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EFFECTS OF ENVIRONMENTAL CHANGE ON BACTERIAL DIVERSITY IN AQUATIC META-ECOSYSTEMS

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presented by
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SUMMARY

One of the core areas of ecological research is to understand species distributions across spatial and environmental gradients. Environmental change can affect spatially structured species distributions and modify ecosystem functioning. Many ecosystem functions, such as biomass production and the decomposition of organic material, are driven by microbes, which are the most abundant organisms on our planet, and essential drivers of ecosystem functions. While being so important for live on earth, we still know very little about the factors governing microbial diversity. One reason for this is that high resolution molecular methods, which allow for describing microbial diversity and community composition, have only been developed within the last few decades. However, even modern sequencing methods still have drawbacks and limitations and there is still no standard method for analyzing microbial communities. Another reason is that natural ecosystems are highly complex, and it is difficult to disentangle the multiple drivers of bacterial diversity in natural populations. One useful way to investigate how environmental change affects aquatic communities across multiple trophic levels is to replicated ecosystems in outdoor mesocosm, as this allows for a balance between realism and experimental control over environmental conditions. The major aim of this thesis was to investigate how bacterial communities are shaped by biogeography and environmental variation. In order to quantify bacterial community responses, I first investigated the effect of variable region of the 16S rRNA on ecological measures of diversity, which were later applied to address ecological questions of bacterial community assembly. I then performed two large-scale outdoor mesocosm experiments (using sets of 300L tanks), each running over several months. I manipulated the food-web structure and environmental conditions of mesocosm ecosystems in order to test for the responses of bacterial communities, and communities at higher trophic levels. In general, my results show that mesocosms offer a good model system for studying how environmental change and trophic interactions affect bacterial community structure and functioning. In the first experiment, I tested how the presence of a consumer (*Daphnia*) affects bacterial and phytoplankton communities within meta-communities with regular dispersal. *Daphnia* strongly reduced the richness of both communities, and affected bacterial community composition, and overall ecosystem functioning (respiration). In the second mesocosm experiment, I investigated how environmental heterogeneity and the fluxes of material and organisms shape bacterial, phytoplankton, and zooplankton communities. Communities showed very different responses to the applied treatments, and I found strong effects on diversity, community composition, as well as on the abundance of individual species. Overall, I show that organisms of higher trophic levels have high impacts on the spatial structure of bacterial communities and bacteria mediated ecosystem functioning.

ZUSAMMENFASSUNG

Die Untersuchung, wie sehr sowohl biogeographische Gradienten, als auch Umweltveränderungen die Ausbreitung und räumliche Verteilung von Organismen beeinflussen, ist ein Kerngebiet der ökologischen Forschung. Umweltveränderungen können sich auf die räumliche Struktur der Verteilung von Organismen auswirken und so auch Ökosystemfunktionen verändern. Kleinstlebewesen (Mikroorganismen) sind nicht nur die häufigsten Lebewesen auf unserem Planeten, sondern auch verantwortlich für viele Ökosystemfunktionen, wie zum Beispiel den Auf- und Abbau von organischem Material. Obwohl das Leben auf unserer Erde ohne Mikroorganismen nicht möglich wäre, wissen wir immer noch sehr wenig darüber, welche Faktoren ihre Vielfalt beeinflussen. Ein Grund dafür ist, dass hochauflösenden molekulare Methoden, welche es uns erlauben, mikrobielle Diversität besser zu untersuchen, erst in den letzten Jahrzehnten entwickelt worden sind. Doch auch hochmoderne molekulare Sequenziermethoden haben noch immer ihre Nachteile und Grenzen, und so gibt es bisher noch keine Standardmethode für die Untersuchung von mikrobiellen Gemeinschaften. Ein weiterer Grund, warum wir immer noch relativ wenig über die mikrobielle Welt wissen, ist die hohe Komplexität von natürlichen Ökosystemen, welche es schwer macht, genau herauszufinden, welche Faktoren wichtig sind die Verteilung von Organismen. Die Durchführung von Mesokosmosexperimenten ist eine gute Möglichkeit, die Auswirkungen von Umwelteinflüssen auf mehrere Trophiestufen in aquatischen Systemen zu untersuchen. Solche Experimente können draussen und in grossen Replikaten durchgeführt werden und ermöglichen eine gute Balance zwischen realistischen Umweltbedingungen und gleichzeitiger Kontrolle einiger Umweltfaktoren. Das Hauptziel meiner Doktorarbeit war es, zu untersuchen, wie sich Umwelteinflüsse und biogeographische Gradienten auf die Bakteriengemeinschaft auswirken. Im ersten Teil meiner Arbeit habe ich mich damit beschäftigt, welchen Einfluss die Verwendung unterschiedlicher hypervariabler Regionen des 16S rRNA-Genes (ein Markergen für die Untersuchung von mikrobiellen Gemeinschaften), auf verschiedene ökologische Analysen von Bakteriengemeinschaften hat. Des weiteren habe ich zwei grosse Mesokosmosexperimente in replizierten 300L Tanks durchgeführt und dabei die Auswirkungen der Zusammensetzung des Nahrungsnetzes auf die Bakteriengemeinschaft sowie höhere Trophiestufen untersucht. Meine Ergebnisse haben gezeigt, dass Mesokosmen ein gutes Modellsystem sind, um zu erforschen, wie Umwelteinflüsse auf trophische Interaktionen und die Zusammensetzung sowie die Funktion von Bakteriengemeinschaften wirken. Im ersten Mesokosmosexperiment habe ich den Einfluss *Daphnien* auf die Bakterien- sowie die Phytoplanktongemeinschaft untersucht und dabei herausgefunden, dass *Daphnien* die Artenvielfalt beider Gemeinschaften stark reduziert haben. Des weiteren haben diese die Artenzusammensetzung der Bakteriengemeinschaft und Ökosystemfunktionen (Respiration) verändert. Im zweiten Mesokosmosexperiment habe ich den Einfluss von Umweltheterogenität, sowie der Verteilung von Organismen und (organischem) Material zwischen verschiedenen Habitaten auf Bakterien-, Phytoplankton- und Zooplanktongemeinschaften erforscht. Die verschiedenen Gemeinschaften haben sehr unterschiedliche Reaktionen auf diese experimentellen Manipulationen gezeigt, wobei ich grosse Einflüsse auf die Diversität, die Artenzusammensetzung, sowie die Häufigkeit einzelner Arten aller

