algorithms trained on the median of pathogen colonization for each treatment. B, D, F. Results derived from algorithms trained on pathogen colonization of individual plants.

The distribution of pathogen colonization was bimodal within experiments 1 and 2 (Figure 5A,B) suggesting two biological groups with low and high pathogen colonization. To test the biological relevance of these bimodal distributions (*i.e.*, observation not by chance), we bootstrapped the data 1,000 times and confirmed global minima of interest in virtually all replicates (Supplemental Table 4) providing justification for binary classification. We thus classified the samples according to the global minimum of pathogen colonization in each distribution into "protected" (pathogen colonization lower than the minimum) and "non-protected" (pathogen colonization equal to or higher than the minimum) to train classifiers (Figure 5A,B; Supplemental Table 5). In addition, we trained regression algorithms to predict the normalized pathogen reduction of a Mini5SynCom, defined as the reduction of $log_{10}$-transformed pathogen cfu $g^{-1}$ plant fresh weight compared to the median axenic infected control of each experiment. As predictors (features), we used presence/absence and also absolute abundances of Mini5SynCom members. The algorithms were either trained using measurements of individual plants or the calculated median values derived from four plants of a box (*i.e.*, Mini5SynCom treatment). All 12 model-algorithm combinations (Supplemental Figure 5) were trained using 10 rounds of 5-fold cross validation (see “Data analysis” in Methods for details). Because all methods involved pseudorandom processes, we repeated all analyses with eight different seeds.

Subsequently, we evaluated the performance of the trained models by generating an independent test dataset (Experiment 3; see “Data analysis” in Methods), a new set of 70 random Mini5SynComs. This test dataset had similar characteristics as the training dataset and was not seen by the algorithms during training (Supplemental Figure 6, Supplemental Table 4,5). Classification using absence/presence or commensal colonization led to substantially better predictions than random classification of samples, with 87-93% of samples (at the level of plant or Mini5SynCom) correctly classified as either “protective” or “not protective” (Figure 5C,D, Supplemental Table 6). The fraction of protective samples in the set of samples predicted as protective (*i.e.*, precision) ranged from 72% to 82%, while random classification showed a precision of 42% and 35% on median and individual values, respectively. The fraction of protective samples that were correctly predicted as protective (*i.e.*, recall) was 94% or 100%, with only one recall at 73%. In comparison, the recalls of the random classifiers were 32%. The fraction of correctly predicted non-protective Mini5SynComs (*i.e.*, specificity) was consistently above 84%, versus less than 69% in random classifications. Regression analyses were also better performing than predictions based on the global average of pathogen colonization (*i.e.*, average calculated from the plant or median mix data) with a root-mean-squared error (RMSE) of ranging from 0.79 to 1.06 for the trained analyses compared to an RMSE of 1.5 of the global average. This translates to an error of one versus two orders of magnitude of trained versus untrained models, respectively (Figure 5E,F; Supplemental Table 7). Regression analyses using absolute colonization of commensal strains as predictors show no improvement over predictions using presence/absence of strains. This suggests that in our system, the presence of strains might be predictive enough of pathogen colonization outcomes.

**Three strains found to be the most important in machine learning algorithms strongly reduce pathogen colonization**

After validating the machine learning approach using strain identity as a feature in microbiome context through the test data set (Figure 5C-F), we wanted to specifically validate the strains most important for the predictions in a targeted manner. All of the 12 analyses converged to support that three strains, i.e., *Acidovorax* Leaf76, *Rhizobium* Leaf68, and *Pseudomonas* Leaf15, were the most important features to predict pathogen colonization (Figure 6A,B; Supplemental Figure 7; Supplemental Table 8). Only one seed used for the GLMNet regression analyses to predict pathogen colonization of individual plants using absolute commensal colonization had a divergent result (Supplemental Figure 7 L).

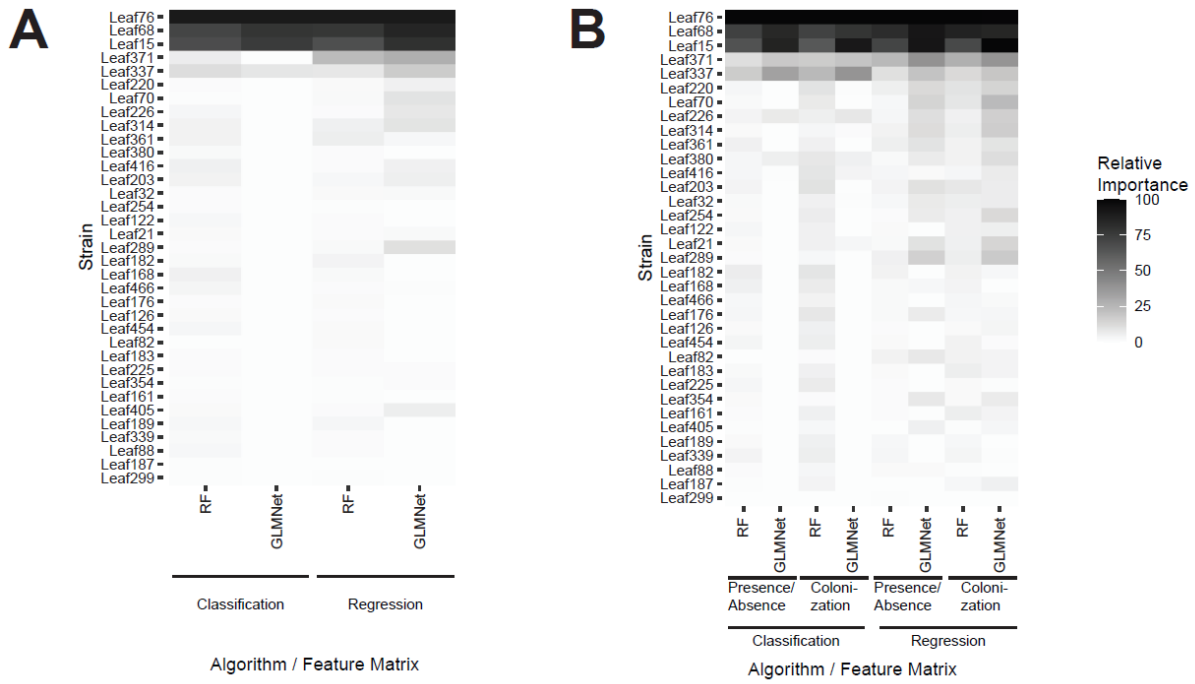


**Figure 6: Relative feature importance of trained machine learning algorithms.** Medians of the relative feature importance calculated across eight seeds; strains are ordered according to their median relative importance across all analyses. A. Relative feature importance derived from algorithms trained on the median of pathogen colonization for each treatment. B. Relative feature importance derived from algorithms trained on pathogen colonization of individual plants. Abbreviations: RF, random forest; GMLNet, elastic net regularized generalized linear model.

We empirically tested the ability of the three strains *Acidovorax* Leaf76, *Rhizobium* Leaf68, and *Pseudomonas* Leaf15 to reduce pathogen colonization. We also included *Rhizobium* Leaf371 which ranked fourth in relative feature importance, which was substantially lower (average of 21%) compared to the top featuring strains (80-100%). We validated the strains individually by testing whether they could significantly reduce pathogen colonization (see "Validation experiment of machine learning results" in Methods). We also tested their synergic effect in binary combinations as well as all four strains together within the same SynCom. To demonstrate that the identified strains provided better protection than other strains, alone or in combination, we assessed the pathogen reduction potential of four randomly selected strains in parallel.

Consistent with the relative feature importance obtained by the machine learning algorithms, the three best-predictive strains *Acidovorax* Leaf76, *Rhizobium* Leaf68, *Pseudomonas* Leaf15 significantly reduced pathogen colonization by two orders of magnitude compared with axenic controls (p-value < 2.4 x $10^{-17}$ after Bonferroni correction in the single best model with delta AIC < 4; Figure 7A; Supplemental Table 10,11). We therefore termed these three strains "pathogen-reducing strains" (PR strains). *Rhizobium* Leaf371 that had lower relative feature importance in the machine learning, showed a significantly reduced pathogen colonization; however, by one order of magnitude less than PR strains (Bonferroni-adjusted p-value = 8.7 x $10^{-6}$), suggesting an intermediate pathogen reduction level. In contrast, the four randomly selected strains did not significantly reduce pathogen colonization, confirming that the strains identified by machine learning reduce pathogen colonization but not the random strains tested (Figure 7B; Supplemental Table 12).

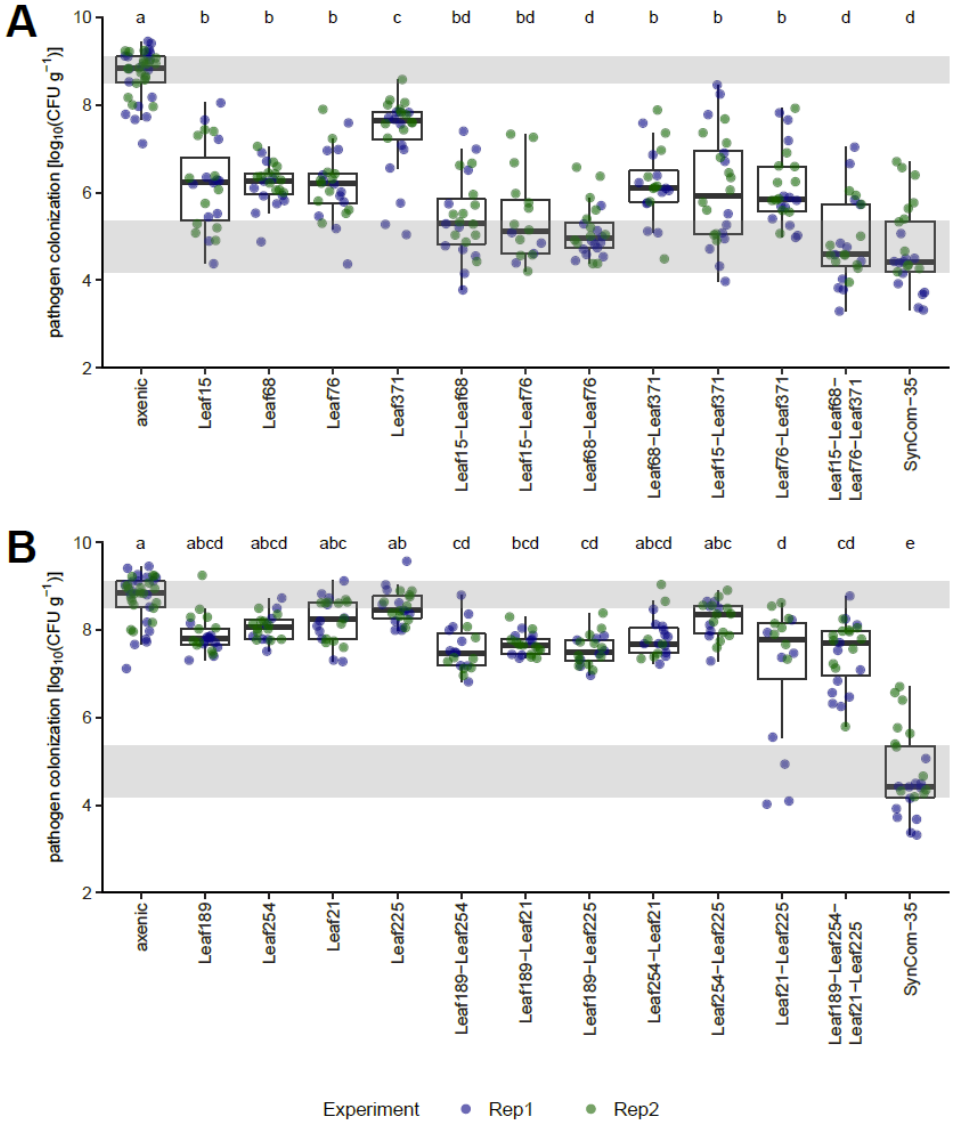


**Figure 7: Relative feature importance of trained machine learning algorithms.** Medians of the relative feature importance calculated across eight seeds; strains are ordered according to their median relative importance across all analyses. A. Relative feature importance derived from algorithms trained on the median of pathogen colonization for each treatment. B. Relative feature importance derived from algorithms trained on pathogen colonization of individual plants. Abbreviations: RF, random forest; GMLNet, elastic net regularized generalized linear model.

Furthermore, *Rhizobium* Leaf68 and *Acidovorax* Leaf76 significantly reduced pathogen colonization by one order of magnitude when applied in combination compared to their individual treatments (p-value < 0.005 after Bonferroni correction; Figure 7A, Supplemental Table 11). Notably, this combination as well as the SynCom of the three PR strains with *Rhizobium* Leaf371 reduced pathogen colonization by about four orders of magnitude, which was not significantly different from the pathogen reduction of the full SynCom (SynCom-35) (Figure 7A, Supplemental Table 11). Five out of the seven random-strain combinations significantly reduced pathogen colonization compared to the axenic controls, however to a maximum of one order of magnitude observed for the four-strain combination (Figure 7B, Supplemental Table 12).

Overall, these experiments confirmed the validity of the machine learning approach to identify important strains and also revealed synergic effects of individual PR strains.

**Refined data analysis and experimental validation reveal combination of strains reducing pathogen colonization**

After experimental validation of the top featuring strains in pathogen reduction, we explored the original data of the screen further to investigate the consistency of the experimentally confirmed features in the data set. For this, we split the median pathogen colonization data of the screen experiments 1 and 2 into two groups. The group "PR Strains" was composed of Mini5SynComs containing the PR strains, *Pseudomonas* Leaf15, *Rhizobium* Leaf68, and *Acidovorax* Leaf76, while the group "Others" contained the remaining Mini5SynComs. We observed a clear division of lower pathogen colonization within the "PR Strains" with a median of 1.94 x $10^5$ cfu $g^{-1}$ plant fresh weight, and a higher colonization level of the "Others" group, median of 6.21 x $10^7$ cfu $g^{-1}$ plant fresh weight (Figure 8A). The "Others" group had a skewed distribution with a hump in the low pathogen colonization region, corresponding to four Mini5SynComs (red circle in Figure 8A). Analysis of the composition of the four circled Mini5SynCom revealed that all contained *Rhizobium* Leaf371, which is consistent with the intermediate pathogen reduction level (Figure 7A). Further, we noted that three out the four Mini5SynComs additionally contained *Arthrobacter* Leaf337 (Figure 8B). *Arthrobacter* Leaf337 was found to be the fifth most important strain in the machine learning (Figure 6) with a slightly lower average relative feature importance (18%) compared to that of *Rhizobium* Leaf371 (21%). From this observation, we visualized the pathogen colonization of plants inoculated with Mini5SynComs containing either *Arthrobacter* Leaf337, *Rhizobium* Leaf371 or their combination (Figure 8C). The communities containing both strains tended to show a lower pathogen colonization than communities containing either strain alone, suggesting a potential additive effect of *Rhizobium* Leaf371 and *Arthrobacter* Leaf337.

To test the hypothesis that *Rhizobium* Leaf371 and *Arthrobacter* Leaf337 together significantly affect pathogen colonization, we conducted an additional experiment. We examined the two strains individually, their binary combination as well as three Mini5SynComs containing both strains (see "Validation experiment of synergetic effect of strains" in Methods). The best two models with no random effect and a random intercept for the experiment effect (delta AIC < 4; see Methods;

Supplemental Table 13) converged regarding the significance of coefficients (Supplemental Table 14). The combination of *Rhizobium* Leaf371 and *Arthrobacter* Leaf337 together, and in combination with other non-PR strains in the validation mixes 1 and 2 significantly improved the pathogen reduction by one order of magnitude compared with treatments of individual strains (p-values ≤ 0.02 after Bonferroni correction; Figure 8D; Supplemental Table 15). Mix6 induced a significant pathogen reduction compared to *Rhizobium* Leaf371 alone (p-value = 0.02 after Bonferroni correction), but not to *Arthrobacter* Leaf337 alone. Overall, the data demonstrate the value of pattern analysis after identifying individual strains through machine learning.

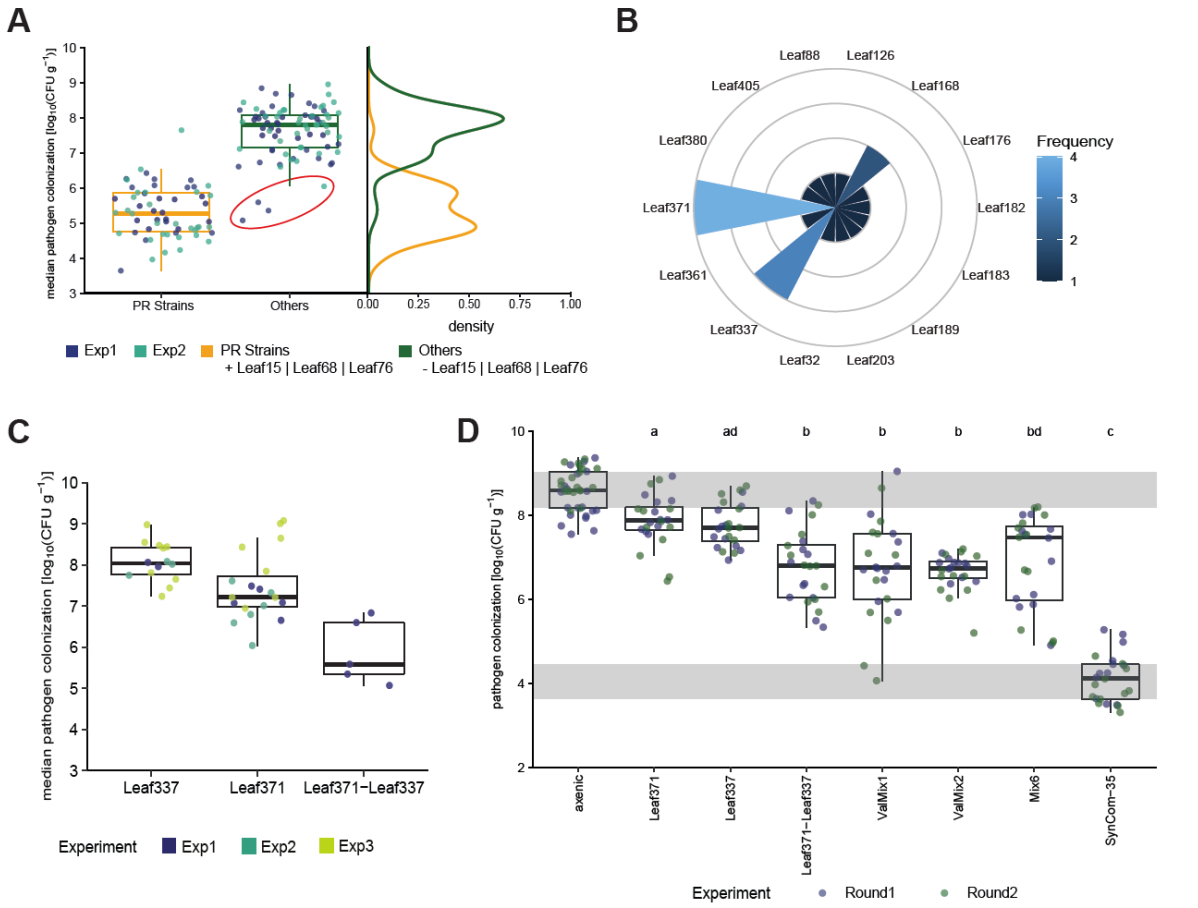


**Figure 8: Identification of additional two strain combination that reduces pathogen colonization.** A. Comparison of the group of treatments that contained at least one of the best-pathogen-reducing strains, Leaf15, Leaf68, or Leaf76 ("PR Strains"), and treatments that did not include those strains ("Others"); The circled Mini5SynComs are those which are included in the tail of the distribution of the "Others" group. Shown are boxplots and density curves of pathogen colonization with each point corresponding to the median of one treatment box. B. Frequency of detection of strains present in the Mini5SynComs of the tail of the "Others" group distribution and circled in panel B. C. Boxplot of the median pathogen colonization with communities containing Leaf371, Leaf337, or their combination in the screen experiments 1 and 2 and test set (Exp3). D. Boxplot of the pathogen colonization with individual strain inoculations, binary combination and SynComs including Leaf371 and/or Leaf337. The interquartile ranges of the axenic and SynCom-35 controls are shaded in grey. Significant differences in *Pst* colonization were estimated with the best model including strain inoculation as fixed effect, and no random effect. Lettering corresponds to significance groups at a 0.05 level after Bonferroni correction with the whole family of pairwise comparisons in this panel. Abbreviations: Exp, experiment.

**Summary of results**

Here, we present an experimental and analytical approach to screen for microbial patterns relevant for a microbiota-associated host trait. We illustrate our approach by investigating the plant protection potential of 35 strains from the *At*-LSPHERE collection combined in 136 randomly composed SynComs of 5 strains each. The pathogen colonization was found to range over 4 orders of magnitudes, and had a bimodal distribution (Figure 3). The pathogen colonization was correlated positively with total commensal colonization, and negatively with community evenness (Figure 4). Through regression and classification analyses using the community composition (presence/absence), we successfully predicted pathogen colonization outcomes (Figure 5). Through these machine learning algorithms, we identified three strains most important for prediction of pathogen colonization outcomes and validated their individual protection potential experimentally (Figure 6). Additionally, we found the three strains to act synergistically to reduce pathogen colonization further compared to their individual inoculation. Through refined data analysis, we found another strain combination to act synergistically to reduce pathogen colonization (Figure 8). In summary, we successfully applied machine learning algorithms on pathogen colonization outcomes of random synthetic community to uncover microbiota patterns important for plant protection, here, strain identity.

# Discussion

The complexity of interactions that take place in host-associated microbiota makes it difficult to uncover causal relationships [48,49]. Therefore, the identification of strains with desired impact on host phenotypes remains a challenging task. Previous studies addressed this challenge by first testing individual strains and then examining a selection of these strains to assess the impact on host phenotypes in a microbial community context [50,51]. The authors statistically modelled host phenotypes in SynComs of two- to five-members based on observed properties of individual strains. Approaches using individual strains, while valuable, may initially include strains whose properties are not conserved at the community level [34]. This is because host phenotypes mediated by the microbiota might be the outcome of complex functions from different community members that are difficult to formalize based on the performance of individual strains. It is, therefore, of interest to explore alternative approaches to identifying features relevant for host phenotypes by starting from the community context. Importantly, it does not require a priori knowledge or hypotheses on the mechanisms underlying microbiota-conferred traits of the host [43].