trophischen Ebenen gemessen habe. Zusammenfassend habe ich mit dieser Arbeit gezeigt, dass die Artenzusammensetzung verschiedener trophischer Stufen einen grossen Einfluss auf die Vielfalt und Zusammensetzung von Bakteriengemeinschaften und deren Ökosystemenfunktionen haben kann.

PUBLICATIONS

This thesis is based on the following papers:

I J. Birtel, J.-C. Walser, S. Pichon, H. Bürgmann, B. Matthews (2015). Estimating Bacterial Diversity for Ecological Studies: Methods, Metrics, and Assumptions. *PLOS ONE*.

II J. Birtel, B. Matthews. Consequences of Grazing Pressure and Dilution on Bacterial and Phytoplankton Community Assembly (*Manuscript*).

III J. Birtel, B. Matthews. Experimental evidence that heterogeneity and dispersal affect the spatial structure at multiple trophic levels in meta-ecosystems (*Manuscript*).

Additional publication, which is not included in this thesis:

N. Czekalski, R. Sigdel, J. Birtel, B. Matthews, H. Bürgmann (2015). Does human activity impact the natural antibiotic resistance background? Abundance of antibiotic resistance genes in 21 lakes. (Accepted at *Environmental International*)

CONTENTS

1

INTRODUCTION

GENERAL BACKGROUND

Understanding the spatial and temporal distribution of species on our planet is a core area of ecological research that has fascinated generations of biologists. In the era of climate change, it is particularly important to investigate if and how ongoing environmental changes will affect the composition and species distributions of natural communities. This knowledge is needed both for the conservation of biodiversity, and for the understanding of how biodiversity changes may affect ecosystem functions and services, such as biomass production and organic matter decomposition. Environmental change is predicted to alter habitats and change community assembly processes across the landscape through changes in, for example, precipitation and temperature. Emerging theory about how communities interact via dispersal and energy (i.e. meta-community and meta-ecosystem theory) is a useful starting point to explore how local and regional processes may explain spatial variation in community composition and ecosystem functioning. Instead of focussing on individual patches, these theories try to integrate how the surrounding patches and environmental conditions interact with local community dynamics (Fig. 1.1).

From meta-communities to meta-ecosystems

A meta-community is defined as a set of local communities which are connected through dispersal of organisms. Metacommunity theory (Leibold et al., 2004) describes four non-exclusive theoretical paradigms which can help identify the processes governing patterns of species distributions and coexistence in spatially structured communities (Leibold and Norberg, 2004; Holyoak et al., 2005; Logue et al., 2011b). These paradigms are species-sorting (SS), mass-effects (ME), patch-dynamics (PD) and neutral-model (NM) (Holyoak et al., 2005), and each describe specific mechanisms which determine species distributions. The species sorting paradigm assumes that local patches are heterogeneous and that patch qualities and niche differentiation drive species occurrence patterns among patches. In the

mass-effects paradigm, high amounts of dispersal between heterogenous patches can create source-sink dynamics which overwhelm differences in niche use and and competitive abilities among species. The patch-dynamics paradigm considers a trade-off between dispersal and local fitness (competition-colonization trade-off), and the neutral-model assumes that all species are equivalent in competitive abilities, movement and fitness, and that stochasticity determines species abundances. None of these paradigms alone can explain community dynamics, but they can be used as a general framework for describing regional species distributions, where the importance of different mechanisms depends on multiple biotic and abiotic characteristics, such as species identities and interactions, food-web structure, ecosystem type, biogeography, and spatial scale (Heino et al., 2014). A recent extension of meta-community theory is the meta-ecosystem concept, which not only focuses on the dispersal of organisms between local patches, but also on the spatially structured flux of organisms, energy and materials (Loreau et al., 2002).

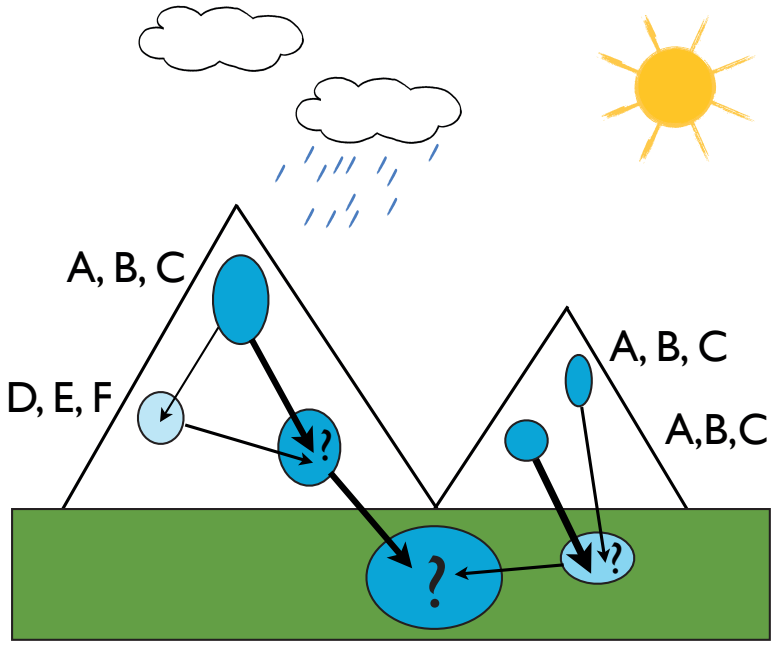

Figure 1.1: ***Conceptual meta-ecosystem illustration.*** *The figure illustrates a network of lakes, which are connected through different quantities of dispersal. What will happen to the local communities (displayed with different letters), when they disperse to a new environment? Who will survive and grow? How do local and regional environmental conditions, and the fluxes of material influence species compositions and ecosystem functioning? How does the food-web structure affect community responses?*