In our study, we showed that screening randomly composed SynComs can be used in combination with statistical modelling to identify community compositions with desired effects on host traits (here pathogen reduction; Figure 1A). We identified several plant protective strains that decrease pathogen colonization when present in commensal communities, alone, or in combinations. Thus, starting with randomly constituted communities allowed direct testing of the robustness of strain effects to biotic variation. In the case of the present study, we identified three strains (PR strains), *Pseudomonas* Leaf15, *Rhizobium* Leaf68, *Acidovorax* Leaf76, that decreased pathogen colonization when present in communities in the phyllosphere of *A. thaliana* (Figure 7).

The present screen was not designed to directly detect plant protection by strain combinations within communities. However, one can tune the parameters “Strain pool size”, “SynCom size”, and the number of tested SynComs to increase the prevalence of specific combinations upon screening and use them as explanatory variables in machine learning analyses (Figure 2). Despite this limitation, we showed synergic effects of the PR strains (Figure 7A), and through data exploration and experimental validation found that *Rhizobium* Leaf371 and *Arthrobacter* Leaf337 had a significant protective effect when present together (Figure 8).

Having found additive effects of the PR strains in this study suggests that these strains may have complementary mechanisms to reduce pathogen colonization, which is an important finding for future SynCom design and application studies. Regarding mechanistic explanations, one way to limit pathogen colonization is for the protective strains to act through the plant by activating the general non-self response (GNSR) of the plant - or more generally the plant immune system, which *Pseudomonas* Leaf15 and *Acidovorax* Leaf76 were shown to be capable of [47]. Protective bacteria can also reduce pathogen invasion by resource competition [50,52,53], or by direct inhibition of the pathogen [18,54,55]. All three PR strains were previously shown to contain type VI secretion system, and *Rhizobium* Leaf68 and *Pseudomonas* Leaf15 were found to protect pattern-triggered-immunity plant mutants [32], which might indicate a contribution of microbe-microbe interactions in pathogen reduction. Further experiments will be needed to characterize the mechanisms behind plant protection identified in the present study.

Screening of random communities also provides an opportunity to investigate additional properties of the microbiota that may condition host protection. Phylogenetic diversity, functional redundancy, community abundance, and evenness are just a few examples. Here, we investigated the two last aspects. Notably, abundance and evenness per se were not as strong as the community composition, *i.e.*, presence and absence of certain strains (Figure 4-6). We found that overall, an increase of one order of magnitude in commensal colonization correlated with a comparable increase in pathogen colonization. This result is in accordance with previous ones and might be explained by the release of plant nutrients due to pathogen colonization which in turn promote commensal growth [56].

Overall, the robustness of the results predicted by machine learning, the assumption-free screen, and the demonstration of causality with empirical validation, make us confident that the screening method is applicable and allows to identify beneficial strains or strain consortia. The knowledge may then be used to design SynCom communities for host applications and investigate the underlying mechanistic basis. Due to the flexibility of the experimental and analytical design, the microbiota screening approach coupled with machine learning is applicable to host-microbiota systems more broadly, beyond the exemplary study system of this study.

# Material and Methods

### Plant growth conditions

In all experiments of the present study, *Arabidopsis thaliana* Col-0 were grown gnotobiotically as described previously [41,44]. Briefly, 140 ml calcined clay (Diamond Pro Calcined Clay Drying Agent) was mixed with 60 ml 0.5× Murashige and Skoog (MS) medium including vitamins, pH 7 (M0222.0050, Duchefa) in gamma-irradiated microboxes (no. O118/80 + OD118 with XXL + (green) filter lid , Saco2). Surface sterilized seeds were stratified at 4 °C for 4 d and 2-3 plants seeded at four spots. Plants were placed in growth chambers set to 22°C and 54% relative humidity with a 11 h photoperiod. Light intensities were set to 180-200 µmol $m^{-2}$ $s^{-1}$ (400-700 nm, PAR) and 5–6 µmol $m^{-2}$ $s^{-1}$ (280-400 nm, UV light). Surplus seedlings were removed from each planted spot to have exactly four plants per box prior to inoculation (10 d). Plants were watered with 500 µl 0.5x MS on days 4, 17, 24 and 31, and harvested on day 38 (5.5 weeks old).

### Inoculation of plant with bacterial suspensions

Throughout this manuscript, the term "inoculation" is used to refer to treatment with commensal strains of the *At*-LSPHERE collection, whereas the term "infection" refers to spraying with the foliar pathogen *P. syringae*. Bacterial strains were streaked out on R2A agar supplemented with 0.5% (v/v) methanol and incubated at 22°C for 6 days. Strains were resuspended individually in 1.5 ml 10 mM $MgCl_2$, and vortexed for 10 min. If required, suspensions were filtered through a sterile 10 µm filter (CellTrics, Sysmex Suisse AG) to remove aggregates. Suspensions were adjusted to $OD_{600}$ of 0.2 and combined in equal ratio to prepare the Mini5SynComs. The mixed Mini5SynCom suspensions were diluted 1:10 and used for inoculation. Axenic seedlings were inoculated by pipetting 500 µl of bacterial suspension or 10 mM $MgCl_2$ onto the plants at 10 days post germination. To control the OD adjusted suspension and the inoculum, tenfold dilution series were prepared, and spotted onto R2A agar supplemented with 0.5% methanol to determine colony-forming units (CFU).

### Plant infection

Infection inoculum of *Pseudomonas syringae* pv. tomato DC3000 *luxCDABE* (*Ps*t) [45] was prepared as described in Innerebner et al. [17]. Briefly, a lawn of *Ps*t was grown on King's B agar [57] at 28°C overnight, resuspended in 10 ml 10mM $MgCl_2$ and $OD_{600}$ adjusted to 0.001. The plants were sprayed at day 24 with either buffer (non-infected controls, NI) or with *Ps*t suspension using a thin-layer chromatography reagent sprayer (Faust Laborbedarf AG). Each box was sprayed 6 times, which corresponded to roughly $10^4$ pathogen CFUs per plant. Pathogen titer was assessed by CFU determination on King's B agar.

**Bacterial plant colonization by colony-forming units**

Except for the pilot experiment (see above), plants were harvested at 14 dpi (corresponding to 38 days old plants). Plant weight and bacterial abundances were measured following Pfeilmeier et al. [44]. Briefly, plants were removed from clay, roots cut off and the whole phyllosphere part transferred into cold pre-weighed 2-ml tubes containing a sterile metal bead (5 mm diameter) and 200 µl 100 sodium phosphate buffer pH 7. Plant fresh weight was recorded, and plants were homogenized by shaking in the TissueLyzer II (Qiagen) for 45 s at 25 Hz. After addition of 600 µl of 100mM phosphate buffer pH 7 to homogenized plants, tubes were vortexed, and a ten-fold dilution series was prepared. To assess total pathogen colonization, 4 µl of the dilution series was spotted onto selective R2A agar containing 50 µg/ml rifampicin. To assess commensal colonization, 50 µl of the $10^{-3}$ and $10^{-4}$ dilutions were spread onto R2A agar supplemented with 0.5% methanol. Plates were incubated at room temperature until CFUs could be counted (2 to 7 days). Mini5SynCom strains were whenever possible counted separately based on colony morphology. When two strains could not be distinguished, 20 colonies were randomly picked and identified by either restreaking on selective R2A agar with 0.5% MeOH (antibiotics added at the following concentrations in µg/ml: kanamycin (50), tetracycline (10), ampicillin (100), colistin (10)) or minimal medium agar containing methanol as sole carbon source [38], or by DNA fingerprinting using the Enterobacterial Repetitive Intergenic Consensus (ERIC) sequences protocol [58]. For ERIC-PCR, 25 µl reactions were set up containing 12.5 µl DreamTaq Green PCR Master Mix (Thermoscience, Cat# K1081), 6.5 µl distilled water, 2.5 µl of each 10 µM primer (ERIC1R and ERIC2 primer [58]), and 1 µl heat-lysed bacterial cells. PCR were performed in a thermocycler (Biometra, T1 thermocycler) with an initial denaturation (95°C, 5 min), followed by 35 cycles of denaturation (95°C, 30 s), annealing (50°C, 1 min) and extension (65°C, 2 min) with a single final extension (65°C, 8 min). PCR products were separated on a 1.5% (w/v) agarose gel supplemented with Gelred (Biotium, Cat# 41003) for 2 h at 80 V and patterns compared to known strains. Some commensals could still not be distinguished and were thus entered into the datafile as "ambiguous". The CFUs of those undistinguishable groups of commensals were still taken into consideration in the calculation of the abundance of entire Mini5SynComs. Final measurements of bacterial colonization were in CFU per gram of plant fresh weight.

**Strain pool size for community screen**

We aimed to have each strain present in about 20 communities on average to gain enough power in statistical analyses. For space and time reasons, we were constrained by using a maximum of 136 microboxes dedicated to the screen of the Mini5SynComs and the rest to controls. We calculated the expected prevalence of a strain across all screened communities as

$$E(X) = \frac{k!\ (n-1)!}{n!\ (k-1)!} \times N$$

with k being the size of the Mini5SynCom (k = 5), *n* the size of the pool of strains to choose from, and *N* the number of screened Mini5SynComs (N = 136). On the left hand of the multiplication sign, the equation corresponds to the number of possible communities that share a specific strain $\binom{n-1}{k-1}$ divided by the total number of possible five-strain communities drawn from a n-strain pool $\binom{n}{k}$. We calculated that the optimal size of the pool was 35 strains or lower (Figure. 2A).

**Synthetic community assembly and controls**

Random communities in pilot and random screen experiments were designed from their specific strain pools (SynCom-137 for pilot experiment, SynCom-35 for Mini5SynCom screen) using a webtool randomizer (www.randomizer.org). The SynCom-35 pool used for the Mini5SynCom screen is a subset of SynCom-137, which was used for the pilot experiment. For the pilot experiment, controls included four microboxes mock-inoculated with buffer instead of inoculation with synthetic communities, four of which were mock-sprayed with buffer instead of pathogen at the infection timepoint (axenic non-infected plants). For each experiment of the random communities screen, following controls were included: one box inoculated with the SynCom-low and another with SynCom-high (two SynComs characterized by low and high pathogen colonization in the pilot experiment), one inoculated with a community comprising all 35 strains of SynCom-35 and nine microboxes that were mock-inoculated with buffer instead of inoculation with synthetic communities, two of which were mock-sprayed with buffer instead of pathogen at the infection timepoint (axenic non-infected plants). For the validation experiments, the same controls were included, but with different amount of replica boxes: 3 boxes for control communities per experimental replicate, 3 axenic non-infected boxes (mock spray during infection), and 5 axenic infected boxes.

**Validation experiment of machine learning results**

We empirically tested the ability of strains identified in machine learning to reduce pathogen colonization. We inoculated each strain to 24 plants (six microboxes) later infected with *Ps*t (see "Plant infection" above). To test for potential synergetic effects, we also inoculated 24 plants with each binary combination of these strains, and all of them together. To demonstrate that the identified strains provided better protection than other strains, we applied the same treatment scheme with the same number of randomly selected strains as additional controls. We compared the pathogen colonization among those treatments, 40 axenic plants infected with *Ps*t, and 24 plants preliminary inoculated with SynCom-35. The microboxes for each treatment were split equally across two experimental runs.

**Validation experiment of synergetic effect of strains**

After further exploration of data, we raised the hypothesis that two strains could have a synergetic effect on pathogen reduction. To empirically test this hypothesis, we inoculated each of these strains and their combination following the same experimental design as for the validation of the machine

learning results. In addition, we included three Mini5SynComs containing those two strains together. One of these Mini5SynComs was taken from the original screen (Mix6) and two additional ones were assembled with three random non-PR strains (ValMix1 and 2). Controls were as in the validation experiment for machine learning results (see "Validation experiment of machine learning results" above).

**Data analysis**

**Data transformation, visualization and exclusion.** Data were analyzed with R 4.2.2 [59]. Main figures were plotted using the R package *ggplot2* within *tidyverse* v1.3.2 [60], along with R package *gridExtra* v2.3 [61] and *ggpubr* v0.6.0 [62]. All colonization (CFU) and luminescence measurements were $\log_{10}$-transformed prior to analysis. Where mentioned in the text, pathogen colonization was normalized within experimental round by subtracting the median of axenic infected control samples. Any data point not included in the closed interval [Q1 – 1.5 IQR, Q3 + 1.5 IQR] was flagged as an outlier, with IQR being the interquartile range. We excluded plants which showed fungal contamination, and among outliers the samples that were disturbed during experimental procedure (*i.e.*, box fell down).

**Pilot Experiment.** Luminescence measurements were used as a proxy for pathogen colonization at 3, 6 and 12 days post infection as described previously [32]. Briefly, microboxes were placed with open lid into the IVIS Spectrum Imaging System (Xenogen). and luminescence was acquired for 30 s at 500 nm wavelength. In the Living Image Software v.4.2., circular region of interests (ROI) were set around each plant, adjusting to bigger plants if necessary, and exporting the total photon flux per ROI. Prior to data analysis, the total flux [p/s] measurements were log10-transformed. Differences in luminescence among random communities and among boxes inoculated with same community were detected using Welch ANOVAs (SI 1, Supplemental Table 1, Supplemental Figure 2, 3). To test whether pathogen luminescence can be detected for the different treatments (*i.e.*, luminescence higher than background luminescence of axenic non-infected plants), we performed one-sided Welch's t-tests.

**Correlation of pathogen colonization with commensal colonization or evenness.** We modelized the colonization of *Pst* (dependent variable) in experiments 1 and 2 with two sets of generalized mixed models. The fixed effects were commensal colonization and Mini5SynCom evenness (Pielou's index) for the first and second sets, respectively. Each group of models included a full version with random intercepts and/or slopes for experiments and microboxes, all nested random structures, and a model with no random effects. When both present, the microbox effect was nested in the experiment effect. Furthermore, microbox grouping was completely confounded with the composition of Mini5SynComs. For each set, we calculated the Akaike information criterion (AIC) with the base R function "AIC" to select the best models (delta AIC < 4) and present the results based on these. We excluded from these analyses all controls and problematic outliers (see above) to only include Mini5SynComs. Because the calculation of the evenness required the abundance of each member of communities, we also excluded

Mini5SynComs with ambiguous values. At the end, 134 and 122 Mini5SynComs were included for the regression of *Ps*t abundance with the abundance and evenness of communities, respectively. Because we did not include any sample with no commensal in analyses, we centered the commensal colonization before analyses to interpret the intercept as the expected *Ps*t colonization at mid-levels of commensal colonization. All models were fitted with the restricted maximum likelihood method with the function *lmer* when random effects were present (package *lme4* v1.1.28 [63]) or the function *gls* for models without random effects (package *nlme* v3.1.157 [64]). We examined the response plots, residual distributions, and residual plots for no deficiency patterns in the fit of models.

**Machine Learning Training of Algorithms.** We trained random forests (RF) and elastic-net regularized generalized linear models (GLMNET) to predict pathogen outcomes based on the presence of the 35 strains (*i.e.*, features) included in Mini5SynComs. The 136 randomly assembled Mini5SynComs screened in experiment 1 and 2 of the screen served as training data (the controls were not used). For classifications, we used the global minima present in the bimodal distributions of the pathogen colonization of experiments 1 and 2, which suggested the presence of two groups with low and high pathogen colonization, respectively. To estimate if these local minima were not the result of stochastic events during population sampling, we bootstrapped the datasets of the two experiments 1,000 times and measured the relative frequency of detection of local minima in the same regions of the density curves. After this control, we classified samples into a protected class (pathogen colonization lower than the minima) and a non-protected class (pathogen colonization equal or higher than the minima) for each distribution. For regression analyses, we predicted the normalized pathogen reduction of a Mini5SynCom. We trained the models on individual plant measurements (544 plants) as well as on aggregated pathogen abundance data (median pathogen CFU/g for one condition; 136 microboxes). Predictors were either presence/absence of Mini5SynCom members or the absolute abundance of these determined by CFU enumeration at the end of the experiment. When strains could not be unambiguously assigned, the recovered CFU were partitioned equally between the non-distinguishable strains (16% of samples (88 plants), or 6.7% of inoculated strains). Models were fitted using the R library *caret* v6.0-93 [65] with the embedded models *randomForest* v4.7-1.1 [66] in conjunction with *e1071* v1.7-13[67] and *glmnet* v4.1-6 [68], for RF and GLMnet, respectively. All model / algorithm combinations (12 in total; Supplemental Figure 5) were tuned using repeated k-fold cross-validation using 10 rounds of 5-fold cross-validation. If individual plant measurements were used instead of aggregated data, measurements were split at the level of the treatment to ensure that all samples of a Mini5SynCom were either in the training or validation set. The metrics used to select the best tuning parameters were kappa and root-mean-squared errors for classification and regression, respectively. Final models were then fitted to all training data using the tuned parameters. Because pseudo-random processes were involved in those procedures, we integrated all results from the repetition of all analyses with eight different starting seeds.

**Performances of trained algorithms.** To minimize data leakage leading to overestimation of model performances, we used an independent test set to assess the quality of our trained classifiers and regression models. To do so, we conducted a separate experiment (experiment 3) following the same procedures as for experiments 1 and 2, with 68 new Mini5SynComs randomly generated from the same pool of strains and including the same controls. Because the two control communities SynCom-High and SynCom-Low had not been used in the training of the different models, we included these controls in the test set. The distribution of pathogen colonization was comparable to the training dataset, and its bimodal shape was not sensitive to perturbation across 1,000 bootstrap replicates (see Results), so the same classification approach was taken. For classification, the performance metrics of trained models were compared with a random classification of the test samples. For regression analyses, we compared the performances of the trained models with those of a model constantly predicting the mean of the entire dataset. Because we were interested in finding the strains being most important to predict plant protection, we extracted the relative importance of strains from all ML analyses using the *varImp*() function implemented in the R library *caret* v6.0-93 [65]. We verified the sensitivity of this relative importance to the uncertainty on the position of minima used in the training set. We re-fitted the RF classifier with presence / absence of strains and individual plant measurements after reclassification of training samples using ten different values of minima sampled randomly between the 5$^{th}$ and 95$^{th}$ percentiles of the bootstrapped distributions of local minima of experiments 1 and 2 (see above).

**Validation experiments.** To test for significant differences in pathogen colonization, we used generalized mixed models after averaging the pathogen colonization per microboxes and avoid this grouping structure in the data. We followed the same procedures as above for the fitting of models, model selections, and diagnostics of fit. All models included the inoculation treatment as a fixed effect with or without random effects. The most complete random structure included random intercepts and slopes for experimental runs. The significance level of contrasts was 0.05 after Bonferroni correction. The families of hypotheses used for those corrections were all binary-combinations of contrasts included in Figure 6A, B and 7C to keep the probability of one or more false positive inferior to 0.05 in each corresponding sections of the manuscript.

**Data and Code availability**

Source code and data is available on https://gitlab.ethz.ch/RandomScreenBE/mini5syncom.git.

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# Supplemental Tables

**Supplemental Table 1:** One sided Welsh t-tests of random communities in pilot experiment conducted using medians of each Mix calculated from $log_{10}$-transformed IVIS luminescence.