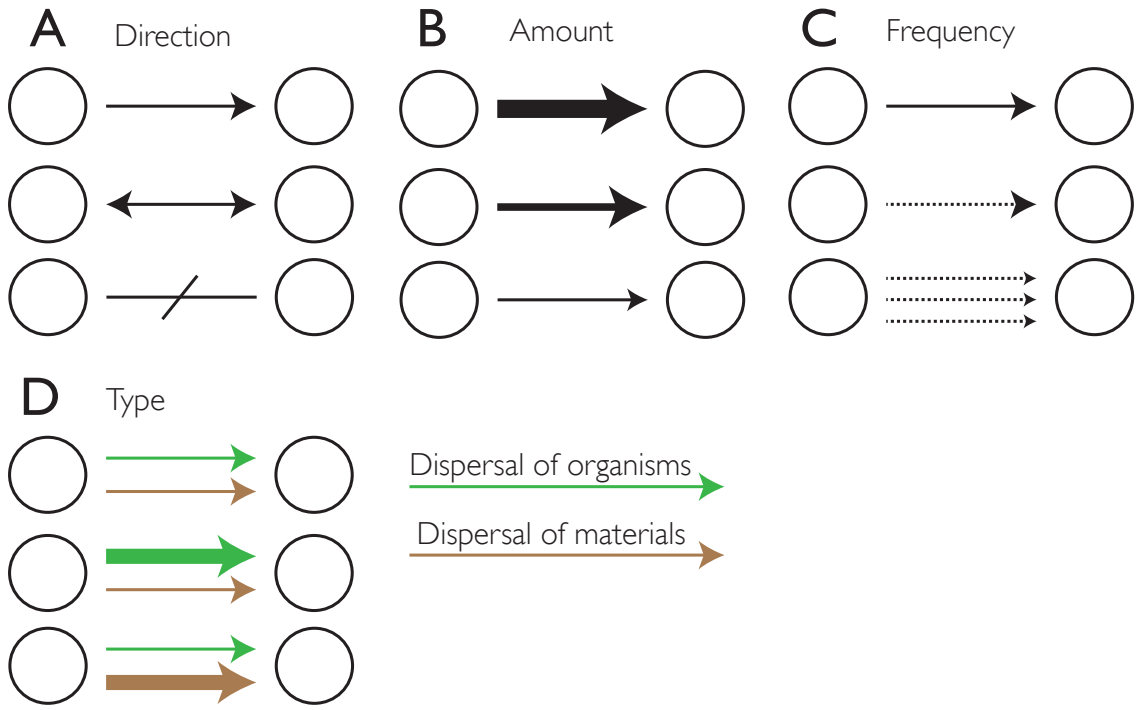

Figure 1.2: ***Conceptual figure of different dispersal characteristics.*** *Amongst others, dispersal between patches can vary in dispersal direction (A), dispersal quantities (B), and frequency of dispersal pulses (C), and can include the dispersal of organisms and other materials, which can be dispersed in different quantities (D).*

Dispersal

One important aspect of meta-community and meta-ecosystem dynamics is the nature of dispersal between local patches, which may vary in direction, amount, and frequency (Fig. 1.2). Dispersal can be either uni- or bi-directional, or non-existent due to insurmountable barriers (Fig. 1.2A). In a network of lakes that are connected through the flow of water (Fig. 1.1), passive dispersers will generally move in the direction of water flow, while motile organisms, and organisms with the ability to fly can move in either direction. Dispersal quantities (Fig. 1.2B) are important for determining source-sink dynamics and can create mass-effects. Finally, dispersal frequencies (Fig. 1.2C) can vary through time, for example due to spring floods and water regulations. Both organisms and other materials can disperse in different quantities (Fig. 1.2D) and vary in direction and frequencies. Due to climate change, higher amounts of precipitation are expected in some parts of the world, while other parts may suffer water shortages (Dore, 2005). Climate change also predicts an increase in flood frequency for some parts of the world (Hirabayashi et al., 2013), which can lead to strong variations in dispersal quantities and frequencies.

The importance of dispersal for meta-community dynamics and diversity has been studied in several theoretical and empirical setups and for various communities (e.g. Logue et al.,

2011b; Eklöf et al., 2012; Vanschoenwinkel et al., 2013; Berga et al., 2014), and studies have revealed differential responses with body size and dispersal mode (Beisner et al., 2006; De Bie et al., 2012; Verreydt et al., 2012). Most studies focussed on the dispersal of organisms, but recent theoretical frameworks have also addressed how the dispersal of material fluxes influences community and ecosystem responses (Massol et al., 2011).

Trophic interactions

Metacommunity dynamics also depend on the trophic structure within and among local habitats. On the one hand, the emergence of species-rich food webs can be explained by metacommunity theory, as intermediate levels of colonization and limited dispersal are expected to maximize food-web complexity (Pillai et al., 2011). Food-web complexity, on the other hand, can influence diversity within metacommunities. The presence of predators has been shown to reduce both local and regional richness across multiple trophic levels (Chase et al., 2009; Berga et al., 2014) and can therefore alter the mechanisms of spatially structured community assembly. For example, Howeth and Leibold (2008) have shown that zooplankton and phytoplankton biomass strongly responded to changes in trophic structure, which were generated by differences in connectivity between patches. Verreydt et al. (2012) has demonstrated that dispersal can lead to metacommunity responses that cascade through food-webs. This is particularly true when keystone species, for example *Daphnia*, are dispersal limited.