| group1 | group2 | TimePoint | estimate | estimate1 | estimate2 | .y. | statistic | p.value | parameter | conf.low | conf.high | method | alternative | p.adj | p.signif [1] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| axenic NI | axenic | 3dpi | -1.323 | 4.860 | 6.183 | LogFlux | -9.017 | 0.001 | 3.103 | #NAME? | -0.982 | Welch Two Sample t-test | less | 0.023 | ** |
| axenic NI | M1 | 3dpi | -0.245 | 4.860 | 5.104 | LogFlux | -2.687 | 0.034 | 3.274 | #NAME? | -0.037 | Welch Two Sample t-test | less | 0.611 | * |
| axenic NI | M10 | 3dpi | -0.081 | 4.860 | 4.941 | LogFlux | -3.457 | 0.008 | 5.434 | #NAME? | -0.035 | Welch Two Sample t-test | less | 0.142 | ** |
| axenic NI | M11 | 3dpi | -0.051 | 4.860 | 4.910 | LogFlux | -1.826 | 0.059 | 5.979 | #NAME? | 0.003 | Welch Two Sample t-test | less | 1.000 | |
| axenic NI | M12 | 3dpi | -0.069 | 4.860 | 4.928 | LogFlux | -2.371 | 0.028 | 5.898 | #NAME? | -0.012 | Welch Two Sample t-test | less | 0.505 | * |
| axenic NI | M13 | 3dpi | -0.064 | 4.860 | 4.924 | LogFlux | -2.166 | 0.037 | 5.826 | #NAME? | -0.006 | Welch Two Sample t-test | less | 0.674 | * |
| axenic NI | M14 | 3dpi | -0.088 | 4.860 | 4.947 | LogFlux | -2.176 | 0.043 | 4.600 | #NAME? | -0.005 | Welch Two Sample t-test | less | 0.777 | * |
| axenic NI | M15 | 3dpi | -0.142 | 4.860 | 5.002 | LogFlux | -4.078 | 0.004 | 5.164 | #NAME? | -0.072 | Welch Two Sample t-test | less | 0.080 | ** |
| axenic NI | M16 | 3dpi | -0.330 | 4.860 | 5.190 | LogFlux | -1.652 | 0.098 | 3.055 | #NAME? | 0.137 | Welch Two Sample t-test | less | 1.000 | |
| axenic NI | M17 | 3dpi | -0.541 | 4.860 | 5.401 | LogFlux | -2.040 | 0.067 | 3.031 | #NAME? | 0.080 | Welch Two Sample t-test | less | 1.000 | |
| axenic NI | M2 | 3dpi | -0.080 | 4.860 | 4.939 | LogFlux | -1.502 | 0.105 | 3.863 | #NAME? | 0.035 | Welch Two Sample t-test | less | 1.000 | |
| axenic NI | M3 | 3dpi | -0.041 | 4.860 | 4.900 | LogFlux | -1.874 | 0.062 | 4.606 | #NAME? | 0.004 | Welch Two Sample t-test | less | 1.000 | |
| axenic NI | M4 | 3dpi | -0.067 | 4.860 | 4.927 | LogFlux | -2.585 | 0.021 | 5.974 | #NAME? | -0.017 | Welch Two Sample t-test | less | 0.375 | * |
| axenic NI | M5 | 3dpi | -0.749 | 4.860 | 5.608 | LogFlux | -2.592 | 0.040 | 3.026 | #NAME? | -0.071 | Welch Two Sample t-test | less | 0.722 | * |
| axenic NI | M6 | 3dpi | -0.455 | 4.860 | 5.315 | LogFlux | -1.912 | 0.075 | 3.039 | #NAME? | 0.102 | Welch Two Sample t-test | less | 1.000 | |
| axenic NI | M7 | 3dpi | -0.115 | 4.860 | 4.975 | LogFlux | -3.511 | 0.007 | 5.427 | #NAME? | -0.050 | Welch Two Sample t-test | less | 0.134 | ** |
| axenic NI | M8 | 3dpi | -0.045 | 4.860 | 4.905 | LogFlux | -2.072 | 0.048 | 4.731 | #NAME? | -0.001 | Welch Two Sample t-test | less | 0.865 | * |
| axenic NI | M9 | 3dpi | -0.361 | 4.860 | 5.221 | LogFlux | -1.818 | 0.082 | 3.056 | #NAME? | 0.103 | Welch Two Sample t-test | less | 1.000 | |
| axenic NI | axenic | 6dpi | -1.181 | 4.894 | 6.075 | LogFlux | -20.700 | 0.000 | 3.501 | #NAME? | -1.054 | Welch Two Sample t-test | less | 0.001 | *** |
| axenic NI | M1 | 6dpi | -0.505 | 4.894 | 5.399 | LogFlux | -4.093 | 0.012 | 3.101 | #NAME? | -0.218 | Welch Two Sample t-test | less | 0.223 | * |
| axenic NI | M10 | 6dpi | -0.042 | 4.894 | 4.936 | LogFlux | -0.898 | 0.211 | 3.782 | #NAME? | 0.059 | Welch Two Sample t-test | less | 1.000 | |
| axenic NI | M11 | 6dpi | -0.145 | 4.894 | 5.040 | LogFlux | -6.862 | 0.000 | 5.907 | #NAME? | -0.104 | Welch Two Sample t-test | less | 0.005 | *** |
| axenic NI | M12 | 6dpi | -0.028 | 4.894 | 4.922 | LogFlux | -1.094 | 0.159 | 5.714 | #NAME? | 0.022 | Welch Two Sample t-test | less | 1.000 | |
| axenic NI | M13 | 6dpi | -0.047 | 4.894 | 4.941 | LogFlux | -1.834 | 0.059 | 5.716 | #NAME? | 0.003 | Welch Two Sample t-test | less | 1.000 | |
| axenic NI | M14 | 6dpi | -0.125 | 4.894 | 5.019 | LogFlux | -6.341 | 0.000 | 5.513 | #NAME? | -0.086 | Welch Two Sample t-test | less | 0.009 | *** |
| axenic NI | M15 | 6dpi | -0.213 | 4.894 | 5.108 | LogFlux | -5.463 | 0.002 | 4.147 | #NAME? | -0.131 | Welch Two Sample t-test | less | 0.044 | ** |
| axenic NI | M16 | 6dpi | -0.382 | 4.894 | 5.277 | LogFlux | -2.357 | 0.049 | 3.058 | #NAME? | -0.003 | Welch Two Sample t-test | less | 0.883 | * |
| axenic NI | M17 | 6dpi | -0.459 | 4.894 | 5.353 | LogFlux | -2.649 | 0.038 | 3.051 | #NAME? | -0.054 | Welch Two Sample t-test | less | 0.682 | * |
| axenic NI | M2 | 6dpi | -0.003 | 4.894 | 4.897 | LogFlux | -0.096 | 0.464 | 5.069 | #NAME? | 0.057 | Welch Two Sample t-test | less | 1.000 | |
| axenic NI | M3 | 6dpi | -0.106 | 4.894 | 5.001 | LogFlux | -2.225 | 0.047 | 3.734 | #NAME? | -0.002 | Welch Two Sample t-test | less | 0.854 | * |
| axenic NI | M4 | 6dpi | -0.110 | 4.894 | 5.004 | LogFlux | -1.612 | 0.098 | 3.346 | #NAME? | 0.044 | Welch Two Sample t-test | less | 1.000 | |
| axenic NI | M5 | 6dpi | -0.596 | 4.894 | 5.490 | LogFlux | -2.485 | 0.044 | 3.026 | #NAME? | -0.034 | Welch Two Sample t-test | less | 0.793 | * |
| axenic NI | M6 | 6dpi | -0.521 | 4.894 | 5.415 | LogFlux | -4.250 | 0.011 | 3.103 | #NAME? | -0.236 | Welch Two Sample t-test | less | 0.201 | * |
| axenic NI | M7 | 6dpi | -0.074 | 4.894 | 4.969 | LogFlux | -1.795 | 0.074 | 4.002 | #NAME? | 0.014 | Welch Two Sample t-test | less | 1.000 | |
| axenic NI | M8 | 6dpi | -0.066 | 4.894 | 4.961 | LogFlux | -1.754 | 0.075 | 4.233 | #NAME? | 0.013 | Welch Two Sample t-test | less | 1.000 | |
| axenic NI | M9 | 6dpi | -0.322 | 4.894 | 5.216 | LogFlux | -4.423 | 0.009 | 3.300 | #NAME? | -0.157 | Welch Two Sample t-test | less | 0.158 | ** |
| axenic NI | axenic | 12dpi | -1.029 | 4.955 | 5.984 | LogFlux | -8.539 | 0.001 | 3.121 | #NAME? | -0.750 | Welch Two Sample t-test | less | 0.026 | ** |
| axenic NI | M1 | 12dpi | -0.258 | 4.955 | 5.213 | LogFlux | -6.264 | 0.001 | 4.174 | #NAME? | -0.171 | Welch Two Sample t-test | less | 0.026 | ** |
| axenic NI | M10 | 12dpi | 0.029 | 4.955 | 4.925 | LogFlux | 1.592 | 0.907 | 4.075 | #NAME? | 0.068 | Welch Two Sample t-test | less | 1.000 | |
| axenic NI | M11 | 12dpi | -0.065 | 4.955 | 5.020 | LogFlux | -1.718 | 0.077 | 4.415 | #NAME? | 0.014 | Welch Two Sample t-test | less | 1.000 | |
| axenic NI | M12 | 12dpi | -0.025 | 4.955 | 4.980 | LogFlux | -1.291 | 0.128 | 4.779 | #NAME? | 0.015 | Welch Two Sample t-test | less | 1.000 | |
| axenic NI | M13 | 12dpi | -0.085 | 4.955 | 5.039 | LogFlux | -4.492 | 0.004 | 4.372 | #NAME? | -0.046 | Welch Two Sample t-test | less | 0.080 | ** |
| axenic NI | M14 | 12dpi | -0.118 | 4.955 | 5.073 | LogFlux | -5.644 | 0.001 | 5.479 | #NAME? | -0.077 | Welch Two Sample t-test | less | 0.016 | *** |
| axenic NI | M15 | 12dpi | -0.239 | 4.955 | 5.194 | LogFlux | -2.363 | 0.047 | 3.173 | #NAME? | -0.006 | Welch Two Sample t-test | less | 0.849 | * |
| axenic NI | M16 | 12dpi | -0.392 | 4.955 | 5.347 | LogFlux | -3.037 | 0.027 | 3.105 | #NAME? | -0.092 | Welch Two Sample t-test | less | 0.482 | * |
| axenic NI | M17 | 12dpi | -0.367 | 4.955 | 5.322 | LogFlux | -2.721 | 0.035 | 3.096 | #NAME? | -0.054 | Welch Two Sample t-test | less | 0.630 | * |
| axenic NI | M2 | 12dpi | 0.003 | 4.955 | 4.952 | LogFlux | 0.095 | 0.536 | 5.311 | #NAME? | 0.062 | Welch Two Sample t-test | less | 1.000 | |
| axenic NI | M3 | 12dpi | -0.168 | 4.955 | 5.122 | LogFlux | -4.823 | 0.003 | 4.706 | #NAME? | -0.097 | Welch Two Sample t-test | less | 0.050 | ** |
| axenic NI | M4 | 12dpi | -0.305 | 4.955 | 5.259 | LogFlux | -4.523 | 0.008 | 3.404 | #NAME? | -0.153 | Welch Two Sample t-test | less | 0.138 | ** |
| axenic NI | M5 | 12dpi | -0.423 | 4.955 | 5.378 | LogFlux | -5.053 | 0.006 | 3.255 | #NAME? | -0.232 | Welch Two Sample t-test | less | 0.110 | ** |
| axenic NI | M6 | 12dpi | -0.412 | 4.955 | 5.367 | LogFlux | -3.865 | 0.014 | 3.155 | #NAME? | -0.166 | Welch Two Sample t-test | less | 0.252 | * |
| axenic NI | M7 | 12dpi | -0.019 | 4.955 | 4.973 | LogFlux | -0.728 | 0.247 | 5.875 | #NAME? | 0.032 | Welch Two Sample t-test | less | 1.000 | |
| axenic NI | M8 | 12dpi | -0.060 | 4.955 | 5.015 | LogFlux | -2.232 | 0.034 | 5.751 | #NAME? | -0.007 | Welch Two Sample t-test | less | 0.621 | * |
| axenic NI | M9 | 12dpi | -0.221 | 4.955 | 5.175 | LogFlux | -5.497 | 0.002 | 4.241 | #NAME? | -0.137 | Welch Two Sample t-test | less | 0.040 | ** |

[1] Significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

**Supplemental Table 2:** SynCom-35 strain collection used in the screening experiment with taxonomical[2] and a priori knowledge information [1,3-6].

| Strain_ID [1,2] | Strain number [1, 2] | Phylum [2] | Familly [2] | Genus [2] | SynCom-35 | SynCom-Low | SynCom-High | previous studies [3,4,5] | mean protection score 13dpi [6, 7] | Pilot Experiment |
|---|---|---|---|---|---|---|---|---|---|---|
| 15 | Leaf15 | Proteobacteria | Pseudomonadaceae | Pseudomonas | YES | | | 3 | 100 | YES |
| 21 | Leaf21 | Proteobacteria | Sphingomonadaceae | Sphingomonas | YES | YES | | 3 | 100 | YES |
| 32 | Leaf32 | Proteobacteria | Sphingomonadaceae | Sphingomonas | YES | | | 3 | 23 | YES |
| 68 | Leaf68 | Proteobacteria | Rhizobiaceae | Rhizobium | YES | YES | | 3, 4 | 98 | YES |
| 70 | Leaf70 | Proteobacteria | Xanthomonadaceae | Stenotrophomonas | YES | | | 3, 4 | 13 | YES |
| 76 | Leaf76 | Proteobacteria | Comamonadaceae | Acidovorax | YES | | | 3 | 36 | |
| 82 | Leaf82 | Bacteroidetes | Flavobacteriaceae | Flavobacterium | YES | | | 3, 4 | 26 | |
| 88 | Leaf88 | Proteobacteria | Methylobacteriaceae | Methylobacterium | YES | | YES | 4, 5 | 19 | YES |
| 122 | Leaf122 | Proteobacteria | Methylobacteriaceae | Methylobacterium | YES | | | | 14 | |
| 126 | Leaf126 | Proteobacteria | Oxalobacteraceae | Duganella | YES | | | 4 | 77 | YES |
| 161 | Leaf161 | Actinobacteria | Microbacteriaceae | Microbacterium | YES | | | | 21 | YES |
| 168 | Leaf168 | Proteobacteria | Caulobacteraceae | Brevundimonas | YES | | | | 0 | YES |
| 176 | Leaf176 | Bacteroidetes | Sphingobacteriaceae | Pedobacter | YES | | | 4 | 11 | YES |
| 182 | Leaf182 | Firmicutes | Paenibacillaceae | Brevibacillus | YES | | | 4 | 0 | YES |
| 183 | Leaf183 | Actinobacteria | Microbacteriaceae | Curtobacterium | YES | | | 4 | 46 | YES |
| 187 | Leaf187 | Firmicutes | Bacillales | Exiguobacterium | YES | | | 4, 5 | 11 | YES |
| 189 | Leaf189 | Bacteroidetes | Cytophagaceae | Dyadobacter | YES | | | 4 | 31 | YES |
| 203 | Leaf203 | Actinobacteria | Microbacteriaceae | Microbacterium | YES | | | 3, 4 | 81 | YES |
| 220 | Leaf220 | Proteobacteria | Comamonadaceae | Variovorax | YES | | YES | 3, 4, 5 | 25 | YES |
| 225 | Leaf225 | Actinobacteria | Nocardiaceae | Rhodococcus | YES | | | 4 | 37 | |
| 226 | Leaf226 | Proteobacteria | Sphingomonadaceae | Sphingomonas | YES | | YES | | 49 | YES |
| 254 | Leaf254 | Actinobacteria | Microbacteriaceae | Frigoribacterium | YES | | | | 43 | YES |
| 289 | Leaf289 | Actinobacteria | Nocardioidaceae | Aeromicrobium | YES | | | 3, 4 | 0 | |
| 299 | Leaf299 | Actinobacteria | Microbacteriaceae | Rathayibacter | YES | | | 5 | 14 | |
| 314 | Leaf314 | Actinobacteria | Microbacteriaceae | Plantibacter | YES | | | | 14 | YES |
| 337 | Leaf337 | Actinobacteria | Micrococcaceae | Arthrobacter | YES | | YES | 4 | 68 | YES |
| 339 | Leaf339 | Proteobacteria | Sphingomonadaceae | Sphingomonas | YES | | | | 2 | YES |
| 354 | Leaf354 | Actinobacteria | Nocardiaceae | Williamsia | YES | | YES | 4 | 35 | YES |
| 361 | Leaf361 | Proteobacteria | Methylobacteriaceae | Methylobacterium | YES | YES | | | 5 | YES |
| 371 | Leaf371 | Proteobacteria | Rhizobiaceae | Rhizobium | YES | | | 4, 5 | 29 | |
| 380 | Leaf380 | Actinobacteria | Geodermatophilaceae | Blastococcus | YES | YES | | 4 | 31 | YES |
| 405 | Leaf405 | Bacteroidetes | Flavobacteriaceae | Chryseobacterium | YES | | | 3, 4, 5 | 10 | YES |
| 416 | Leaf416 | Proteobacteria | Methylophilaceae | Methylophilus | YES | | | 3, 4 | 16 | |
| 454 | Leaf454 | Proteobacteria | Aurantimonadaceae | Aurantimonas | YES | | | | 13 | YES |
| 466 | Leaf466 | Proteobacteria | Methylobacteriaceae | Methylobacterium | YES | YES | | | 2 | YES |
| 2 | Leaf2 | Proteobacteria | Sphingomonadaceae | Novosphingobium | | | | | 18 | |
| 3 | Leaf3 | Actinobacteria | Sanguibacteraceae | Sanguibacter | | | | | 17 | YES |
| 4 | Leaf4 | Proteobacteria | Sphingomonadaceae | Sphingomonas | | | | | 15 | |
| 5 | Leaf5 | Proteobacteria | Sphingomonadaceae | Sphingomonas | | | | | 22 | |
| 10 | Leaf10 | Proteobacteria | Sphingomonadaceae | Sphingomonas | | | | | 28 | |
| 13 | Leaf13 | Firmicutes | Bacillaceae | Bacillus | | | | | 8 | YES |
| 17 | Leaf17 | Proteobacteria | Sphingomonadaceae | Sphingomonas | | | | | 7 | YES |
| 26 | Leaf26 | Proteobacteria | Sphingomonadaceae | Sphingobium | | | | | 22 | YES |
| 28 | Leaf28 | Proteobacteria | Sphingomonadaceae | Sphingomonas | | | | | 21 | |
| 30 | Leaf30 | Proteobacteria | Sphingomonadaceae | Sphingomonas | | | | | 32 | |
| 33 | Leaf33 | Proteobacteria | Sphingomonadaceae | Sphingomonas | | | | | 1 | YES |
| 34 | Leaf34 | Proteobacteria | Sphingomonadaceae | Sphingomonas | | | | | 18 | YES |
| 41 | Leaf41 | Bacteroidetes | Sphingobacteriaceae | Pedobacter | | | | | 0 | |
| 42 | Leaf42 | Proteobacteria | Sphingomonadaceae | Sphingomonas | | | | | 4 | YES |
| 49 | Leaf49 | Firmicutes | Bacillaceae | Bacillus | | | | | 56 | |
| 50 | Leaf50 | Proteobacteria | Enterobacteriaceae | Serratia | | | | | 0 | |
| 51 | Leaf51 | Proteobacteria | Enterobacteriaceae | Serratia | | | | | 93 | |
| 53 | Leaf53 | Proteobacteria | Enterobacteriaceae | Erwinia | | | | | 99 | YES |
| 58 | Leaf58 | Proteobacteria | Pseudomonadaceae | Pseudomonas | | | | | 100 | YES |
| 59 | Leaf59 | Proteobacteria | Pseudomonadaceae | Pseudomonas | | | | | 100 | |
| 61 | Leaf61 | Proteobacteria | Oxalobacteraceae | Duganella | | | | | 71 | |
| 62 | Leaf62 | Proteobacteria | Sphingomonadaceae | Sphingomonas | | | | | 3 | |
| 64 | Leaf64 | Proteobacteria | Hyphomicrobiaceae | Devosia | | | | | 9 | YES |
| 67 | Leaf67 | Proteobacteria | Sphingomonadaceae | Sphingomonas | | | | | 38 | |
| 69 | Leaf69 | Actinobacteria | Micrococcaceae | Arthrobacter | | | | | 28 | |
| 72 | Leaf72 | Firmicutes | Paenibacillaceae | Paenibacillus | | | | | 20 | |
| 73 | Leaf73 | Proteobacteria | Comamonadaceae | Acidovorax | | | | | 16 | |
| 75 | Leaf75 | Firmicutes | Bacilli | Bacillus | | | | | NA | |
| 78 | Leaf78 | Proteobacteria | Comamonadaceae | Acidovorax | | | | | 30 | |
| 83 | Leaf83 | Proteobacteria | Pseudomonadaceae | Pseudomonas | | | | | 8 | YES |
| 85 | Leaf85 | Proteobacteria | Methylobacteriaceae | Methylobacterium | | | | | 0 | YES |
| 86 | Leaf86 | Proteobacteria | Methylobacteriaceae | Methylobacterium | | | | | 0 | YES |
| 87 | Leaf87 | Proteobacteria | Methylobacteriaceae | Methylobacterium | | | | | 0 | |
| 90 | Leaf90 | Proteobacteria | Methylobacteriaceae | Methylobacterium | | | | | 16 | |
| 91 | Leaf91 | Proteobacteria | Methylobacteriaceae | Methylobacterium | | | | | 0 | |
| 99 | Leaf99 | Proteobacteria | Methylobacteriaceae | Methylobacterium | | | | | 8 | |
| 106 | Leaf106 | Proteobacteria | Methylobacteriaceae | Methylobacterium | | | | | 18 | |
| 118 | Leaf118 | Proteobacteria | Methylobacteriaceae | Methylobacterium | | | | | 18 | YES |
| 127 | Leaf127 | Proteobacteria | Pseudomonadaceae | Pseudomonas | | | | | 84 | |
| 129 | Leaf129 | Proteobacteria | Pseudomonadaceae | Pseudomonas | | | | | 99 | |
| 130 | Leaf130 | Proteobacteria | Moraxellaceae | Acinetobacter | | | | | 90 | YES |
| 131 | Leaf131 | Proteobacteria | Xanthomonadaceae | Xanthomonas | | | | | 62 | YES |
| 132 | Leaf132 | Bacteroidetes | Sphingobacteriaceae | Pedobacter | | | | | 2 | |
| 137 | Leaf137 | Actinobacteria | Micrococcaceae | Arthrobacter | | | | | 93 | |
| 139 | Leaf139 | Proteobacteria | Oxalobacteraceae | Massilia | | | | | 7 | |
| 141 | Leaf141 | Actinobacteria | Micrococcaceae | Arthrobacter | | | | | 70 | YES |
| 145 | Leaf145 | Actinobacteria | Micrococcaceae | Arthrobacter | | | | | 92 | YES |
| 151 | Leaf151 | Actinobacteria | Microbacteriaceae | Microbacterium | | | | | 74 | |
| 160 | Leaf160 | Proteobacteria | Comamonadaceae | Acidovorax | | | | | 79 | YES |
| 164 | Leaf164 | Actinobacteria | Microbacteriaceae | Rathayibacter | | | | | 25 | YES |
| 177 | Leaf177 | Proteobacteria | Burkholderiaceae | Burkholderia | | | | | 99 | YES |
| 180 | Leaf180 | Bacteroidetes | Flavobacteriaceae | Chryseobacterium | | | | | 13 | |
| 185 | Leaf185 | Actinobacteria | Microbacteriaceae | Rathayibacter | | | | | 46 | |
| 201 | Leaf201 | Bacteroidetes | Flavobacteriaceae | Chryseobacterium | | | | | 7 | YES |
| 208 | Leaf208 | Proteobacteria | Sphingomonadaceae | Sphingomonas | | | | | 13 | |
| 216 | Leaf216 | Bacteroidetes | Sphingobacteriaceae | Pedobacter | | | | | 28 | YES |
| 222 | Leaf222 | Actinobacteria | Microbacteriaceae | Agromyces | | | | | 9 | |
| 231 | Leaf231 | Proteobacteria | Sphingomonadaceae | Sphingomonas | | | | | 32 | |
| 233 | Leaf233 | Actinobacteria | Nocardiaceae | Rhodococcus | | | | | 75 | YES |
| 234 | Leaf234 | Actinobacteria | Micrococcaceae | Arthrobacter | | | | | 11 | |
| 242 | Leaf242 | Proteobacteria | Sphingomonadaceae | Sphingomonas | | | | | 39 | |
| 245 | Leaf245 | Actinobacteria | Nocardioidaceae | Aeromicrobium | | | | | 0 | YES |
| 250 | Leaf250 | Bacteroidetes | Sphingobacteriaceae | Pedobacter | | | | | 0 | |
| 257 | Leaf257 | Proteobacteria | Sphingomonadaceae | Sphingomonas | | | | | 78 | |
| 261 | Leaf261 | Actinobacteria | Microbacteriaceae | Curtobacterium | | | | | 36 | YES |

Supplemental Table 2 continued

| | | | | | | |
|---|---|---|---|---|---|---|
| 262 | Leaf262 | Proteobacteria | Rhizobiaceae | Rhizobium | 87 | YES |
| 263 | Leaf263 | Actinobacteria | Microbacteriaceae | Clavibacter | 10 | |
| 264 | Leaf264 | Actinobacteria | Microbacteriaceae | Leifsonia | 5 | |
| 265 | Leaf265 | Proteobacteria | Comamonadaceae | Pseudorhodoferax | 43 | |
| 267 | Leaf267 | Proteobacteria | Comamonadaceae | Variovorax | 16 | YES |
| 274 | Leaf274 | Proteobacteria | Comamonadaceae | Pseudorhodoferax | 48 | YES |
| 280 | Leaf280 | Proteobacteria | Caulobacteraceae | Brevundimonas | 0 | YES |
| 285 | Leaf285 | Actinobacteria | Nocardioidaceae | Nocardioides | 1 | |
| 288 | Leaf288 | Actinobacteria | Microbacteriaceae | Microbacterium | 13 | |
| 304 | Leaf304 | Actinobacteria | Microbacteriaceae | Frondihabitans | 40 | |
| 306 | Leaf306 | Proteobacteria | Rhizobiaceae | Rhizobium | 21 | |
| 311 | Leaf311 | Proteobacteria | Rhizobiaceae | Rhizobium | 80 | |
| 313 | Leaf313 | Bacteroidetes | Flavobacteriaceae | Chryseobacterium | 0 | |
| 324 | Leaf324 | Proteobacteria | Aurantimonadaceae | Aureimonas | 29 | |
| 326 | Leaf326 | Deinococcus-Thermus | Deinococcaceae | Deinococcus | 11 | YES |
| 334 | Leaf334 | Actinobacteria | Cellulomonadaceae | Cellulomonas | 19 | |
| 335 | Leaf335 | Actinobacteria | Microbacteriaceae | Agreia | 54 | |
| 336 | Leaf336 | Actinobacteria | Microbacteriaceae | Leifsonia | 25 | |
| 343 | Leaf343 | Proteobacteria | Sphingomonadaceae | Sphingomonas | 25 | YES |
| 344 | Leaf344 | Proteobacteria | Bradyrhizobiaceae | Bosea | 21 | |
| 350 | Leaf350 | Actinobacteria | Nocardioidaceae | Aeromicrobium | 10 | YES |
| 351 | Leaf351 | Actinobacteria | Microbacteriaceae | Microbacterium | 69 | YES |
| 357 | Leaf357 | Proteobacteria | Sphingomonadaceae | Sphingomonas | 4 | |
| 359 | Leaf359 | Bacteroidetes | Flavobacteriaceae | Flavobacterium | 0 | |
| 363 | Leaf363 | Proteobacteria | Caulobacteraceae | Brevundimonas | 4 | |
| 369 | Leaf369 | Actinobacteria | Geodermatophilaceae | Geodermatophilus | 42 | |
| 391 | Leaf391 | Proteobacteria | Rhizobiaceae | Rhizobium | 39 | |
| 394 | Leaf394 | Bacteroidetes | Flavobacteriaceae | Chryseobacterium | 0 | |
| 396 | Leaf396 | Proteobacteria | Bradyrhizobiaceae | Bradyrhizobium | 12 | YES |
| 400 | Leaf400 | Proteobacteria | Comamonadaceae | Acidovorax | 11 | YES |
| 406 | Leaf406 | Firmicutes | Bacillaceae | Bacillus | 10 | YES |
| 412 | Leaf412 | Proteobacteria | Sphingomonadaceae | Sphingomonas | 21 | |
| 420 | Leaf420 | Proteobacteria | Hyphomicrobiaceae | Devosia | 12 | |
| 427 | Leaf427 | Proteobacteria | Aurantimonadaceae | Aurantimonas | 45 | |
| 434 | Leaf434 | Proteobacteria | Pseudomonadaceae | Pseudomonas | 100 | YES |
| 443 | Leaf443 | Proteobacteria | Aurantimonadaceae | Aurantimonas | 6 | |
| 456 | Leaf456 | Proteobacteria | Methylobacteriaceae | Methylobacterium | 5 | YES |

[1] S. Pfeilmeier et al., The plant NADPH oxidase RBOHD is required for microbiota homeostasis in leaves. Nat Microbiol 6, 852-864 (2021).

[2] Y. Bai et al., Functional overlap of the Arabidopsis leaf and root microbiota. Nature 528, 364-369 (2015).

[3] B. A. Maier et al., A general non-self response as part of plant immunity. Nat Plants 7, 696-705 (2021).

[4] C. I. Carlström et al., Synthetic microbiota reveal priority effects and keystone strains in the Arabidopsis phyllosphere. Nat Ecol Evol 3, 1445-1454 (2019).

[5] M. Schäfer, C. M. Vogel, M. Bortfeld-Miller, M. Mittelviefhaus, J. A. Vorholt, Mapping phyllosphere microbiota interactions in planta to establish genotype–phenotype relationships. Nature Microbiology 7, 856-867 (2022).

[6] C. M. Vogel, D. B. Potthoff, M. Schafer, N. Barandun, J. A. Vorholt, Protective role of the Arabidopsis leaf microbiota against a bacterial pathogen. Nat Microbiol 6, 1537-1548 (2021).

[7] "Protection score based on distribution of disease relative to control plants with 0 corresponding to no improvement in plant phenotype relative to axenic controls and 100 corresponding to all plants completely healfth"

**Supplemental Table 3:** Best linear mixed models with the $log_{10}$-transformed pathogen colonization as dependent variable, and either the $log_{10}$-transformed commensal colonization (centred), or evenness as fixed effects. Best models are those with a delta AIC (Akaike information criterion) bellow four. Most complete models included box and experimental random effects with the first nested in the second. Measurement units and transformations of variables are indicated between squared brackets. Abbreviations: AIC, Akaike information criterion; LogCFUgFW, $log_{10}$-transformed colonization in colony forming units per gram of plant fresh weight.

**Formula:** Pst [LogCFUgFW] ~ Commensals [centered LogCFUgFW] + (Commensals [centered LogCFUgFW] | Box)

**fit:** Restricted maximum likelihood (REML)
**t-tests:** Satterthwaite's method
**REML criterion at convergence:** 1471
**AIC:** 1483.048484
**Delta AIC:** 0

**Scaled residuals:**

| Minimum | Quartile 1 | Median | Quartile 3 | Maximum |
|---|---|---|---|---|
| -3.3245 | -0.4591 | 0.0557 | 0.5127 | 3.4797 |

**Random effects:**

| Groups | Name | Variance | Standard deviation | Correlation |
|---|---|---|---|---|
| Box | Intercept | 1.2208 | 1.1049 | |
| | Commensals [centered LogCFUgFW] | 0.3514 | 0.5928 | -0.5 |
| Residual | | 0.4937 | 0.7026 | |

**Number of observation:** 531

**groups:**

| | |
|---|---|
| Box | 134 |

**Fixed effects:**

| | Estimate | Standard error | Degrees of freedom | t-value | P-value |
|---|---|---|---|---|---|
| Intercept | 6.7091 | 0.1009 | 130.3883 | 66.46 | <2e-16 |
| Commensals [centered LogCFUgFW] | 1.4451 | 0.1327 | 73.2999 | 10.89 | <2e-16 |

**Correlation of Fixed Effects:** -0.194

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**Formula:** Pst [LogCFUgFW] ~ Commensals [centered LogCFUgFW] + (1 | Experiment) + (Commensals [centered LogCFUgFW] | Experiment:Box)

**Linear mixed model fit:** Restricted maximum likelihood (REML)
**t-tests:** Satterthwaite's method
**REML criterion at convergence:** 1471
**AIC:** 1485.048484
**Delta AIC:** 1.999999999

**Scaled residuals:**

| Minimum | Quartile 1 | Median | Quartile 3 | Maximum |
|---|---|---|---|---|
| -3.3245 | -0.4591 | 0.0557 | 0.5127 | 3.4797 |

**Random effects:**

| Groups | Name | Variance | Standard deviation | Correlation |
|---|---|---|---|---|
| Experiment:Box | Intercept | 1.2208 | 1.1049 | |
| | Commensals [centered LogCFUgFW] | 0.3514 | 0.5928 | -0.5 |
| metadata__Experiment | Intercept | 0 | 0 | |
| Residual | | 0.4937 | 0.7026 | |

**Number of obs:** 531

**groups:**

| | |
|---|---|
| Experiment:Box | 134 |
| Experiment | 2 |

**Fixed effects:**

| | Estimate | Standard error | Degrees of freedom | t-value | P-value |
|---|---|---|---|---|---|
| Intercept | 6.7091 | 0.1009 | 130.3886 | 66.46 | <2e-16 |
| Commensals [centered LogCFUgFW] | 1.4451 | 0.1327 | 73.3 | 10.89 | <2e-16 |

**Correlation of Fixed Effects:** -0.194

**Supplemental Table 4:** Percentage of bootstrapped datasets with a detected minima used to classify samples between pathogen protected and non-protected. Data were bootstrapped 1,000 times.

| Experiment | Frequency with medians per microbox | Frequency with measurements per plant |
|---|---|---|
| Experiment 1 | 0.938 | 0.972 |
| Experiment 2 | 0.988 | 0.999 |
| Experiment 3 | 0.991 | 0.927 |

**Supplemental Table 5:** Pathogen colonization [$\log_{10}$(CFU $g^{-1}$ fresh weight)] corresponding to the local minima in density curves of the different experiments. Measurements were either individual plant measurements or medians of plant measurements for each box.

| Experiment | Median per microbox | Individual plants |
|---|---|---|
| Experiment 1 | 6.463583274 | 6.275468375 |
| Experiment 2 | 6.064341892 | 5.73234855 |
| Experiment 3 | 6.471816486 | 6.420945727 |

**Supplemental Table 6:** Performances of the classification analyses. The results are the medians calculated across the eight seeds used to initiate pseudo-random processes. Abbreviations: ML, machine learning; GLMNet, elastic-net regularized generalized linear models; RF, random forest; NA, non-applicable.

| Pathogen colonization prediction | Predicted variable on | Feature Matrix | ML Algorithm | Accuracy | Kappa | Specificity | Precision | Recall | F1 | Balanced Accuracy | True Positive | False Positive | True Negative | False Negative |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Classification | Individual | Commensal Colonization | GLMNet | 0.870 | 0.720 | 0.838 | 0.723 | 0.942 | 0.818 | 0.890 | 81 | 31 | 160 | 5 |
| Classification | Individual | Commensal Colonization | RF | 0.839 | 0.623 | 0.887 | 0.746 | 0.733 | 0.739 | 0.810 | 63 | 21.5 | 169.5 | 23 |
| Classification | Individual | Presence/ Absence | GLMNet | 0.870 | 0.720 | 0.838 | 0.723 | 0.942 | 0.818 | 0.890 | 81 | 31 | 160 | 5 |
| Classification | Individual | Presence/ Absence | RF | 0.870 | 0.720 | 0.838 | 0.723 | 0.942 | 0.818 | 0.890 | 81 | 31 | 160 | 5 |
| Classification | Median | Presence/ Absence | GLMNet | 0.929 | 0.847 | 0.894 | 0.821 | 1.000 | 0.902 | 0.947 | 23 | 5 | 42 | 0 |
| Classification | Median | Presence/ Absence | RF | 0.929 | 0.847 | 0.894 | 0.821 | 1.000 | 0.902 | 0.947 | 23 | 5 | 42 | 0 |
| Classification | Median | NA | Random | 0.514 | -0.016 | 0.664 | 0.424 | 0.321 | 0.364 | 0.492 | 9.75 | 13.25 | 26.25 | 20.75 |
| Classification | Individual | NA | Random | 0.564 | 0.013 | 0.694 | 0.355 | 0.319 | 0.336 | 0.506 | 30.5 | 55.5 | 125.75 | 65.25 |

**Supplemental Table 7:** Performances of the regression analyses. The results are the medians calculated across the eight seeds used to initiate pseudo-random processes. The global-average algorithm consists in predicting the pathogen colonization to be equal to the average colonization of the entire dataset. Abbreviations: RMSE, root-mean-squared error; ML, machine learning; GLMNet, elastic-net regularized generalized linear models; RF, random forest; NA, non-applicable.

| Pathogen colonization prediction | Predicted variable on | Feature Matrix | ML Algorithm | RMSE |
|---|---|---|---|---|
| Regression | Individual | CommensalColonization | GLMNet | 1.055 |
| Regression | Individual | CommensalColonization | RF | 0.972 |
| Regression | Individual | PresenceAbsence | GLMNet | 1.045 |
| Regression | Individual | PresenceAbsence | RF | 0.998 |
| Regression | Median | PresenceAbsence | GLMNet | 0.868 |
| Regression | Median | PresenceAbsence | RF | 0.788 |
| Regression | Median | NA | Global average | 1.495 |
| Regression | Individual | NA | Global average | 1.557 |

**Supplemental Table 8:** Relative importance of strains in all machine learning analyses. The results are the medians calculated across the eight seeds used for each model-method combination. Abbreviations: ML, machine learning; GLMNet, elastic-net regularized generalized linear models; RF, random forest.

| Pathogen colonization prediction | Predicted variable on | Feature Matrix | ML Algorithm | Strain | Relative Feature Importance |
|---|---|---|---|---|---|
| Classification | Median | Presence/ Absence | RF | Leaf122 | 2.400 |
| Classification | Individual | Presence/ Absence | RF | Leaf122 | 2.425 |
| Regression | Median | Presence/ Absence | RF | Leaf122 | 1.318 |
| Regression | Individual | Presence/ Absence | RF | Leaf122 | 1.573 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf122 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf122 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf122 | 0.000 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf122 | 0.000 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf122 | 4.868 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf122 | 0.000 |
| Regression | Individual | Commensal Colonization | RF | Leaf122 | 4.364 |
| Classification | Individual | Commensal Colonization | RF | Leaf122 | 4.375 |
| Classification | Median | Presence/ Absence | RF | Leaf126 | 1.426 |
| Classification | Individual | Presence/ Absence | RF | Leaf126 | 1.543 |
| Regression | Median | Presence/ Absence | RF | Leaf126 | 0.658 |
| Regression | Individual | Presence/ Absence | RF | Leaf126 | 0.771 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf126 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf126 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf126 | 0.000 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf126 | 0.001 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf126 | 3.143 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf126 | 0.000 |
| Regression | Individual | Commensal Colonization | RF | Leaf126 | 1.477 |
| Classification | Individual | Commensal Colonization | RF | Leaf126 | 4.476 |
| Classification | Median | Presence/ Absence | RF | Leaf15 | 75.106 |
| Classification | Individual | Presence/ Absence | RF | Leaf15 | 65.585 |
| Regression | Median | Presence/ Absence | RF | Leaf15 | 73.880 |
| Regression | Individual | Presence/ Absence | RF | Leaf15 | 71.260 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf15 | 83.573 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf15 | 86.320 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf15 | 88.325 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf15 | 92.948 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf15 | 100.000 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf15 | 91.312 |
| Regression | Individual | Commensal Colonization | RF | Leaf15 | 69.267 |
| Classification | Individual | Commensal Colonization | RF | Leaf15 | 62.728 |
| Classification | Median | Presence/ Absence | RF | Leaf161 | 0.666 |
| Classification | Individual | Presence/ Absence | RF | Leaf161 | 1.245 |
| Regression | Median | Presence/ Absence | RF | Leaf161 | 0.237 |
| Regression | Individual | Presence/ Absence | RF | Leaf161 | 1.058 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf161 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf161 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf161 | 0.000 |

Supplemental Table 8 continued

| | | | | | |
|---|---|---|---|---|---|
| Regression | Individual | Presence/ Absence | GLMNet | Leaf161 | 0.000 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf161 | 3.523 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf161 | 0.000 |
| Regression | Individual | Commensal Colonization | RF | Leaf161 | 5.262 |
| Classification | Individual | Commensal Colonization | RF | Leaf161 | 4.775 |
| Classification | Median | Presence/ Absence | RF | Leaf168 | 4.804 |
| Classification | Individual | Presence/ Absence | RF | Leaf168 | 4.687 |
| Regression | Median | Presence/ Absence | RF | Leaf168 | 1.831 |
| Regression | Individual | Presence/ Absence | RF | Leaf168 | 1.865 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf168 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf168 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf168 | 0.000 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf168 | 2.148 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf168 | 0.000 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf168 | 0.000 |
| Regression | Individual | Commensal Colonization | RF | Leaf168 | 3.438 |
| Classification | Individual | Commensal Colonization | RF | Leaf168 | 6.571 |
| Classification | Median | Presence/ Absence | RF | Leaf176 | 0.717 |
| Classification | Individual | Presence/ Absence | RF | Leaf176 | 2.345 |
| Regression | Median | Presence/ Absence | RF | Leaf176 | 1.567 |
| Regression | Individual | Presence/ Absence | RF | Leaf176 | 1.662 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf176 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf176 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf176 | 0.000 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf176 | 6.534 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf176 | 2.471 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf176 | 0.000 |
| Regression | Individual | Commensal Colonization | RF | Leaf176 | 2.098 |
| Classification | Individual | Commensal Colonization | RF | Leaf176 | 7.498 |
| Classification | Median | Presence/ Absence | RF | Leaf182 | 1.840 |
| Classification | Individual | Presence/ Absence | RF | Leaf182 | 5.657 |
| Regression | Median | Presence/ Absence | RF | Leaf182 | 4.044 |
| Regression | Individual | Presence/ Absence | RF | Leaf182 | 4.056 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf182 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf182 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf182 | 0.000 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf182 | 0.000 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf182 | 2.202 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf182 | 0.000 |
| Regression | Individual | Commensal Colonization | RF | Leaf182 | 4.048 |
| Classification | Individual | Commensal Colonization | RF | Leaf182 | 8.109 |
| Classification | Median | Presence/ Absence | RF | Leaf183 | 0.853 |
| Classification | Individual | Presence/ Absence | RF | Leaf183 | 1.764 |
| Regression | Median | Presence/ Absence | RF | Leaf183 | 0.968 |
| Regression | Individual | Presence/ Absence | RF | Leaf183 | 1.408 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf183 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf183 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf183 | 0.000 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf183 | 0.000 |

Supplemental Table 8 continued

| | | | | | |
|---|---|---|---|---|---|
| Regression | Individual | Commensal Colonization | GLMNet | Leaf183 | 3.710 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf183 | 0.000 |
| Regression | Individual | Commensal Colonization | RF | Leaf183 | 5.253 |
| Classification | Individual | Commensal Colonization | RF | Leaf183 | 4.271 |
| Classification | Median | Presence/ Absence | RF | Leaf187 | 0.451 |
| Classification | Individual | Presence/ Absence | RF | Leaf187 | 0.357 |
| Regression | Median | Presence/ Absence | RF | Leaf187 | 0.000 |
| Regression | Individual | Presence/ Absence | RF | Leaf187 | 0.162 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf187 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf187 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf187 | 0.000 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf187 | 0.000 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf187 | 4.661 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf187 | 0.000 |
| Regression | Individual | Commensal Colonization | RF | Leaf187 | 1.976 |
| Classification | Individual | Commensal Colonization | RF | Leaf187 | 3.393 |
| Classification | Median | Presence/ Absence | RF | Leaf189 | 2.432 |
| Classification | Individual | Presence/ Absence | RF | Leaf189 | 1.405 |
| Regression | Median | Presence/ Absence | RF | Leaf189 | 2.906 |
| Regression | Individual | Presence/ Absence | RF | Leaf189 | 2.089 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf189 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf189 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf189 | 0.000 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf189 | 0.001 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf189 | 0.000 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf189 | 0.000 |
| Regression | Individual | Commensal Colonization | RF | Leaf189 | 2.029 |
| Classification | Individual | Commensal Colonization | RF | Leaf189 | 4.522 |
| Classification | Median | Presence/ Absence | RF | Leaf203 | 4.291 |
| Classification | Individual | Presence/ Absence | RF | Leaf203 | 3.547 |
| Regression | Median | Presence/ Absence | RF | Leaf203 | 2.419 |
| Regression | Individual | Presence/ Absence | RF | Leaf203 | 3.464 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf203 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf203 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf203 | 5.725 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf203 | 9.562 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf203 | 5.634 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf203 | 0.000 |
| Regression | Individual | Commensal Colonization | RF | Leaf203 | 7.560 |
| Classification | Individual | Commensal Colonization | RF | Leaf203 | 9.435 |
| Classification | Median | Presence/ Absence | RF | Leaf21 | 1.601 |
| Classification | Individual | Presence/ Absence | RF | Leaf21 | 1.498 |
| Regression | Median | Presence/ Absence | RF | Leaf21 | 1.096 |
| Regression | Individual | Presence/ Absence | RF | Leaf21 | 1.632 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf21 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf21 | 0.014 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf21 | 1.973 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf21 | 9.384 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf21 | 13.227 |

Supplemental Table 8 continued

| | | | | | |
|---|---|---|---|---|---|
| Classification | Individual | Commensal Colonization | GLMNet | Leaf21 | 2.528 |
| Regression | Individual | Commensal Colonization | RF | Leaf21 | 4.527 |
| Classification | Individual | Commensal Colonization | RF | Leaf21 | 4.127 |
| Classification | Median | Presence/ Absence | RF | Leaf220 | 1.307 |
| Classification | Individual | Presence/ Absence | RF | Leaf220 | 2.613 |
| Regression | Median | Presence/ Absence | RF | Leaf220 | 1.680 |
| Regression | Individual | Presence/ Absence | RF | Leaf220 | 4.904 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf220 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf220 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf220 | 4.721 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf220 | 11.819 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf220 | 13.748 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf220 | 0.235 |
| Regression | Individual | Commensal Colonization | RF | Leaf220 | 9.037 |
| Classification | Individual | Commensal Colonization | RF | Leaf220 | 8.932 |
| Classification | Median | Presence/ Absence | RF | Leaf225 | 1.360 |
| Classification | Individual | Presence/ Absence | RF | Leaf225 | 2.457 |
| Regression | Median | Presence/ Absence | RF | Leaf225 | 0.755 |
| Regression | Individual | Presence/ Absence | RF | Leaf225 | 0.704 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf225 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf225 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf225 | 0.963 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf225 | 0.000 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf225 | 0.273 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf225 | 0.000 |
| Regression | Individual | Commensal Colonization | RF | Leaf225 | 1.340 |
| Classification | Individual | Commensal Colonization | RF | Leaf225 | 6.001 |
| Classification | Median | Presence/ Absence | RF | Leaf226 | 2.917 |
| Classification | Individual | Presence/ Absence | RF | Leaf226 | 3.734 |
| Regression | Median | Presence/ Absence | RF | Leaf226 | 1.329 |
| Regression | Individual | Presence/ Absence | RF | Leaf226 | 2.485 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf226 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf226 | 6.931 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf226 | 8.451 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf226 | 10.522 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf226 | 15.680 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf226 | 7.508 |
| Regression | Individual | Commensal Colonization | RF | Leaf226 | 4.403 |
| Classification | Individual | Commensal Colonization | RF | Leaf226 | 4.824 |
| Classification | Median | Presence/ Absence | RF | Leaf254 | 0.796 |
| Classification | Individual | Presence/ Absence | RF | Leaf254 | 1.334 |
| Regression | Median | Presence/ Absence | RF | Leaf254 | 0.312 |
| Regression | Individual | Presence/ Absence | RF | Leaf254 | 0.656 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf254 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf254 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf254 | 0.000 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf254 | 6.250 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf254 | 11.747 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf254 | 0.000 |