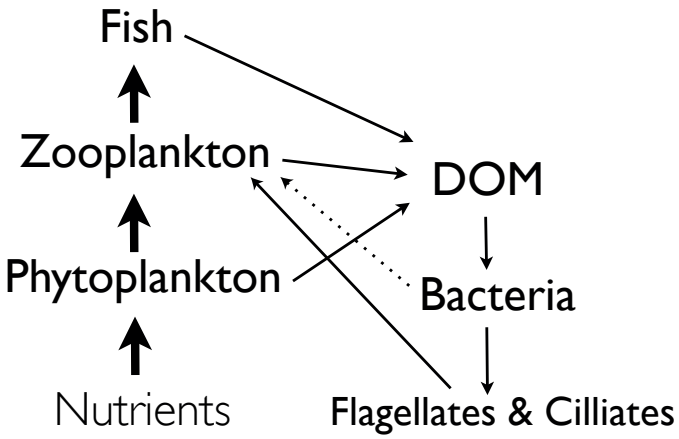

Figure 1.3: ***Conceptual figure of a simple aquatic food web.***

Bacterial communities

Microbial communities are important drivers of many key ecosystem processes, such as the cycling of carbon and nitrogen (Falkowski et al., 2008). The abundance and composition of microbial taxa affects ecosystem functions and services, while environmental change can affect species distributions (Petchey et al., 1999; Langenheder et al., 2010; Venail and Vives, 2013). However, ecological theory describing species distributions has been developed mainly for plants and animals and can only partly be applied for bacterial communities due to their unique properties such as asexual reproduction and horizontal gene transfer (Prosser et al., 2007). Short generation times, small size, and recent advances in molecular techniques, though, make bacterial communities more popular model systems, to help bridge the gap between ecological theory and microbial ecology (Jessup et al., 2004).
Several metacommunity studies have investigated the importance of local environmental and spatial distance for species distributions, and included bacterial communities (some examples are shown in Table 1.1, and see also Logue et al. 2011b), but the importance of environmental parameters and spatial structure for bacterial community responses remains unclear. This may partly be due to the use of community fingerprinting methods that differentially resolve bacterial diversity (such as ARISA and T-RFLP). Such methods only consider dominant species (Orcutt et al., 2009), while species diversity may be hidden in the rare and closely related species. Recent studies have started using next generation sequencing techniques to characterize bacterial communities so as to understand how environmental and spatial factors control bacterial communities. Combining high resolution sequencing methods and spatially structured food-web experiments might help to understand how natural bacterial communities respond to environmental change, and how this might affect ecosystem functioning.

Testing ecological theory in mesocosms

One way to study metacommunity dynamics in near-natural, but controlled conditions is to use mesocosms (Fig. 1.4). While natural ecosystems are highly variable and complex systems, mesocosms offer a bridge between small scale lab experiments in microcosms, and natural systems (Stewart et al., 2013). Mesocosms are outdoor experimental units closely simulating environmental conditions, which can be replicated and provide more realistic conditions, that are not possible in the lab (Odum, 1984). Mesocosms have been used to study components of environmental change, metacommunity structure, and trophic interactions at multiple levels of complexity, at different scales, and in different habitats (Stewart et al., 2013). Since mesocosms can be highly controlled, but still resemble near-

natural ecosystems, they can serve as useful means for studying effects of environmental change. For example, it is possible to manipulate dispersal events, nutrient conditions, temperature, and food-web complexity.

AIMS OF THIS THESIS

In this thesis, I explore the following questions in aquatic systems:

- How do biogeography and environmental variation influence diversity and ecosystem functioning?
- How does food-web structure in general, and predation in particular, shape metacommunity diversity?
- How does environmental heterogeneity affect the spatial structure of diversity across multiple trophic levels?

To address these questions, I have conducted a lake survey across Switzerland, as well as two mesocosm experiments. The main focus of my work was to study bacterial community responses to environmental change, but I also measured phytoplankton and zooplankton community responses. Methods to characterize bacterial communities have evolved rapidly over the last decades, from simple fingerprinting techniques to the sequencing of entire communities. Because of recent advances in method developments, **chapter I** focusses on how the hypervariable region of the 16S rRNA, a standard gene for comparing bacterial community composition, influences measures of bacterial diversity and community composition. For **chapter II**, I have tested for the joint effects of grazing and the abundance of bacterial cells on bacterial and phytoplankton communities using a mesocosm experiment. **Chapter III** describes a second mesocosm experiment in which I explored the effects of resource heterogeneity and dispersal regime on bacteria, phytoplankton, and zooplankton communities. In **chapters II and III**, the bacterial community is characterized by the method developed in **chapter I**.