Supplemental Table 8 continued

| | | | | | |
|---|---|---|---|---|---|
| Regression | Individual | Commensal Colonization | RF | Leaf254 | 4.253 |
| Classification | Individual | Commensal Colonization | RF | Leaf254 | 5.770 |
| Classification | Median | Presence/ Absence | RF | Leaf289 | 0.710 |
| Classification | Individual | Presence/ Absence | RF | Leaf289 | 1.110 |
| Regression | Median | Presence/ Absence | RF | Leaf289 | 1.880 |
| Regression | Individual | Presence/ Absence | RF | Leaf289 | 4.163 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf289 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf289 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf289 | 10.941 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf289 | 15.246 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf289 | 17.484 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf289 | 0.000 |
| Regression | Individual | Commensal Colonization | RF | Leaf289 | 5.483 |
| Classification | Individual | Commensal Colonization | RF | Leaf289 | 2.075 |
| Classification | Median | Presence/ Absence | RF | Leaf299 | 0.428 |
| Classification | Individual | Presence/ Absence | RF | Leaf299 | 0.093 |
| Regression | Median | Presence/ Absence | RF | Leaf299 | 0.460 |
| Regression | Individual | Presence/ Absence | RF | Leaf299 | 0.425 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf299 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf299 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf299 | 0.000 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf299 | 0.000 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf299 | 0.000 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf299 | 0.000 |
| Regression | Individual | Commensal Colonization | RF | Leaf299 | 0.000 |
| Classification | Individual | Commensal Colonization | RF | Leaf299 | 0.000 |
| Classification | Median | Presence/ Absence | RF | Leaf314 | 4.285 |
| Classification | Individual | Presence/ Absence | RF | Leaf314 | 1.551 |
| Regression | Median | Presence/ Absence | RF | Leaf314 | 5.102 |
| Regression | Individual | Presence/ Absence | RF | Leaf314 | 3.906 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf314 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf314 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf314 | 9.523 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf314 | 11.263 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf314 | 16.466 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf314 | 0.691 |
| Regression | Individual | Commensal Colonization | RF | Leaf314 | 5.467 |
| Classification | Individual | Commensal Colonization | RF | Leaf314 | 2.912 |
| Classification | Median | Presence/ Absence | RF | Leaf32 | 1.325 |
| Classification | Individual | Presence/ Absence | RF | Leaf32 | 1.807 |
| Regression | Median | Presence/ Absence | RF | Leaf32 | 1.533 |
| Regression | Individual | Presence/ Absence | RF | Leaf32 | 2.007 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf32 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf32 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf32 | 2.133 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf32 | 6.726 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf32 | 5.705 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf32 | 0.301 |
| Regression | Individual | Commensal Colonization | RF | Leaf32 | 5.521 |

Supplemental Table 8 continued

| | | | | | |
|---|---|---|---|---|---|
| Classification | Individual | Commensal Colonization | RF | Leaf32 | 4.128 |
| Classification | Median | Presence/ Absence | RF | Leaf337 | 12.089 |
| Classification | Individual | Presence/ Absence | RF | Leaf337 | 16.652 |
| Regression | Median | Presence/ Absence | RF | Leaf337 | 8.490 |
| Regression | Individual | Presence/ Absence | RF | Leaf337 | 10.053 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf337 | 8.551 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf337 | 33.044 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf337 | 18.272 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf337 | 19.940 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf337 | 18.754 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf337 | 38.353 |
| Regression | Individual | Commensal Colonization | RF | Leaf337 | 11.774 |
| Classification | Individual | Commensal Colonization | RF | Leaf337 | 24.250 |
| Classification | Median | Presence/ Absence | RF | Leaf339 | 1.896 |
| Classification | Individual | Presence/ Absence | RF | Leaf339 | 3.533 |
| Regression | Median | Presence/ Absence | RF | Leaf339 | 0.932 |
| Regression | Individual | Presence/ Absence | RF | Leaf339 | 2.819 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf339 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf339 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf339 | 0.000 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf339 | 0.000 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf339 | 0.366 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf339 | 0.000 |
| Regression | Individual | Commensal Colonization | RF | Leaf339 | 3.342 |
| Classification | Individual | Commensal Colonization | RF | Leaf339 | 5.501 |
| Classification | Median | Presence/ Absence | RF | Leaf354 | 0.296 |
| Classification | Individual | Presence/ Absence | RF | Leaf354 | 1.310 |
| Regression | Median | Presence/ Absence | RF | Leaf354 | 0.398 |
| Regression | Individual | Presence/ Absence | RF | Leaf354 | 1.084 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf354 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf354 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf354 | 1.132 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf354 | 7.118 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf354 | 6.604 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf354 | 0.000 |
| Regression | Individual | Commensal Colonization | RF | Leaf354 | 1.466 |
| Classification | Individual | Commensal Colonization | RF | Leaf354 | 1.033 |
| Classification | Median | Presence/ Absence | RF | Leaf361 | 4.360 |
| Classification | Individual | Presence/ Absence | RF | Leaf361 | 4.418 |
| Regression | Median | Presence/ Absence | RF | Leaf361 | 6.036 |
| Regression | Individual | Presence/ Absence | RF | Leaf361 | 4.862 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf361 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf361 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf361 | 2.368 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf361 | 8.895 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf361 | 8.542 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf361 | 0.000 |
| Regression | Individual | Commensal Colonization | RF | Leaf361 | 4.005 |
| Classification | Individual | Commensal Colonization | RF | Leaf361 | 4.296 |

Supplemental Table 8 continued

| | | | | | |
|---|---|---|---|---|---|
| Classification | Median | Presence/ Absence | RF | Leaf371 | 6.267 |
| Classification | Individual | Presence/ Absence | RF | Leaf371 | 10.289 |
| Regression | Median | Presence/ Absence | RF | Leaf371 | 25.313 |
| Regression | Individual | Presence/ Absence | RF | Leaf371 | 23.975 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf371 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf371 | 18.048 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf371 | 30.716 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf371 | 39.019 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf371 | 38.376 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf371 | 19.634 |
| Regression | Individual | Commensal Colonization | RF | Leaf371 | 27.455 |
| Classification | Individual | Commensal Colonization | RF | Leaf371 | 16.596 |
| Classification | Median | Presence/ Absence | RF | Leaf380 | 1.831 |
| Classification | Individual | Presence/ Absence | RF | Leaf380 | 2.812 |
| Regression | Median | Presence/ Absence | RF | Leaf380 | 0.817 |
| Regression | Individual | Presence/ Absence | RF | Leaf380 | 1.738 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf380 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf380 | 4.980 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf380 | 0.000 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf380 | 5.909 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf380 | 10.778 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf380 | 4.686 |
| Regression | Individual | Commensal Colonization | RF | Leaf380 | 3.762 |
| Classification | Individual | Commensal Colonization | RF | Leaf380 | 7.512 |
| Classification | Median | Presence/ Absence | RF | Leaf405 | 1.427 |
| Classification | Individual | Presence/ Absence | RF | Leaf405 | 0.400 |
| Regression | Median | Presence/ Absence | RF | Leaf405 | 0.781 |
| Regression | Individual | Presence/ Absence | RF | Leaf405 | 0.000 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf405 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf405 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf405 | 5.867 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf405 | 4.735 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf405 | 2.959 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf405 | 0.000 |
| Regression | Individual | Commensal Colonization | RF | Leaf405 | 0.452 |
| Classification | Individual | Commensal Colonization | RF | Leaf405 | 1.984 |
| Classification | Median | Presence/ Absence | RF | Leaf416 | 4.987 |
| Classification | Individual | Presence/ Absence | RF | Leaf416 | 3.000 |
| Regression | Median | Presence/ Absence | RF | Leaf416 | 1.362 |
| Regression | Individual | Presence/ Absence | RF | Leaf416 | 2.608 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf416 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf416 | 0.371 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf416 | 4.668 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf416 | 1.427 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf416 | 6.022 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf416 | 3.723 |
| Regression | Individual | Commensal Colonization | RF | Leaf416 | 2.816 |
| Classification | Individual | Commensal Colonization | RF | Leaf416 | 8.306 |
| Classification | Median | Presence/ Absence | RF | Leaf454 | 2.634 |

Supplemental Table 8 continued

| | | | | | |
|---|---|---|---|---|---|
| Classification | Individual | Presence/ Absence | RF | Leaf454 | 3.276 |
| Regression | Median | Presence/ Absence | RF | Leaf454 | 1.388 |
| Regression | Individual | Presence/ Absence | RF | Leaf454 | 1.476 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf454 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf454 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf454 | 0.000 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf454 | 0.000 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf454 | 1.163 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf454 | 0.000 |
| Regression | Individual | Commensal Colonization | RF | Leaf454 | 3.911 |
| Classification | Individual | Commensal Colonization | RF | Leaf454 | 5.275 |
| Classification | Median | Presence/ Absence | RF | Leaf466 | 3.587 |
| Classification | Individual | Presence/ Absence | RF | Leaf466 | 2.225 |
| Regression | Median | Presence/ Absence | RF | Leaf466 | 1.256 |
| Regression | Individual | Presence/ Absence | RF | Leaf466 | 1.954 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf466 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf466 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf466 | 0.382 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf466 | 0.000 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf466 | 1.736 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf466 | 0.000 |
| Regression | Individual | Commensal Colonization | RF | Leaf466 | 2.619 |
| Classification | Individual | Commensal Colonization | RF | Leaf466 | 4.027 |
| Classification | Median | Presence/ Absence | RF | Leaf68 | 78.935 |
| Classification | Individual | Presence/ Absence | RF | Leaf68 | 72.882 |
| Regression | Median | Presence/ Absence | RF | Leaf68 | 85.650 |
| Regression | Individual | Presence/ Absence | RF | Leaf68 | 81.967 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf68 | 86.568 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf68 | 84.056 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf68 | 94.227 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf68 | 92.114 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf68 | 84.919 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf68 | 78.114 |
| Regression | Individual | Commensal Colonization | RF | Leaf68 | 86.670 |
| Classification | Individual | Commensal Colonization | RF | Leaf68 | 72.500 |
| Classification | Median | Presence/ Absence | RF | Leaf70 | 0.270 |
| Classification | Individual | Presence/ Absence | RF | Leaf70 | 1.914 |
| Regression | Median | Presence/ Absence | RF | Leaf70 | 1.841 |
| Regression | Individual | Presence/ Absence | RF | Leaf70 | 1.974 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf70 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf70 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf70 | 9.851 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf70 | 13.623 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf70 | 23.284 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf70 | 0.000 |
| Regression | Individual | Commensal Colonization | RF | Leaf70 | 7.836 |
| Classification | Individual | Commensal Colonization | RF | Leaf70 | 6.641 |
| Classification | Median | Presence/ Absence | RF | Leaf76 | 100.000 |
| Classification | Individual | Presence/ Absence | RF | Leaf76 | 100.000 |

Supplemental Table 8 continued

| | | | | | |
|---|---|---|---|---|---|
| Regression | Median | Presence/ Absence | RF | Leaf76 | 100.000 |
| Regression | Individual | Presence/ Absence | RF | Leaf76 | 100.000 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf76 | 100.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf76 | 100.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf76 | 100.000 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf76 | 100.000 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf76 | 99.516 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf76 | 100.000 |
| Regression | Individual | Commensal Colonization | RF | Leaf76 | 100.000 |
| Classification | Individual | Commensal Colonization | RF | Leaf76 | 100.000 |
| Classification | Median | Presence/ Absence | RF | Leaf82 | 0.000 |
| Classification | Individual | Presence/ Absence | RF | Leaf82 | 0.000 |
| Regression | Median | Presence/ Absence | RF | Leaf82 | 1.398 |
| Regression | Individual | Presence/ Absence | RF | Leaf82 | 3.901 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf82 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf82 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf82 | 0.000 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf82 | 7.105 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf82 | 3.684 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf82 | 0.000 |
| Regression | Individual | Commensal Colonization | RF | Leaf82 | 3.905 |
| Classification | Individual | Commensal Colonization | RF | Leaf82 | 1.157 |
| Classification | Median | Presence/ Absence | RF | Leaf88 | 2.192 |
| Classification | Individual | Presence/ Absence | RF | Leaf88 | 1.015 |
| Regression | Median | Presence/ Absence | RF | Leaf88 | 0.823 |
| Regression | Individual | Presence/ Absence | RF | Leaf88 | 1.418 |
| Classification | Median | Presence/ Absence | GLMNet | Leaf88 | 0.000 |
| Classification | Individual | Presence/ Absence | GLMNet | Leaf88 | 0.000 |
| Regression | Median | Presence/ Absence | GLMNet | Leaf88 | 0.000 |
| Regression | Individual | Presence/ Absence | GLMNet | Leaf88 | 1.296 |
| Regression | Individual | Commensal Colonization | GLMNet | Leaf88 | 0.000 |
| Classification | Individual | Commensal Colonization | GLMNet | Leaf88 | 0.000 |
| Regression | Individual | Commensal Colonization | RF | Leaf88 | 0.426 |
| Classification | Individual | Commensal Colonization | RF | Leaf88 | 2.539 |

**Supplemental Table 9:** Relative importance of strains in random forest when varying the global minima used to define the classes in the training dataset (experiments 1 and 2). The dependent variable is the pathogen colonization measured on individual plants, and the independent variables are presence-absence of strains in Mini5SynCom. The local minima consisted in 10 random sampling of the bootstrapped distributions of global minima in experiments 1 and 2.
Abbreviation: Rep, replicate.

| Strain | Rep1 | Rep2 | Rep3 | Rep4 | Rep5 | Rep6 | Rep7 | Rep8 | Rep9 | Rep10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Leaf76 | 100.000 | 100.000 | 100.000 | 100.000 | 100.000 | 100.000 | 100.000 | 100.000 | 100.000 | 100.000 |
| Leaf68 | 70.968 | 85.406 | 75.802 | 68.195 | 62.949 | 61.152 | 63.915 | 65.444 | 64.140 | 78.977 |
| Leaf15 | 65.534 | 79.412 | 75.217 | 63.656 | 62.573 | 59.263 | 61.411 | 59.792 | 64.832 | 77.836 |
| Leaf337 | 15.825 | 9.253 | 17.351 | 12.005 | 15.793 | 16.565 | 20.174 | 16.913 | 17.117 | 17.871 |
| Leaf371 | 12.148 | 11.550 | 16.169 | 12.094 | 13.298 | 11.756 | 15.620 | 12.942 | 11.611 | 14.571 |
| Leaf361 | 4.189 | 8.257 | 5.361 | 3.993 | 5.086 | 4.777 | 3.415 | 3.905 | 4.526 | 5.867 |
| Leaf203 | 4.109 | 4.184 | 4.758 | 3.329 | 2.977 | 5.055 | 4.360 | 4.479 | 3.152 | 4.812 |
| Leaf454 | 4.001 | 2.731 | 3.935 | 3.137 | 2.475 | 3.230 | 3.289 | 3.772 | 2.816 | 3.812 |
| Leaf416 | 3.903 | 2.358 | 3.498 | 2.899 | 2.599 | 5.667 | 5.902 | 5.159 | 3.002 | 3.113 |
| Leaf168 | 3.841 | 0.174 | 4.382 | 3.881 | 6.104 | 4.009 | 2.115 | 3.323 | 5.150 | 4.934 |
| Leaf182 | 3.698 | 35.465 | 6.561 | 6.335 | 8.297 | 5.153 | 5.160 | 2.569 | 7.945 | 5.968 |
| Leaf225 | 3.451 | 0.603 | 3.744 | 2.533 | 2.442 | 3.750 | 4.591 | 3.531 | 2.725 | 3.572 |
| Leaf122 | 3.146 | 0.598 | 3.903 | 2.129 | 2.743 | 3.085 | 2.375 | 2.472 | 2.337 | 4.238 |
| Leaf466 | 3.079 | 7.451 | 3.681 | 1.449 | 3.449 | 4.008 | 4.545 | 3.806 | 3.417 | 2.693 |
| Leaf380 | 2.672 | 0.011 | 1.871 | 2.604 | 0.882 | 4.209 | 3.046 | 3.951 | 2.165 | 1.691 |
| Leaf339 | 2.663 | 0.387 | 2.568 | 2.955 | 1.954 | 3.934 | 4.216 | 4.486 | 2.995 | 1.968 |
| Leaf314 | 2.377 | 0.698 | 2.129 | 1.146 | 1.000 | 3.074 | 3.201 | 3.252 | 1.381 | 2.489 |
| Leaf254 | 2.268 | 1.560 | 2.066 | 0.599 | 1.477 | 2.280 | 3.314 | 2.833 | 1.217 | 2.444 |
| Leaf189 | 2.145 | 6.963 | 4.436 | 2.274 | 1.373 | 4.529 | 5.330 | 3.499 | 1.840 | 4.528 |
| Leaf226 | 2.117 | 2.944 | 3.271 | 4.934 | 3.373 | 2.812 | 3.136 | 2.552 | 3.682 | 2.896 |
| Leaf220 | 1.975 | 7.618 | 1.976 | 3.314 | 1.575 | 2.132 | 2.669 | 1.974 | 2.051 | 1.845 |
| Leaf70 | 1.730 | 1.457 | 2.668 | 1.186 | 2.045 | 4.079 | 3.053 | 2.098 | 2.642 | 2.371 |
| Leaf176 | 1.726 | 3.881 | 2.460 | 2.027 | 1.940 | 2.079 | 2.035 | 1.751 | 2.302 | 1.793 |
| Leaf32 | 1.605 | 3.274 | 2.154 | 3.975 | 2.059 | 2.273 | 1.520 | 1.349 | 2.692 | 1.908 |
| Leaf161 | 1.575 | 0.187 | 2.141 | 1.966 | 0.723 | 1.922 | 2.119 | 2.381 | 1.236 | 1.733 |
| Leaf289 | 1.351 | 2.804 | 1.885 | 2.087 | 1.113 | 0.869 | 1.503 | 1.511 | 0.894 | 2.104 |
| Leaf21 | 1.254 | 0.332 | 1.855 | 0.649 | 0.579 | 2.151 | 2.395 | 2.136 | 1.543 | 1.698 |
| Leaf126 | 1.194 | 1.525 | 1.343 | 1.423 | 1.583 | 2.138 | 2.181 | 1.931 | 1.939 | 1.209 |
| Leaf183 | 1.182 | 11.320 | 2.856 | 2.830 | 2.690 | 2.815 | 2.162 | 1.197 | 2.387 | 2.998 |
| Leaf354 | 1.146 | 0.850 | 1.083 | 1.001 | 1.650 | 1.763 | 1.117 | 1.642 | 2.330 | 0.668 |
| Leaf88 | 0.746 | 7.051 | 1.275 | 1.742 | 0.975 | 1.204 | 1.933 | 1.550 | 0.960 | 1.200 |
| Leaf187 | 0.601 | 1.629 | 0.194 | 0.434 | 0.111 | 0.887 | 0.665 | 1.188 | 0.217 | 0.444 |
| Leaf405 | 0.328 | 0.000 | 0.292 | 0.000 | 0.118 | 0.681 | 0.461 | 1.037 | 0.974 | 0.145 |
| Leaf299 | 0.176 | 2.742 | 0.184 | 0.556 | 0.000 | 0.489 | 1.274 | 1.413 | 0.000 | 0.000 |
| Leaf82 | 0.000 | 1.264 | 0.000 | 2.227 | 0.177 | 0.000 | 0.000 | 0.000 | 0.526 | 0.046 |

**Supplemental Table 10:** Model selection with Akaike information criterion for regressions analyses of the validation experiment of machine-learning results. Pathogen colonization was the dependent variable, strain inoculation with pathogen-reducing and random-strains was a fixed effect, and the replicates of the experiment was a random effect. Each data point is the average of pathogen colonization for the four plants of one box. Abbreviations: AIC, Akaike information criterion.

| Dependent variable | Fixed effect | Random structure | AIC | delta AIC |
|---|---|---|---|---|
| $Log_{10}$-transformed pst colonization | Strain inoculation | random intercept for experiment | 258.335 | 0.000 |
| $Log_{10}$-transformed pst colonization | Strain inoculation | no random effect | 265.197 | 6.863 |
| $Log_{10}$-transformed pst colonization | Strain inoculation | random slope for experiment | 925.508 | 667.173 |
| $Log_{10}$-transformed pst colonization | Strain inoculation | random intercept and slope for experiment | 925.517 | 667.182 |

**Supplemental Table 11:** Contrasts of the treatments presented in Figure 6A. The significance-group lettering was based on a 0.05 significance level after Bonferroni correction calculated from the family of all pairwise comparisons of treatments presented in this panel of the Figure 6. Abbreviation: Bonf., Bonferroni.

| Treatment 1 | Treatment 2 | Estimate | Standard error | t value | p-value | Bonf. corrected p-value |
|---|---|---|---|---|---|---|
| axenic | Leaf15 | -2.541 | 0.233 | -10.904 | 0.000 | 0.000 |
| axenic | Leaf15Leaf371 | -2.595 | 0.233 | -11.134 | 0.000 | 0.000 |
| axenic | Leaf15Leaf68 | -3.238 | 0.233 | -13.893 | 0.000 | 0.000 |
| axenic | Leaf15Leaf68Leaf76Leaf371 | -3.807 | 0.233 | -16.336 | 0.000 | 0.000 |
| axenic | Leaf15Leaf76 | -3.352 | 0.268 | -12.526 | 0.000 | 0.000 |
| axenic | Leaf371 | -1.314 | 0.233 | -5.639 | 0.000 | 0.000 |
| axenic | Leaf68 | -2.489 | 0.233 | -10.681 | 0.000 | 0.000 |
| axenic | Leaf68Leaf371 | -2.375 | 0.233 | -10.190 | 0.000 | 0.000 |
| axenic | Leaf68Leaf76 | -3.588 | 0.233 | -15.395 | 0.000 | 0.000 |
| axenic | Leaf76 | -2.507 | 0.233 | -10.758 | 0.000 | 0.000 |
| axenic | Leaf76Leaf371 | -2.578 | 0.233 | -11.063 | 0.000 | 0.000 |
| axenic | SynCom35 | -3.973 | 0.233 | -17.049 | 0.000 | 0.000 |
| Leaf68 | Leaf76 | -0.018 | 0.261 | -0.069 | 0.945 | 73.694 |
| Leaf68 | Leaf371 | 1.175 | 0.261 | 4.509 | 0.000 | 0.001 |
| Leaf68 | Leaf15Leaf68 | -0.749 | 0.261 | -2.873 | 0.005 | 0.374 |
| Leaf68 | Leaf15Leaf76 | -0.863 | 0.292 | -2.956 | 0.004 | 0.291 |
| Leaf68 | Leaf15Leaf371 | -0.106 | 0.261 | -0.406 | 0.686 | 53.488 |
| Leaf68 | Leaf68Leaf76 | -1.099 | 0.261 | -4.217 | 0.000 | 0.004 |
| Leaf68 | Leaf68Leaf371 | 0.114 | 0.261 | 0.439 | 0.661 | 51.593 |
| Leaf68 | Leaf76Leaf371 | -0.089 | 0.261 | -0.342 | 0.733 | 57.169 |
| Leaf68 | Leaf15Leaf68Leaf76Leaf371 | -1.318 | 0.261 | -5.058 | 0.000 | 0.000 |
| Leaf68 | Leaf15 | -0.052 | 0.261 | -0.200 | 0.842 | 65.690 |
| Leaf68 | SynCom35 | -1.484 | 0.261 | -5.696 | 0.000 | 0.000 |
| Leaf76 | Leaf371 | 1.193 | 0.261 | 4.579 | 0.000 | 0.001 |
| Leaf76 | Leaf15Leaf68 | -0.731 | 0.261 | -2.804 | 0.006 | 0.458 |
| Leaf76 | Leaf15Leaf76 | -0.845 | 0.292 | -2.894 | 0.004 | 0.351 |
| Leaf76 | Leaf15Leaf371 | -0.088 | 0.261 | -0.336 | 0.737 | 57.508 |
| Leaf76 | Leaf68Leaf76 | -1.081 | 0.261 | -4.147 | 0.000 | 0.005 |
| Leaf76 | Leaf68Leaf371 | 0.132 | 0.261 | 0.508 | 0.612 | 47.745 |
| Leaf76 | Leaf76Leaf371 | -0.071 | 0.261 | -0.273 | 0.786 | 61.277 |
| Leaf76 | Leaf15Leaf68Leaf76Leaf371 | -1.300 | 0.261 | -4.989 | 0.000 | 0.000 |
| Leaf76 | Leaf15 | -0.034 | 0.261 | -0.130 | 0.897 | 69.939 |
| Leaf76 | SynCom35 | -1.466 | 0.261 | -5.627 | 0.000 | 0.000 |
| Leaf371 | Leaf15Leaf68 | -1.924 | 0.261 | -7.382 | 0.000 | 0.000 |
| Leaf371 | Leaf15Leaf76 | -2.038 | 0.292 | -6.982 | 0.000 | 0.000 |
| Leaf371 | Leaf15Leaf371 | -1.281 | 0.261 | -4.915 | 0.000 | 0.000 |
| Leaf371 | Leaf68Leaf76 | -2.274 | 0.261 | -8.726 | 0.000 | 0.000 |
| Leaf371 | Leaf68Leaf371 | -1.061 | 0.261 | -4.070 | 0.000 | 0.007 |
| Leaf371 | Leaf76Leaf371 | -1.264 | 0.261 | -4.851 | 0.000 | 0.000 |
| Leaf371 | Leaf15Leaf68Leaf76Leaf371 | -2.493 | 0.261 | -9.567 | 0.000 | 0.000 |
| Leaf371 | Leaf15 | -1.227 | 0.261 | -4.709 | 0.000 | 0.001 |
| Leaf371 | SynCom35 | -2.659 | 0.261 | -10.205 | 0.000 | 0.000 |
| Leaf15Leaf68 | Leaf15Leaf76 | -0.114 | 0.292 | -0.391 | 0.696 | 54.323 |
| Leaf15Leaf68 | Leaf15Leaf371 | 0.643 | 0.261 | 2.468 | 0.015 | 1.168 |
| Leaf15Leaf68 | Leaf68Leaf76 | -0.350 | 0.261 | -1.344 | 0.182 | 14.163 |
| Leaf15Leaf68 | Leaf68Leaf371 | 0.863 | 0.261 | 3.312 | 0.001 | 0.095 |
| Leaf15Leaf68 | Leaf76Leaf371 | 0.660 | 0.261 | 2.531 | 0.013 | 0.985 |
| Leaf15Leaf68 | Leaf15Leaf68Leaf76Leaf371 | -0.569 | 0.261 | -2.185 | 0.031 | 2.402 |
| Leaf15Leaf68 | Leaf15 | 0.697 | 0.261 | 2.674 | 0.009 | 0.665 |
| Leaf15Leaf68 | SynCom35 | -0.736 | 0.261 | -2.823 | 0.006 | 0.433 |
| Leaf15Leaf76 | Leaf15Leaf371 | 0.757 | 0.292 | 2.594 | 0.011 | 0.830 |
| Leaf15Leaf76 | Leaf68Leaf76 | -0.236 | 0.292 | -0.808 | 0.420 | 32.793 |
| Leaf15Leaf76 | Leaf68Leaf371 | 0.977 | 0.292 | 3.348 | 0.001 | 0.084 |
| Leaf15Leaf76 | Leaf76Leaf371 | 0.774 | 0.292 | 2.651 | 0.009 | 0.709 |
| Leaf15Leaf76 | Leaf15Leaf68Leaf76Leaf371 | -0.455 | 0.292 | -1.560 | 0.121 | 9.472 |
| Leaf15Leaf76 | Leaf15 | 0.811 | 0.292 | 2.778 | 0.006 | 0.494 |

Supplemental Table 11 continued

| | | | | | | |
|---|---|---|---|---|---|---|
| Leaf15Leaf76 | SynCom35 | -0.621 | 0.292 | -2.129 | 0.035 | 2.748 |
| Leaf15Leaf371 | Leaf68Leaf76 | -0.993 | 0.261 | -3.811 | 0.000 | 0.017 |
| Leaf15Leaf371 | Leaf68Leaf371 | 0.220 | 0.261 | 0.845 | 0.400 | 31.199 |
| Leaf15Leaf371 | Leaf76Leaf371 | 0.017 | 0.261 | 0.064 | 0.949 | 74.053 |
| Leaf15Leaf371 | Leaf15Leaf68Leaf76Leaf371 | -1.212 | 0.261 | -4.652 | 0.000 | 0.001 |
| Leaf15Leaf371 | Leaf15 | 0.054 | 0.261 | 0.206 | 0.837 | 65.293 |
| Leaf15Leaf371 | SynCom35 | -1.378 | 0.261 | -5.291 | 0.000 | 0.000 |
| Leaf68Leaf76 | Leaf68Leaf371 | 1.213 | 0.261 | 4.656 | 0.000 | 0.001 |
| Leaf68Leaf76 | Leaf76Leaf371 | 1.010 | 0.261 | 3.875 | 0.000 | 0.013 |
| Leaf68Leaf76 | Leaf15Leaf68Leaf76Leaf371 | -0.219 | 0.261 | -0.841 | 0.402 | 31.338 |
| Leaf68Leaf76 | Leaf15 | 1.047 | 0.261 | 4.017 | 0.000 | 0.008 |
| Leaf68Leaf76 | SynCom35 | -0.385 | 0.261 | -1.480 | 0.142 | 11.042 |
| Leaf68Leaf371 | Leaf76Leaf371 | -0.203 | 0.261 | -0.781 | 0.436 | 34.033 |
| Leaf68Leaf371 | Leaf15Leaf68Leaf76Leaf371 | -1.432 | 0.261 | -5.497 | 0.000 | 0.000 |
| Leaf68Leaf371 | Leaf15 | -0.166 | 0.261 | -0.639 | 0.524 | 40.897 |
| Leaf68Leaf371 | SynCom35 | -1.599 | 0.261 | -6.135 | 0.000 | 0.000 |
| Leaf76Leaf371 | Leaf15Leaf68Leaf76Leaf371 | -1.229 | 0.261 | -4.716 | 0.000 | 0.000 |
| Leaf76Leaf371 | Leaf15 | 0.037 | 0.261 | 0.142 | 0.887 | 69.182 |
| Leaf76Leaf371 | SynCom35 | -1.395 | 0.261 | -5.354 | 0.000 | 0.000 |
| Leaf15Leaf68Leaf76Leaf371 | Leaf15 | 1.266 | 0.261 | 4.858 | 0.000 | 0.000 |
| Leaf15Leaf68Leaf76Leaf371 | SynCom35 | -0.166 | 0.261 | -0.638 | 0.525 | 40.916 |
| Leaf15 | SynCom35 | -1.432 | 0.261 | -5.497 | 0.000 | 0.000 |

**Supplemental Table 12:** Contrast of the treatments presented in Figure 6B. The significance-group lettering was based on a 0.05 significance level after Bonferroni correction calculated from the family of all pairwise comparisons of treatments presented in this panel of the Figure 6. Abbreviation: Bonf., Bonferroni.

| Treatment 1 | Treatment 2 | Estimate | Standard error | t value | p-value | Bonf. corrected p-value |
|---|---|---|---|---|---|---|
| axenic | Leaf189 | -0.813 | 0.233 | -3.487 | 0.001 | 0.053 |
| axenic | Leaf189Leaf21 | -1.029 | 0.247 | -4.162 | 0.000 | 0.005 |
| axenic | Leaf189Leaf225 | -1.168 | 0.247 | -4.722 | 0.000 | 0.000 |
| axenic | Leaf189Leaf254 | -1.119 | 0.247 | -4.523 | 0.000 | 0.001 |
| axenic | Leaf189Leaf254Leaf21Leaf225 | -1.240 | 0.233 | -5.322 | 0.000 | 0.000 |
| axenic | Leaf21 | -0.486 | 0.233 | -2.085 | 0.039 | 3.055 |
| axenic | Leaf21Leaf225 | -1.550 | 0.267 | -5.804 | 0.000 | 0.000 |
| axenic | Leaf225 | -0.143 | 0.233 | -0.613 | 0.541 | 42.223 |
| axenic | Leaf254 | -0.640 | 0.247 | -2.589 | 0.011 | 0.841 |
| axenic | Leaf254Leaf21 | -0.846 | 0.247 | -3.420 | 0.001 | 0.066 |
| axenic | Leaf254Leaf225 | -0.461 | 0.247 | -1.865 | 0.065 | 5.038 |
| axenic | SynCom35 | -3.973 | 0.233 | -17.049 | 0.000 | 0.000 |
| Leaf189 | Leaf254 | 0.172 | 0.273 | 0.631 | 0.529 | 41.288 |
| Leaf189 | Leaf21 | 0.327 | 0.261 | 1.254 | 0.212 | 16.541 |
| Leaf189 | Leaf225 | 0.670 | 0.261 | 2.571 | 0.011 | 0.883 |
| Leaf189 | Leaf189Leaf254 | -0.306 | 0.273 | -1.119 | 0.265 | 20.694 |
| Leaf189 | Leaf189Leaf21 | -0.217 | 0.273 | -0.792 | 0.430 | 33.512 |
| Leaf189 | Leaf189Leaf225 | -0.355 | 0.273 | -1.298 | 0.197 | 15.331 |
| Leaf189 | Leaf254Leaf21 | -0.033 | 0.273 | -0.121 | 0.904 | 70.530 |
| Leaf189 | Leaf254Leaf225 | 0.352 | 0.273 | 1.286 | 0.201 | 15.667 |
| Leaf189 | Leaf21Leaf225 | -0.737 | 0.291 | -2.530 | 0.013 | 0.989 |
| Leaf189 | Leaf189Leaf254Leaf21Leaf225 | -0.428 | 0.261 | -1.641 | 0.103 | 8.061 |
| Leaf189 | SynCom35 | -3.161 | 0.261 | -12.130 | 0.000 | 0.000 |
| Leaf254 | Leaf21 | 0.154 | 0.273 | 0.565 | 0.573 | 44.710 |
| Leaf254 | Leaf225 | 0.497 | 0.273 | 1.820 | 0.071 | 5.554 |
| Leaf254 | Leaf189Leaf254 | -0.478 | 0.286 | -1.674 | 0.097 | 7.543 |
| Leaf254 | Leaf189Leaf21 | -0.389 | 0.285 | -1.363 | 0.175 | 13.676 |
| Leaf254 | Leaf189Leaf225 | -0.527 | 0.285 | -1.848 | 0.067 | 5.229 |
| Leaf254 | Leaf254Leaf21 | -0.205 | 0.286 | -0.719 | 0.474 | 36.946 |
| Leaf254 | Leaf254Leaf225 | 0.179 | 0.285 | 0.627 | 0.531 | 41.457 |
| Leaf254 | Leaf21Leaf225 | -0.909 | 0.303 | -3.003 | 0.003 | 0.253 |
| Leaf254 | Leaf189Leaf254Leaf21Leaf225 | -0.600 | 0.273 | -2.195 | 0.030 | 2.344 |
| Leaf254 | SynCom35 | -3.333 | 0.273 | -12.193 | 0.000 | 0.000 |
| Leaf21 | Leaf225 | 0.343 | 0.261 | 1.317 | 0.190 | 14.849 |
| Leaf21 | Leaf189Leaf254 | -0.633 | 0.273 | -2.315 | 0.022 | 1.738 |
| Leaf21 | Leaf189Leaf21 | -0.543 | 0.273 | -1.988 | 0.049 | 3.824 |
| Leaf21 | Leaf189Leaf225 | -0.682 | 0.273 | -2.494 | 0.014 | 1.088 |
| Leaf21 | Leaf254Leaf21 | -0.360 | 0.273 | -1.316 | 0.191 | 14.863 |
| Leaf21 | Leaf254Leaf225 | 0.025 | 0.273 | 0.090 | 0.928 | 72.397 |
| Leaf21 | Leaf21Leaf225 | -1.064 | 0.291 | -3.652 | 0.000 | 0.030 |
| Leaf21 | Leaf189Leaf254Leaf21Leaf225 | -0.754 | 0.261 | -2.895 | 0.004 | 0.350 |
| Leaf21 | SynCom35 | -3.487 | 0.261 | -13.385 | 0.000 | 0.000 |
| Leaf225 | Leaf189Leaf254 | -0.976 | 0.273 | -3.570 | 0.001 | 0.040 |
| Leaf225 | Leaf189Leaf21 | -0.887 | 0.273 | -3.243 | 0.002 | 0.119 |
| Leaf225 | Leaf189Leaf225 | -1.025 | 0.273 | -3.749 | 0.000 | 0.021 |
| Leaf225 | Leaf254Leaf21 | -0.703 | 0.273 | -2.571 | 0.011 | 0.883 |
| Leaf225 | Leaf254Leaf225 | -0.318 | 0.273 | -1.165 | 0.246 | 19.219 |
| Leaf225 | Leaf21Leaf225 | -1.407 | 0.291 | -4.830 | 0.000 | 0.000 |
| Leaf225 | Leaf189Leaf254Leaf21Leaf225 | -1.098 | 0.261 | -4.212 | 0.000 | 0.004 |
| Leaf225 | SynCom35 | -3.831 | 0.261 | -14.702 | 0.000 | 0.000 |
| Leaf189Leaf254 | Leaf189Leaf21 | 0.089 | 0.286 | 0.312 | 0.755 | 58.910 |
| Leaf189Leaf254 | Leaf189Leaf225 | -0.049 | 0.286 | -0.172 | 0.864 | 67.391 |
| Leaf189Leaf254 | Leaf254Leaf21 | 0.273 | 0.285 | 0.956 | 0.341 | 26.583 |
| Leaf189Leaf254 | Leaf254Leaf225 | 0.657 | 0.286 | 2.301 | 0.023 | 1.802 |
| Leaf189Leaf254 | Leaf21Leaf225 | -0.431 | 0.303 | -1.423 | 0.157 | 12.256 |
| Leaf189Leaf254 | Leaf189Leaf254Leaf21Leaf225 | -0.122 | 0.273 | -0.445 | 0.657 | 51.249 |
| Leaf189Leaf254 | SynCom35 | -2.855 | 0.273 | -10.443 | 0.000 | 0.000 |
| Leaf189Leaf21 | Leaf189Leaf225 | -0.138 | 0.285 | -0.485 | 0.629 | 49.044 |

Supplemental Table 12 continued

| | | | | | | |
|---|---|---|---|---|---|---|
| Leaf189Leaf21 | Leaf254Leaf21 | 0.184 | 0.286 | 0.643 | 0.522 | 40.687 |
| Leaf189Leaf21 | Leaf254Leaf225 | 0.568 | 0.285 | 1.991 | 0.049 | 3.802 |
| Leaf189Leaf21 | Leaf21Leaf225 | -0.520 | 0.303 | -1.718 | 0.088 | 6.884 |
| Leaf189Leaf21 | Leaf189Leaf254Leaf21Leaf225 | -0.211 | 0.273 | -0.772 | 0.442 | 34.458 |
| Leaf189Leaf21 | SynCom35 | -2.944 | 0.273 | -10.769 | 0.000 | 0.000 |
| Leaf189Leaf225 | Leaf254Leaf21 | 0.322 | 0.286 | 1.127 | 0.262 | 20.439 |
| Leaf189Leaf225 | Leaf254Leaf225 | 0.707 | 0.285 | 2.475 | 0.015 | 1.145 |
| Leaf189Leaf225 | Leaf21Leaf225 | -0.382 | 0.303 | -1.261 | 0.210 | 16.343 |
| Leaf189Leaf225 | Leaf189Leaf254Leaf21Leaf225 | -0.073 | 0.273 | -0.266 | 0.791 | 61.695 |
| Leaf189Leaf225 | SynCom35 | -2.806 | 0.273 | -10.263 | 0.000 | 0.000 |
| Leaf254Leaf21 | Leaf254Leaf225 | 0.385 | 0.286 | 1.345 | 0.181 | 14.114 |
| Leaf254Leaf21 | Leaf21Leaf225 | -0.704 | 0.303 | -2.325 | 0.022 | 1.694 |
| Leaf254Leaf21 | Leaf189Leaf254Leaf21Leaf225 | -0.395 | 0.273 | -1.444 | 0.151 | 11.810 |
| Leaf254Leaf21 | SynCom35 | -3.128 | 0.273 | -11.441 | 0.000 | 0.000 |
| Leaf254Leaf225 | Leaf21Leaf225 | -1.089 | 0.303 | -3.595 | 0.000 | 0.037 |
| Leaf254Leaf225 | Leaf189Leaf254Leaf21Leaf225 | -0.779 | 0.273 | -2.850 | 0.005 | 0.400 |
| Leaf254Leaf225 | SynCom35 | -3.512 | 0.273 | -12.848 | 0.000 | 0.000 |
| Leaf21Leaf225 | Leaf189Leaf254Leaf21Leaf225 | 0.309 | 0.291 | 1.062 | 0.290 | 22.643 |
| Leaf21Leaf225 | SynCom35 | -2.424 | 0.291 | -8.320 | 0.000 | 0.000 |
| Leaf189Leaf254Leaf21Leaf225 | SynCom35 | -2.733 | 0.261 | -10.489 | 0.000 | 0.000 |

**Supplemental Table 13:** Model selection with Akaike information criterion for regressions analyses of the validation of combination of strains reducing pathogen colonization. Pathogen colonization was the dependent variable, strain inoculations was a fixed effect, and the replicates of the experiment was the random effect. Each data point is the average of pathogen colonization for the four plants of one box. Abbreviations: AIC, Akaike information criterion.

| Dependent variable | Fixed effect | Random structure | AIC | deltaAIC |
|---|---|---|---|---|
| $Log_{10}$-transformed pst colonization | Strain inoculation | no random effect | 84.010 | 0.000 |
| $Log_{10}$-transformed pst colonization | Strain inoculation | random intercept for experiment | 86.010 | 2.000 |
| $Log_{10}$-transformed pst colonization | Strain inoculation | random slope for experiment | 137.755 | 53.745 |
| $Log_{10}$-transformed pst colonization | Strain inoculation | random intercept and slope for experiment | 137.755 | 53.745 |

**Supplemental Table 14:** Results of the regression analyses to validate the additive effect of Leaf371 and Leaf337 on pathogen reduction for the two best models with delta AIC inferior to four.

**Model formula** pst ~ Inoculation

**Generalized least squares fit:** REML

**Coefficients:**

| | Value | Standard error | t-value | p-value |
|---|---|---|---|---|
| Intercept | 8.589634 | 0.1547944 | 55.4906 | 0 |
| Inoculation Leaf337 | -0.833854 | 0.2527782 | -3.29876 | 0.0021 |
| Inoculation Leaf337-Leaf371 | -1.798839 | 0.2527782 | -7.11627 | 0 |
| Inoculation Leaf371 | -0.714265 | 0.2527782 | -2.82566 | 0.0074 |
| Inoculation mix6 | -1.697471 | 0.2527782 | -6.71526 | 0 |
| Inoculation validation mix1 | -1.854182 | 0.2527782 | -7.33521 | 0 |
| Inoculation validation mix2 | -1.944013 | 0.2527782 | -7.69059 | 0 |

**Correlation:**

| | Intercept | Inoculation Leaf337 | Inoculation Leaf337-Leaf371 | Inoculation Leaf371 | Inocculatio mix6 | Inoculation validation mix1 |
|---|---|---|---|---|---|---|
| Inoculation Leaf337 | -0.612 | | | | | |
| Inoculation Leaf337-Leaf371 | -0.612 | 0.375 | | | | |
| Inoculation Leaf371 | -0.612 | 0.375 | 0.375 | | | |
| Inoculation mix6 | -0.612 | 0.375 | 0.375 | 0.375 | | |
| Inoculation validation mix1 | -0.612 | 0.375 | 0.375 | 0.375 | 0.375 | |
| Inoculation validation mix2 | -0.612 | 0.375 | 0.375 | 0.375 | 0.375 | 0.375 |

**Standardized residuals:**

| Minimum | Quartile 1 | Median | Quartile 3 | Maximum |
|---|---|---|---|---|
| -2.7479585 | -0.5435194 | 0.1914377 | 0.5275292 | 1.9943498 |

**Residual standard error:** 0.4895029
**Degrees of freedom:** 46 total; 39 residual

---

**Model formula:** pst ~ Inoculation + (1 | Experiment)

**Linear mixed model fit:** REML
**t-tests:** Satterthwaite's method

**REML criterion at convergence:** 68

**Scaled residuals:**

| Minimum | Quartile 1 | Median | Quartile 3 | Maximum |
|---|---|---|---|---|
| -2.748 | -0.5435 | 0.1914 | 0.5275 | 1.9944 |

**Random effects:**

| Groups | Name | Variance | Standard deviation |
|---|---|---|---|
| | Experiment (Intercept) | 0 | 0 |
| Residual | | 0.2396 | 0.4895 |

**Number of observation:** 46

**groups:**

| | |
|---|---|
| Experiment | 2 |

**Fixed effects:**

| | Estimate | Standard error | degrees of freedom | t value | P-value |
|---|---|---|---|---|---|
| Intercept | 8.5896 | 0.1548 | 39 | 55.491 | < 2e-16 |
| Inoculation Leaf337 | -0.8339 | 0.2528 | 39 | -3.299 | 0.00208 |
| Inoculation Leaf337-Leaf371 | -1.7988 | 0.2528 | 39 | -7.116 | 1.48E-08 |
| Inoculation Leaf371 | -0.7143 | 0.2528 | 39 | -2.826 | 0.0074 |
| Inoculation mix6 | -1.6975 | 0.2528 | 39 | -6.715 | 5.28E-08 |
| Inoculation validation mix1 | -1.8542 | 0.2528 | 39 | -7.335 | 7.46E-09 |
| Inoculation validation mix2 | -1.944 | 0.2528 | 39 | -7.691 | 2.46E-09 |

**Correlation of Fixed Effects:**

| | Intercept | Inoculation L337 | Inoculation Leaf337-Leaf371 | Inoculation Leaf371 | Inoculation mix6 | Inoculation validation mix1 |
|---|---|---|---|---|---|---|
| Inoculation Leaff337 | -0.612 | | | | | |
| Inoculation Leaf337-Leaf371 | -0.612 | 0.375 | | | | |
| Inoculation Leaff371 | -0.612 | 0.375 | 0.375 | | | |
| Inoculation mix6 | -0.612 | 0.375 | 0.375 | 0.375 | | |
| Inoculation validation mix1 | -0.612 | 0.375 | 0.375 | 0.375 | 0.375 | |
| Inoculation validation mix2 | -0.612 | 0.375 | 0.375 | 0.375 | 0.375 | 0.375 |

**Supplemental Table 14:** Results of the regression analyses to validate the additive effect of Leaf371 and Leaf337 on pathogen reduction for the two best models with delta AIC inferior to four.