METHODS

Variable region comparison

Unlike phytoplankton and zooplankton taxa, which can be characterized morphologically using microscopy, the use of morphological differentiation for species identification is not possible for bacterial communities due to the small size the vast diversity of bacterial taxa. The 16S rRNA has become a standard marker gene for determining bacterial community

composition. This gene is highly conserved in prokaryotes, because it codes for ribosomes, which are essential cell organelles, but certain parts of this gene are hypervariable among species. Ideally, we would be comparing whole 16S rRNA sequences when comparing and characterizing bacterial communities, but due to technical limitations, this is not yet possible and researchers have to restrict themselves to one or several of the hypervariable regions of the 16S rRNA gene. As regions vary in their abilities to identify and resolve taxa, **chapter I** focusses on how this influences ecological measures of diversity, which will then be applied in the subsequent chapters.

Mesocosm experiments

The two mesocosm experiments conducted for this thesis were performed in large, replicated outdoor tanks (Fig. 1.4). Each experiment ran for several months, was sampled regularly over the course of the experiment, and more intensively at the end of the experiment. To create metacommunities and determine the spatial structure of diversity (β-diversity), water was dispersed within mesocosm treatments (300L tanks).

In the first mesocosm experiment (**chapter II**), I tested how the presence of *Daphnia*, and the initial abundance of bacterial cells interactivity affect bacterial and phytoplankton diversity and community composition (Fig. 1.5A). The effect of *Daphnia* grazing for bacterial and phytoplankton communities was investigated because results from previous experiments using larger organisms suggests that predation can significantly reduce local and regional richness and community dissimilarity of prey communities (Chase et al., 2009). Some comparative studies (Table 1.1) of bacterial communities have included upper trophic levels as determinants of bacterial community composition, but the influence of other trophic levels on bacterial communities remains unclear. In addition to the grazing treatment, we diluted the bacterial community in half of the metacommunities at the beginning of the experiment in order to remove rare taxa and investigate how this might affect the community assembly of both the bacterial and phytoplankton community.

In the second mesocosm experiment (**chapter III**), I tested for more complex trophic interactions in spatially structured environments, and for the importance of meta-ecosystem fluxes. I crossed a nutrient loading regime, in which I created either homogenous or heterogenous metacommunities, with a dispersal regime, in which organisms were either dispersed live or killed prior to dispersal. The dead dispersal regime was applied to test for the importance of material fluxes, relative to the fluxes of material and organisms in the live dispersal.

Figure 1.4: **Pictures of the mesocosm experiments from 2011 (left; chapter II) and 2012 (right; chapter III).**

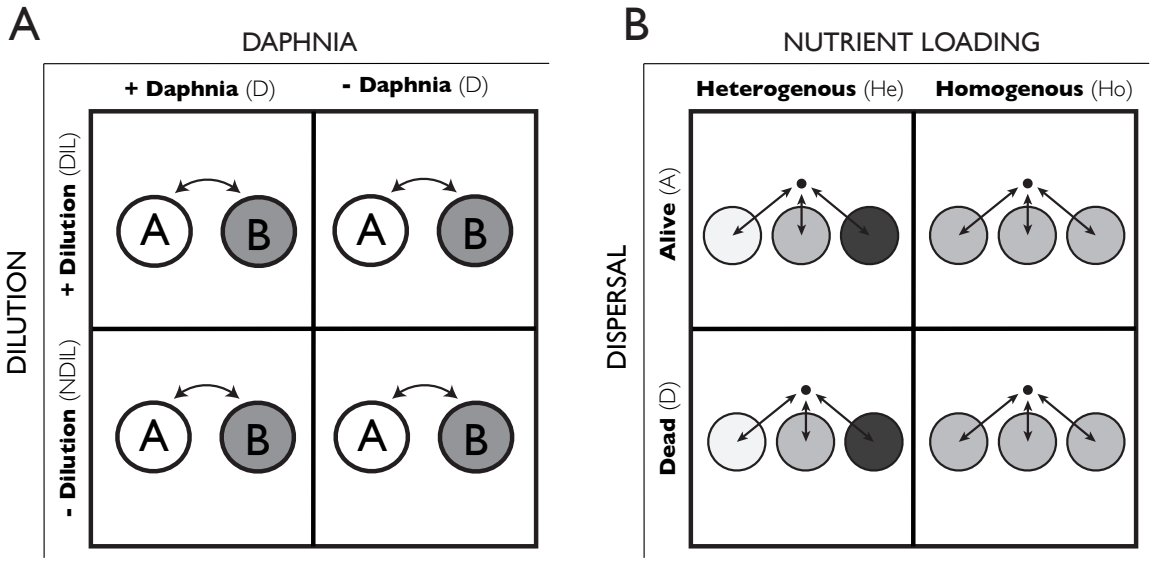

Figure 1.5: ***Experimental design of the mesocosm experiments from 2011 (A; chapter II) and 2012 (B; chapter III).*** *Chapter II describes the results of the 2011 experiment, in which the presence of Daphnia was crossed with the dilution of microbial cells, and chapter III describes the results of the 2012 experiment, in which nutrient heterogeneity and dispersal mode were altered.*