**Model formula** pst ~ Inoculation

**Generalized least squares fit:** REML

**Coefficients:**

| | Value | Standard error | t-value | p-value |
|---|---|---|---|---|
| Intercept | 8.589634 | 0.1547944 | 55.4906 | 0 |
| Inoculation Leaf337 | -0.833854 | 0.2527782 | -3.29876 | 0.0021 |
| Inoculation Leaf337-Leaf371 | -1.798839 | 0.2527782 | -7.11627 | 0 |
| Inoculation Leaf371 | -0.714265 | 0.2527782 | -2.82566 | 0.0074 |
| Inoculation mix6 | -1.697471 | 0.2527782 | -6.71526 | 0 |
| Inoculation validation mix1 | -1.854182 | 0.2527782 | -7.33521 | 0 |
| Inoculation validation mix2 | -1.944013 | 0.2527782 | -7.69059 | 0 |

**Correlation:**

| | Intercept | Inoculation Leaf337 | Inoculation Leaf337-Leaf371 | Inoculation Leaf371 | Inocculatio mix6 | Inoculation validation mix1 |
|---|---|---|---|---|---|---|
| Inoculation Leaf337 | -0.612 | | | | | |
| Inoculation Leaf337-Leaf371 | -0.612 | 0.375 | | | | |
| Inoculation Leaf371 | -0.612 | 0.375 | 0.375 | | | |
| Inoculation mix6 | -0.612 | 0.375 | 0.375 | 0.375 | | |
| Inoculation validation mix1 | -0.612 | 0.375 | 0.375 | 0.375 | 0.375 | |
| Inoculation validation mix2 | -0.612 | 0.375 | 0.375 | 0.375 | 0.375 | 0.375 |

**Standardized residuals:**

| Minimum | Quartile 1 | Median | Quartile 3 | Maximum |
|---|---|---|---|---|
| -2.7479585 | -0.5435194 | 0.1914377 | 0.5275292 | 1.9943498 |

**Residual standard error:** 0.4895029
**Degrees of freedom:** 46 total; 39 residual

---

**Model formula:** pst ~ Inoculation + (1 | Experiment)

**Linear mixed model fit:** REML
**t-tests:** Satterthwaite's method

**REML criterion at convergence:** 68

**Scaled residuals:**

| Minimum | Quartile 1 | Median | Quartile 3 | Maximum |
|---|---|---|---|---|
| -2.748 | -0.5435 | 0.1914 | 0.5275 | 1.9944 |

**Random effects:**

| Groups | Name | Variance | Standard deviation |
|---|---|---|---|
| | Experiment (Intercept) | 0 | 0 |
| Residual | | 0.2396 | 0.4895 |

**Number of observation:** 46

**groups:**

| | |
|---|---|
| Experiment | 2 |

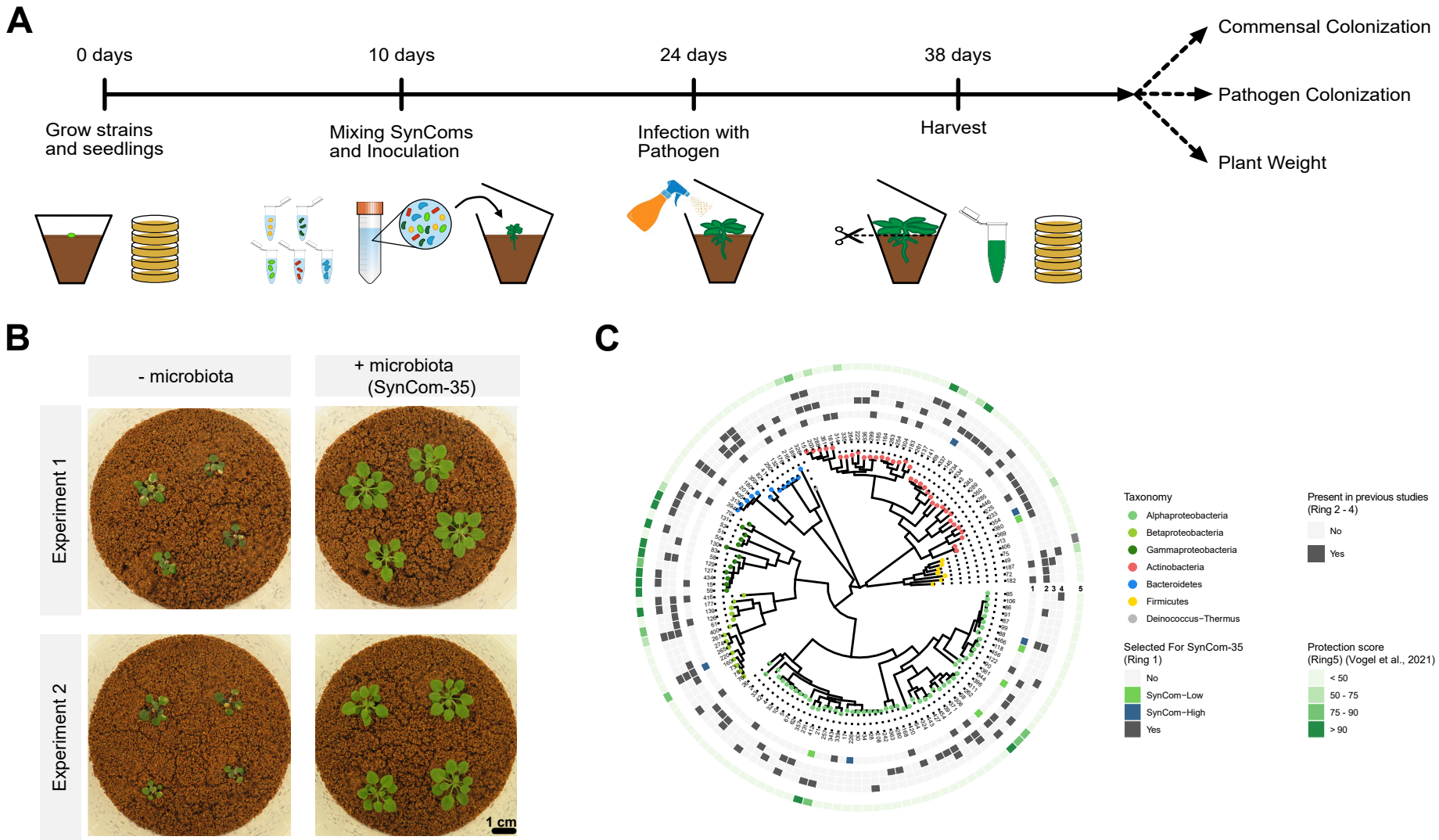
**Fixed effects:**

| | Estimate | Standard error | degrees of freedom | t value | P-value |
|---|---|---|---|---|---|
| Intercept | 8.5896 | 0.1548 | 39 | 55.491 | < 2e-16 |
| Inoculation Leaf337 | -0.8339 | 0.2528 | 39 | -3.299 | 0.00208 |
| Inoculation Leaf337-Leaf371 | -1.7988 | 0.2528 | 39 | -7.116 | 1.48E-08 |
| Inoculation Leaf371 | -0.7143 | 0.2528 | 39 | -2.826 | 0.0074 |
| Inoculation mix6 | -1.6975 | 0.2528 | 39 | -6.715 | 5.28E-08 |
| Inoculation validation mix1 | -1.8542 | 0.2528 | 39 | -7.335 | 7.46E-09 |
| Inoculation validation mix2 | -1.944 | 0.2528 | 39 | -7.691 | 2.46E-09 |

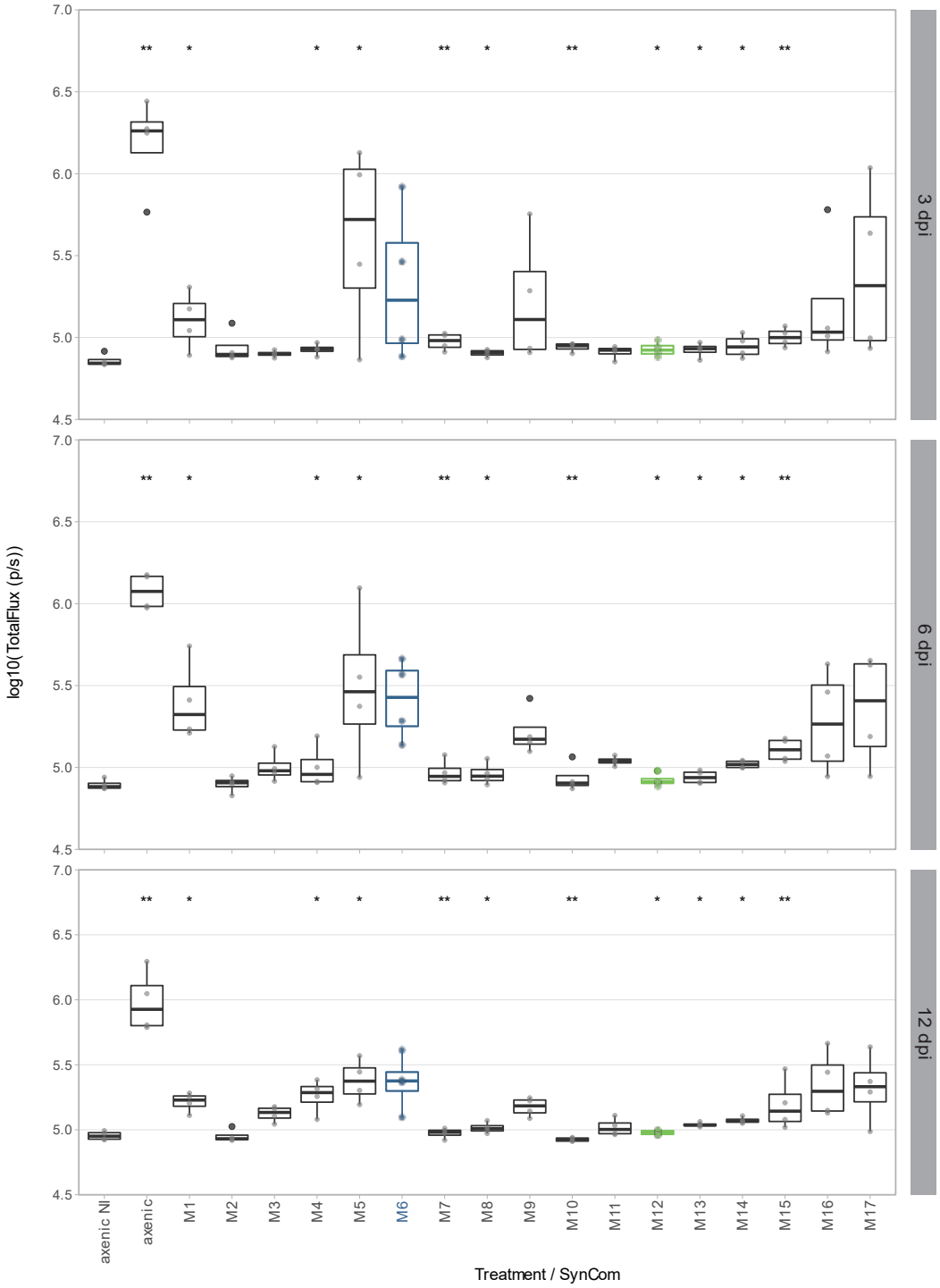
**Correlation of Fixed Effects:**

| | Intercept | Inoculation L337 | Inoculation Leaf337-Leaf371 | Inoculation Leaf371 | Inoculation mix6 | Inoculation validation mix1 |
|---|---|---|---|---|---|---|
| Inoculation Leaff337 | -0.612 | | | | | |
| Inoculation Leaf337-Leaf371 | -0.612 | 0.375 | | | | |
| Inoculation Leaff371 | -0.612 | 0.375 | 0.375 | | | |
| Inoculation mix6 | -0.612 | 0.375 | 0.375 | 0.375 | | |
| Inoculation validation mix1 | -0.612 | 0.375 | 0.375 | 0.375 | 0.375 | |
| Inoculation validation mix2 | -0.612 | 0.375 | 0.375 | 0.375 | 0.375 | 0.375 |

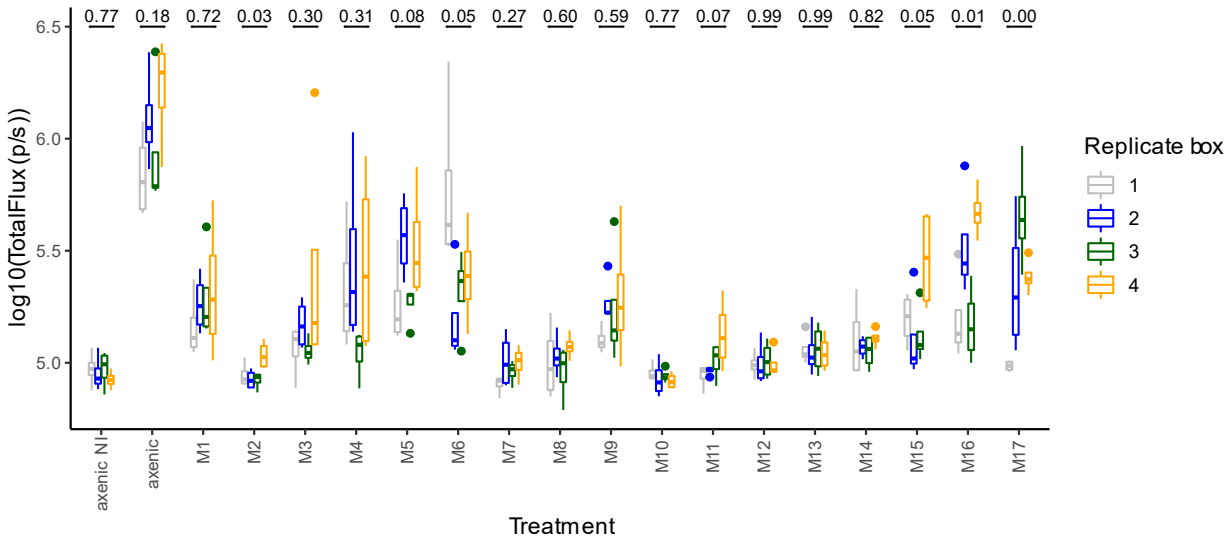
# Supplemental Figures



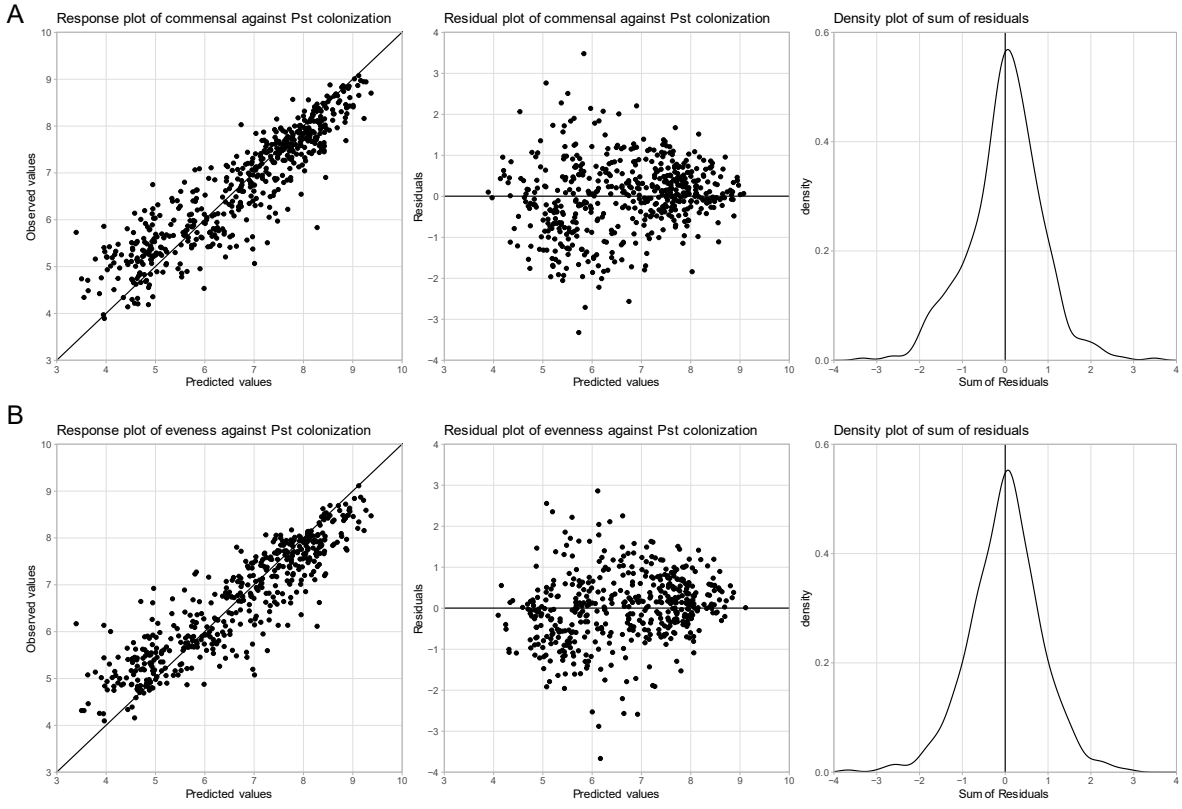

**Supplemental Figure 1: Experimental design, host traits of interest, and bacteria collection.** A. Experimental procedure for plant treatments. B. Illustration of the influence of the phyllosphere microbiota on plant phenotype. C. Phylogenetic tree of the SynCom-137 [44] including single ASVs of the *At*-LSPHERE with information of strain selection in surrounding rings. Ring 1 shows whether a strain was included in this study. Rings 2 to 4 illustrate whether a strain was included in previous studies that used *At*-LSPHERE strains, in order Carlström et al. [41], Schäfer et al. [46], and Maier et al. [47]. Ring 5 illustrates plant protectio as analysed in a previous study [32].



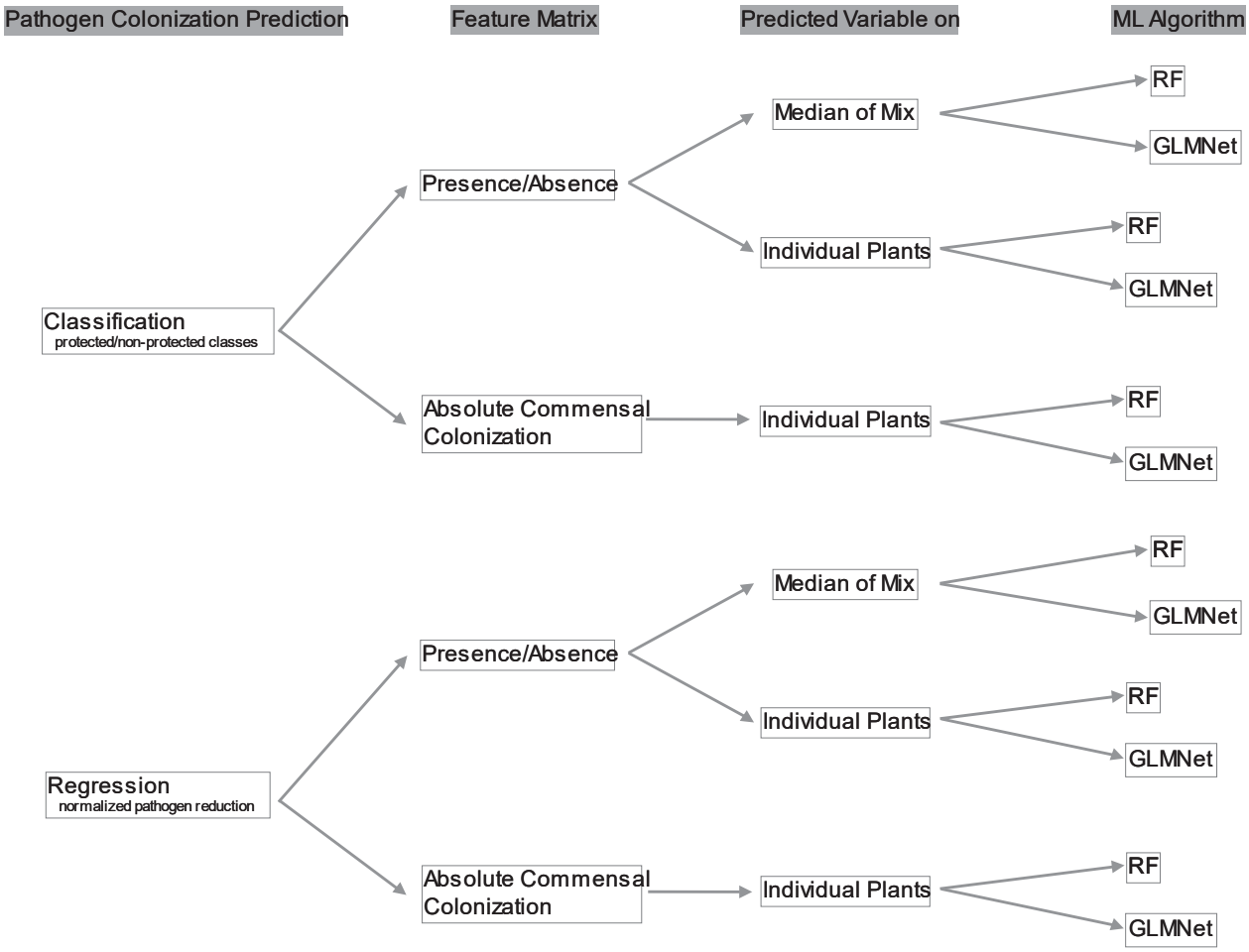

**Supplemental Figure 2: Luminescence measurements in the pilot experiment for the two controls axenic non-infected (axenic NI) and axenic infected (axenic), and the 17 randomly assembled Mini5SynCom (M1 to M17).** Each data point corresponds to the median of the luminescence of four plants in a microbox. Significance levels for mean comparisons between the axenic non-infected control and all other treatments were obtained with one-sided Welsh's tests (see Table S1). M6 and M12 were included in the Mini5SynCom screen (SynCom-Low and SynCom-High, respectively), and are coloured accordingly. Abbreviations: dpi, days post infection. Significance code: NS > 0.05; * ≤ 0.5; ** ≤ 0.01: *** ≤ 0.001; **** ≤ 0.0001.