Table 1.1: **Examples of environmental & spatial factors tested in microbial metacommunity studies**. *x = parameter was tested, but no significant effect was detected, xs = significant effect of parameter was detected. The table also includes the method which has been used for measuring bacterial community composition.*

	Study system/ Reference							
	freshwater lakes[1]	freshwater lakes[2]	freshwater lakes[3]	rock pools[4]	rock pools[5]	rock pools[6]	salt lakes[7]	microcosms[8]
Abiotic parameters								
Water color				xs	x			
Water transparency		xs						
Salinity				xs		x	x	
Total phosphorus		x	x	x	x	x		
Total nitrogen		xs	x	x				
pH	xs	xs	x	x	x		xs	
DOC			x	x				
DIC			x					
Absorbance						x		
Conductivity		xs			x		x	
Secchi depth		x	x					
Temperature	xs	x			x	xs	xs	
Water retention time	xs							
Area	x	xs						
Volume			x	x				
Depth		xs	x					
Environmental heterogeneity						x		
Geographic distance		x					x	
Concentration of different ions							xs	
Biotic parameters								
Chlorophyll a		x		xs	x	xs		xs
Total phytoplankton biomass		xs	x					
Total Zooplankton biomass		xs	x					
Heterotrophic nanoflagellate density		xs			x			xs
Daphnia concentration						x		
% Cyanobacteria		x						
% Bosmina		xs						
% Daphnia		x						
Cilliate biomass		x						
Dinoflagellate biomass		xs						
Calanoid copepod biomass		xs						
Bacterial abundance		x			x			x
Abundance of several bacterial groups								xs
Bacterial growth efficiency								xs
Respiration								xs
Local vs. regional factors								
Local (environmental) factors	x	xs	xs			xs	xs	
Spatial factors	x	x	x			xs	xs	
Method for measuring bacterial community composition								
Reverse blot hybridization	x							
DGGE		x	x					
T-RFLP				x		x		
454-Sequencing					x		x	x

References: 1: Lindström et al. (2005), 2: Van der Gucht et al. (2007), 3: Beisner et al. (2006), 4: Langenheder and Ragnarsson (2007), 5: Langenheder and Székely (2011), 6: Langenheder et al. (2011) 7: Pagaling et al. (2009), 8: Berga et al. (2014).

2

CHAPTER I: VARIABLE REGION COMPARISON

ESTIMATING BACTERIAL DIVERSITY FOR ECOLOGICAL STUDIES: METHODS, METRICS, AND ASSUMPTIONS

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ABSTRACT

Methods to estimate microbial diversity have developed rapidly in an effort to understand the distribution and diversity of microorganisms in natural environments. For bacterial communities, the 16S rRNA gene is the phylogenetic marker gene of choice, but most studies select only a specific region of the 16S rRNA to estimate bacterial diversity. Whereas biases derived from from DNA extraction, primer choice and PCR amplification are well documented, we here address how the choice of variable region can influence a wide range of standard ecological metrics, such as species richness, phylogenetic diversity, β-diversity and rank-abundance distributions. We have used Illumina paired-end sequencing to estimate the bacterial diversity of 20 natural lakes across Switzerland derived from three trimmed variable 16S rRNA regions (V3, V4, V5). Species richness, phylogenetic diversity, community composition, β-diversity, and rank-abundance distributions differed significantly between 16S rRNA regions. Overall, patterns of diversity quantified by the V3 and V5 regions were more similar to one another than those assessed by the V4 region. Similar results were obtained when analyzing the datasets with different sequence similarity thresholds used during sequences clustering and when the same analysis was used on a reference dataset of sequences from the Greengenes database. In addition we also measured species richness from the same lake samples using ARISA Fingerprinting, but did not find a strong relationship between species richness estimated by Illumina and ARISA. We conclude that the selection of 16S rRNA region significantly influences the estimation of bacterial diversity and species distributions and that caution is warranted when comparing data from different variable regions as well as when using different sequencing techniques.