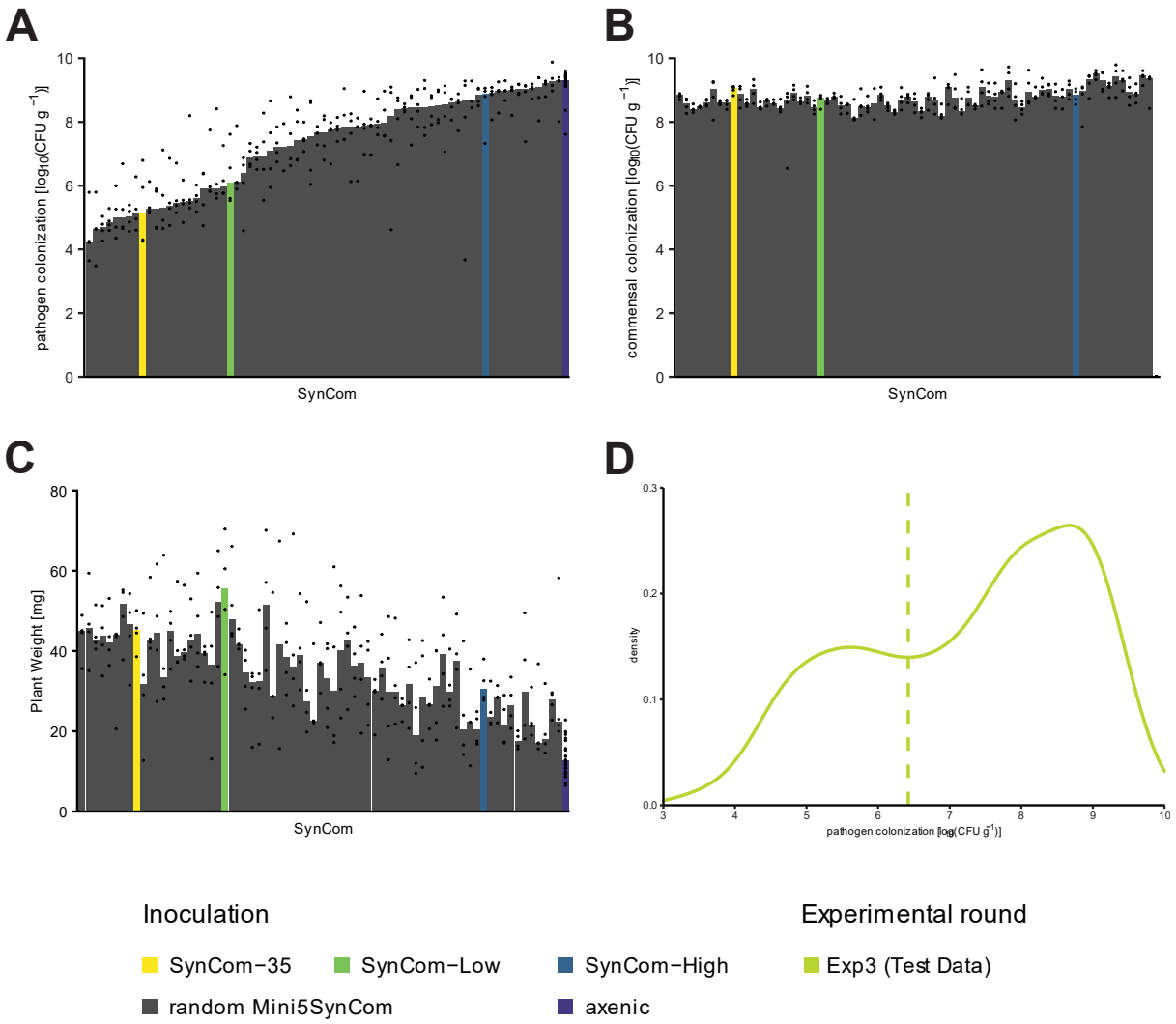

**Supplemental Figure 3: Boxplot of the $log_{10}$-transformed luminescence presented for each Mini5SynCom and the axenic infected (axenic) and axenic non-infected (axenic NI) controls of the pilot experiment.** P-values are presented at the top of each treatment and were calculated with ANOVAs with replicate box as independent variable.



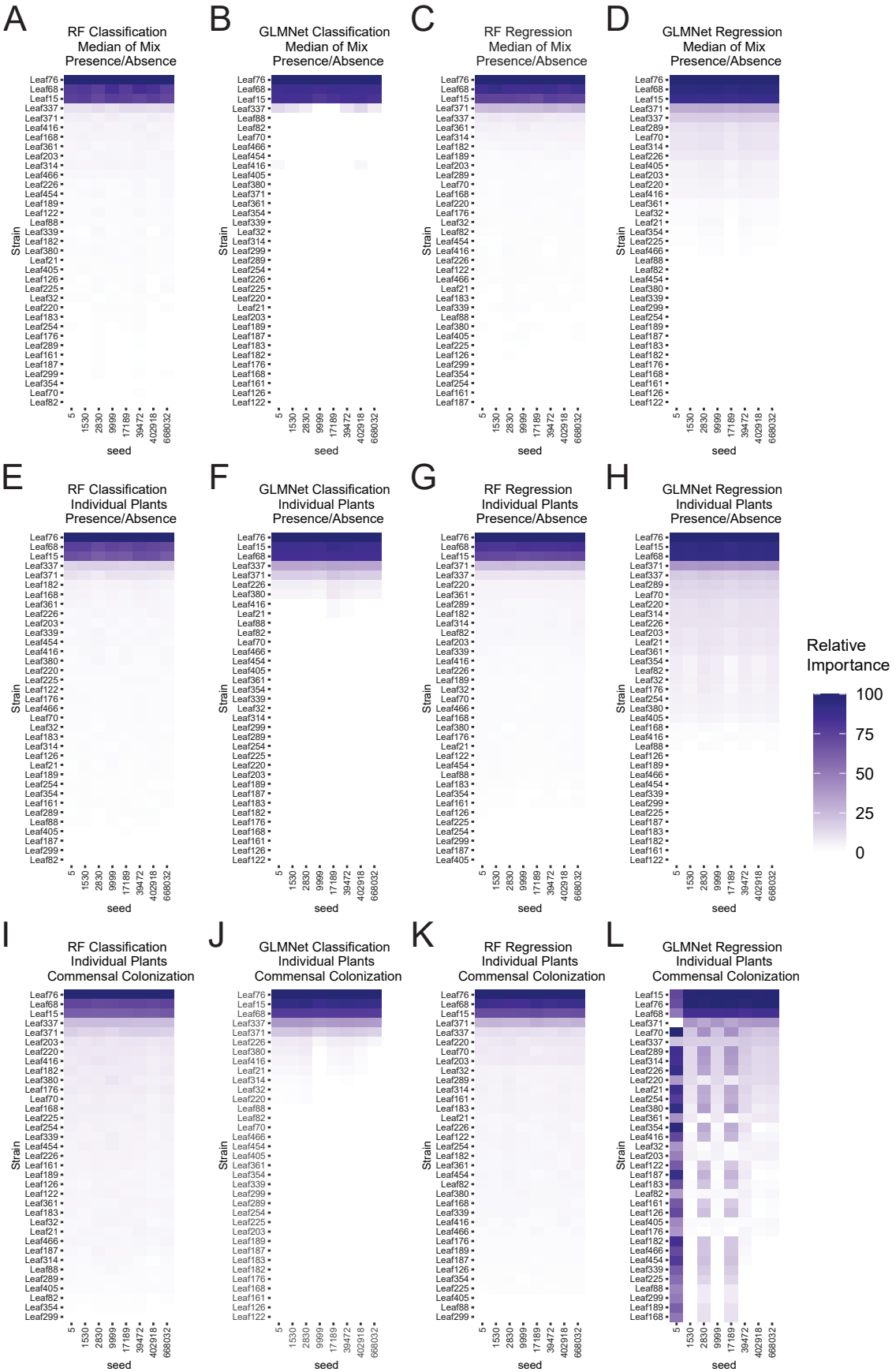

**Supplemental Figure 4: Diagnostic plots of the best linear mixed models for regression analyses with pathogen colonization as dependent variable, and random intercepts and slopes for the box effect.** A. Overall Mini5SynCom commensal colonization as fixed effect. B. Evenness of Mini5SynComs as fixed effect.




**Supplemental Figure 5: Diagram of the different algorithms used in machine learning.** First level is the predicted variable (class or value). Second level is the type of predictive variables used (presence/absence or absolute colonization of Mini5SynCom commensals). Third level is the type of measurements used to define the predicted variable (median of Mini5SynCom or individual plant samples). Last level is the method used (GLMNet or RF).




**Supplemental Figure 6: Strain colonization and plant weight in the test dataset (experiment 3).** A-C. Each bar represents the median for each treatment; points are individual-plant measurements; x-axes represent individual treatments coloured according to experiment and they follow same sorting in all panels. A. Pathogen colonization. B. Overall Mini5Syncom commensal colonization. C. Plant weight. D. Density curve of the pathogen colonization. Abbreviation: Exp3, experiment 3.




**Supplemental Figure 7: Relative feature importance across all analysis with eight different seeds for each model / algorithm combination.** Above each plot the algorithm, the commensal measurement, and the type of predicted variables are indicated. The strains are ordered according to the median of their relative importance across the eight seeds for each model / algorithm combination. Abbreviations: RF, random forest; GLMNet, elastic-net regularized generalized linear models.

# Chapter V

# Discussion
# and
# Outlook

This thesis focused on improving our understanding of altered host protection against invasion by a foliar pathogen, *Pseudomonas syringae* pathovar *tomato* DC3000, caused by changes in the plant-associated microbiota native to the model plant *Arabidopsis thaliana*, as a community function. In the three research chapters presented here, we highlighted the importance of a diverse healthy microbiome in conferring plant protection, while also showing indication that the presence of a selected few competitive strains is crucial. In Chapter II, we investigated the stability of the microbiota through passaging the microbiota over several consecutive passages. We showed that the microbiota is stable after a first shift that occurred during the initial plant colonization. Our data also suggest that the pathogen had a stabilizing effect on the microbiota. Additionally, we showed that we can select for differently composed microbiota through selection on plant phenotype in low diversity communities. However, we found that highly complex communities are fully protective, and the apparent high redundancy in protection as a function of the microbiota made it impossible to select for nonprotective or higher protective phenotypes, attesting the robustness of complex microbiota. In Chapter III, we focused on manipulating the inoculum composition of the synthetic communities to change pathogen colonization after community establishment. We found that the complex communities, SynCom-210 and SynCom-223, were robust to perturbation of both changes in strain abundance and presence in the inoculum. The only reduction of protection found was upon removal of more than half of the strains from the inoculum, when dropping out all Proteobacteria strains. When manipulating strain presence in lower complex SynComs (SynCom-15 [1]), we found that exchanging only 2 strains in the inoculum could bring pathogen colonization to the level of SynCom-210. We hypothesised that beneficial microbes would be more competitive than microbes with lower beneficial traits. In binary competition as well as in community compositions, strains with higher protection scores [2] were found to be more abundant on the plant compared to strains with lower protection scores of the same genus. In Chapter IV, we set up a screen for microbiota patterns relevant for plant protection and found that strain identity in the communities was the most predictive of pathogen colonization outcome. We identified three strains out of a set of 35 to be most important for plant protection, namely *Pseudomonas* Leaf15, *Rhizobium* Leaf68 and *Acidovorax* Leaf76. Through *in silico* data validation, we found a strain combination to reduce pathogen colonization in synergy, namely *Rhizobium* Leaf371 and *Arthrobacter* Leaf337.

In all chapters, we found that a high diversity of the SynCom negatively affected pathogen colonization, i.e. was more suppressive. This was in line with previous research, where invaders had lower colonization capacity when the resident microbiota was more diverse [3-6]. However, we also showed that lower complex communities (SynCom-48p, SynCom-15±2, SynCom-15±5, SynCom8p) can have similar pathogen colonization compared to a community of more than 200 strains (SynCom-210), suggesting that a full function can be reached also with fewer strains. In Chapter IV, we found that three most important strains have similar protection potential to a 35-member community. This suggests that diversity alone does not explain protection potential. Community diversity might increase the probability of the presence of beneficial strains, and the presence of beneficial strains might be more

important than general community diversity. In Chapter III, a community composed of only a fourth of the strains of SynCom-210, SynCom-48p, consisting of protective strains had a comparable pathogen colonization to SynCom-210, and a lower pathogen colonization than SynCom-175np being composed of three times as many, though all were thought to be non-protective by themselves. Previous studies hypothesised that strain abundances in established microbiota might be more important for plant protection than the strain presence [7], suggesting that beneficial microbes need to be competitive in the community to exhibit an effect. In a different system, it was suggested that rare, but competitive strains contribute to suppressive soils, i.e., soils with low disease occurrence [8]. We could show that in our system, strains with high protection scores are more abundant *in planta* compared to strains with low protection scores by themselves (Chapter III). This finding was also supported by the negative correlation of commensal colonization and pathogen colonization found in a tripartite screen of plant-commensal-pathogen [2]. Additionally, in Chapter III, we also show that protective strains are more competitive in competition, which we tested in combination of two commensal strains and in community context. In Chapter IV, the strain identity was the most predictive of pathogen colonization outcomes. We found that total commensal colonization was positively correlated with pathogen colonization, while community evenness was negatively correlated. However, in our system, both of these correlations had high standard deviation, making prediction unreliable. Taken together, we can assume that the identity of a strain, and its function within the community, are most important for pathogen colonization outcomes.

We could show the merits of SynCom experiments coupled with a gnotobiotic system, where we could directly test the absence of strains and effects on pathogen colonization by the inoculated SynCom, and experimentally validate hypothesis found by previous experiments. This was especially highlighted in Chapter IV, where machine learning algorithms suggested three strains to be the main predictors of pathogen colonization, which we validated experimentally and showed pathogen reduction by these three pathogen reducing (PR) strains experimentally. However, in Chapter II, we also highlighted the difficulties of working with complex SynComs, where changes in ASV abundance did not lead to consistent changes in plant phenotype in the presented passaging experiments. We still highlight the importance of investigating the microbe-associated traits in more complex settings, as we found *Acidovorax* Leaf76 in Chapter IV to be one of the pathogen reducing strains, while in a previous screen, albeit using a different plant growth system, it was not found to be protective individually [2]. We also found that *Rhizobium* Leaf371 and *Arthrobacter* Leaf337 were improving protection potential when combined. In Chapter III, we saw that combination of all protective strains into SynCom-48p versus combination of all non-protective strains into SynCom-175np only had small effects on pathogen colonization. Since we found the non-protective *Acidovorax* Leaf76 being one of the pathogen reducing strains in Chapter IV, the question whether there were additional strains with protective potential in SynCom-175np.

In Chapter III, we also observed that strains with higher protection scores were associated with higher competitiveness. The only exception of a low protection score being competitive in a community context was *Rhizobium* Leaf386. Compared to its abundance in SynCom-8np, where only one *Arthrobacter* strain was present, namely Leaf69, *Rhizobium* Leaf386 increased 44-fold in abundance in SynCom-16All, where *Arthrobacter* Leaf137 was also present. Further investigation needs to be made into the mechanisms of the *Rhizobium – Arthrobacter* combination, and how widespread the synergism of this interaction is in the two genera.

In Chapter II, we found several strains (or rather ASVs) to have higher abundances in samples associated with higher pathogen load. When comparing the healthy selection to sick selection or unchallenged to non-selective passaging (mock-infected versus infected), we found *Rhizobium* Leaf311 and *Sanguibacter* Leaf3 to be increased in abundance of the higher pathogen samples (sick or non-selective passaging). Further investigation in which community differences in terms of ASV changes between samples of high and low pathogen abundance could be made in the future. We showed a high plant-to-plant-variation, also in Chapter III, and combining the dataset would give us an extensive dataset to work with. Specifically, we could look into which ASVs correlate with pathogen abundance using a Mantel's test [9] or which ASVs correspond to certain treatments through correspondence analysis [10]. We could also investigate if the selection lines are more dissimilar within each selection compared to between selections to investigate how consistent the selection in Chapter II was [10,11]. While correlating the difference in community composition with different pathogen abundance outcomes might tell us which ASVs are differently abundant, it will not give us insight if these ASVs have changed abundance prior to infection or due to infection. Currently, we compare the community composition of two different treatments (i.e. two populations), and not the community composition of one plant over time. To address the direct link of changes in ASVs upon pathogen infection, the microbiota composition of a plant could be tracked over the course of infection by harvesting single leaves at different timepoints, i.e. before infection and 14 days post infection. In a previous study, the community composition 3 and 5 weeks after inoculation was stable (PERMANOVA 3.22 %, p-value 0.0328) [12]. The two timepoints analysed coincide with the infection timepoint and harvest timepoint used in this study. However, appropriate control conditions must be included in such a time-series experiment. If single leaves are harvested, the wounding of the plant could lead to shifts in the microbiota, and altered pathogen virulence, since *Ps*t can enter and infect through wounds [13]. However, such an experiment could lead to further insights into which ASVs respond to pathogen infection and in what manner they do so.

It was previously suggested that the community composition might change over time, the community function as a whole is maintained and stable [14]. For plant protection, this would mean that the maintenance of presence of protective functions, i.e. antibiosis and resource competition against the pathogen, is more important than the presence of strains in general. In Chapter II, we saw that the community composition of the low-complex community, SynCom-15, did not change when selecting for plant phenotypes, but the overall plant phenotype (healthy versus diseased) was consistent over

selections. In this case, we could analyse whether the lack of compositional changes was made up by functional changes within the strains. Through isolation of the passaged strains and comparison to the non-passaged ancestors, we could gain insight into how fast mutation and evolution occurs in our system. In competition and protection assays we could investigate how much the passaged strains might have improved, individually and as a community, in terms of colonization capacity and protection against the pathogen. Additionally, we could investigate if the protection against *Ps*t infection is also effective against other foliar pathogens, like bacterial pathogens of the genus *Xanthomonas* [15] or fungal pathogen *Botrytis cinerea* [16].

We still lack insights into the mechanisms of the strains we found to be important for plant protection. But our approaches showed that we could consistently pinpoint important strains, and some features, like competitive colonization. Our approaches reduced both false-negatives and false-positives strain identity findings. We showed that the identified beneficial strains (Chapter IV) did not inhibit each other as was shown before [17]. Looking for important patterns reduces the chances of finding behaviour changes, like a switch from being non-protective individually to protective in a community setting, as was seen for *Rhodococcus* Leaf278 [2]. This change could be due to character displacement upon competition and interaction with other strains, as was shown previously [18]. Additionally, indirect interactions can also be found, where the microbiota reduces so-called "pathogen helpers", strains that would facilitate pathogen invasion [19].

The mechanisms with which the microbiota members can reduce pathogen invasion are many and hard to determine. A previous study has shown that a small community of commensal *Pseudomonas* can protect the plant against a community of pathogenic *Pseudomonas* by modulating the plant response, when pathogenic strains were present [20]. The plant reacted differently to the presence of only commensal and a combination of commensal and pathogenic strains. In the rhizosphere, the plant can attract beneficial microbes upon pathogen challenge [21,22]. This raises the question to what extend this is true in the phyllosphere. In Chapter II, we found that the microbiota of healthy selected plants was distinct from sick selected plants from the first selection in the parental passage. However, disease phenotypes associated with these selections was inconsistent. This might be due to the high plant-to-plant variation of pathogen colonization in each treatment that we also saw in Chapter III. Other mechanisms of protection against pathogens include direct antibiosis through antibiotic compounds or killing through the type 6 secretion system (T6SS) [23]. The diversity of the microbiota makes a comparison of function between low and high protection hard, but it was found that in the Rhizobium genus, the protective strains have the T6SS, while non-protective do not [2]. Antibiotic activity against *Ps*t was shown for only a few strains of the *At*-LSPHERE *in vitro* [24], though it was suggested that phyllosphere strains need more cues additional to pathogen presence to exhibit antibiotic activities [25]. Some studies have investigated biofilm formation of potential beneficial strains [22,26,27] as a way to hinder pathogen invasion. Resource competition was shown to be an efficient way to limit pathogen growth, as well as a good predictor on pathogen invasion [28-31]. Communities with high carbon source utilization overlap with the pathogen were

shown to be better at limiting pathogen growth, while that was achieved through higher diversity, the best outcome was seen for commensals that used many carbon sources without competing against each other. For the *At*-LSPHERE, efforts to map carbon source utilization and predict competition outcomes have been made, and could be an interesting tool to add to the prediction algorithms presented in Chapter IV (Schäfer, Pacheco et al., 2023, in revision).

Further investigation into the application potential of beneficial microbes needs to be done. One of the questions unanswered in this thesis is in which order the strains need to arrive. Could we use beneficial strains as a probiotic prevention and if the arrive after the pathogen, would they still suppress or facilitate infection, as was previously shown? [32,33].

The current approaches to restore plant protection through isolation, screening, formulation and application of microbial inoculants and synthetic consortia have inconsistent success, in part due to microbe-microbe interactions, plant genotype, climate, existing microbiome and other physicochemical characteristics of the environment [34]. However, by exploiting the potential of the native microbiota, there is an opportunity to identify competitive and persisting biocontrol strains [35].

This thesis aimed to improve the knowledge of the protection potential of the native microbiota of *Arabidopsis thaliana*. We could show that pathogen colonization was reduced by a diverse synthetic community, but certain key strains were able to reduce the pathogen in less diverse settings. We have set up a experimental and analytical pipeline to screen for microbiota patterns. In our system, the strain identity seems to be the best predictor of pathogen colonization outcomes and therefor the presence of key strains seem to be most important prerequisite for protection. In the future, investigation into genomic and metabolomic functions of these strains might identify further causal relationships between microbiota composition and protection potential.

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