INTRODUCTION

One of the central goals of microbial ecology is to measure and understand the distribution of diversity across spatial and temporal gradients. Ecologists are increasingly interested in using microbial communities to test a wide range of classic ecological hypotheses (Prosser et al., 2007; Nemergut et al., 2013; Martiny et al., 2006; Horner-Devine et al., 2004). In the field of biogeography and macro-ecology, for example, microbial communities have been used in numerous comparative and experimental studies to test whether environmental properties could explain patterns of microbial diversity over a range of spatial scales (Griffiths et al., 2011; Jankowski et al., 2014; Horner-Devine et al., 2003). In metacommunity ecology, there is a growing interest in the relative importance of dispersal and environmental conditions for explaining patterns of microbial diversity (Beisner et al., 2006; De Bie et al., 2012; Declerck et al., 2012) and community assembly (Ofiteru et al., 2010; Burke et al., 2011). Furthermore, in studies of biodiversity and ecosystem function (Cardinale et al., 2012a), microbial communities are rapidly becoming model systems to explore how the composition (*i.e.* species richness and functional diversity) and abundance of microbial taxa can affect specific ecosystem functions and services (Venail and Vives, 2013; Petchey et al., 1999; Petchey and Gaston, 2006). Rank-abundance distributions of microbial communities have also been used to discriminate between alternate models of community assembly (Ofiteru et al., 2010) and to understand how the rare biosphere might be functionally important (Pedrós-Alió, 2011; Besemer et al., 2012). While the rapid development of methods to quantify microbial communities indeed shows great promise for testing ecological theory, it is increasingly important to evaluate how estimates of diversity vary due to technical and methodological considerations.

Before the era of molecular techniques, microbial communities were commonly identified using microscopy or cultivation (LeChevallier et al., 1980), but these methods are known for only capturing a fraction of the microbial taxa present in the environment (Staley and Konopka, 1985). Over the past three decades, microbial ecologists have increasingly been using the 16S rRNA (Olsen et al., 1986; Ward et al., 1990) as a marker gene to differentiate among microbial taxa, and the growing number of sequences in publicly accessible reference databases makes taxa identifications from 16S rRNA sequences more reliable. The characterization of microbial communities through 16S rRNA sequences has become a standard method in microbial ecology and a growing number of open-source sequence analysis tools (such as "mothur" (Schloss et al., 2009), "QIIME" (Caporaso et al., 2010), or "RDP" (Cole et al., 2013)) facilitate the analysis of the large amount of sequences produced by modern massive parallel sequencing methods.

Methods to characterize microbial communities through 16S rRNA sequences have developed rapidly. To reduce costs and time, the classic approach of creating clone banks (Pace et al., 1986; Schmidt et al., 1991) followed by Sanger sequencing (Sanger et al., 1977) has been replaced by next-generation sequencing (NGS) technologies (Schuster, 2007) that produce huge amounts of sequences in very short amounts of time. This development has vastly increased our understanding of environmental microbial communities (*e.g.* Pinto et al. 2012) and medically relevant microbiomes (*e.g.* Grice and Segre 2011; Turnbaugh et al. 2006). However, one of the drawbacks of NGS approaches is the limited read length and that sequencing the complete 16S rRNA gene of entire communities is still costly and methodologically complicated. NGS research is therefore commonly restricted to one or a few of the nine variable regions of the 16S rRNA gene. It is well known that different variable regions of the 16S rRNA gene vary in their abilities to identify and resolve microbial taxa (Kim et al., 2011; Vasileiadis et al., 2012; Guo et al., 2013; Vinje et al., 2014; Schloss, 2010; Klindworth et al., 2012; Engelbrektson et al., 2010; Huse et al., 2012), but there is no consensus about how to choose the best region to characterize microbial communities, and how robust a particular ecological conclusion is based on the choice of region.

Here, using data from 20 bacterial community samples from Swiss lakes (Figure S1), we focus on how the choice of variable region of the 16S rRNA gene influences common biodiversity metrics, including species richness (SR), community composition, phylogenetic diversity (PD) (Cadotte et al., 2010), the relationship between SR and PD and environmental gradients (Green et al., 2008), β-Diversity (Anderson et al., 2011) and rank-abundance distributions (Preston, 1948; Aoki, 1995) (Fig. 2.1). We have used both Illumina MiSeq sequencing of the 16S rRNA gene between the V3 and V5 regions and a community Fingerprinting technique (ARISA = Applied automated Ribosomal Intergenic Spacer Analysis; Fisher and Triplett 1999), which uses the intergenic spacer region between the 16S and the 23S rRNA for determining bacterial diversity. We have used a set of natural lake samples to explore variation in the composition of the microbial communities, and to better understand how different variable regions of the 16S rRNA gene affect patterns of diversity, and furthermore applied the same analysis pipeline to reference data from the Greengenes database in order to compare our conclusions from natural samples to an existing database of sequences.

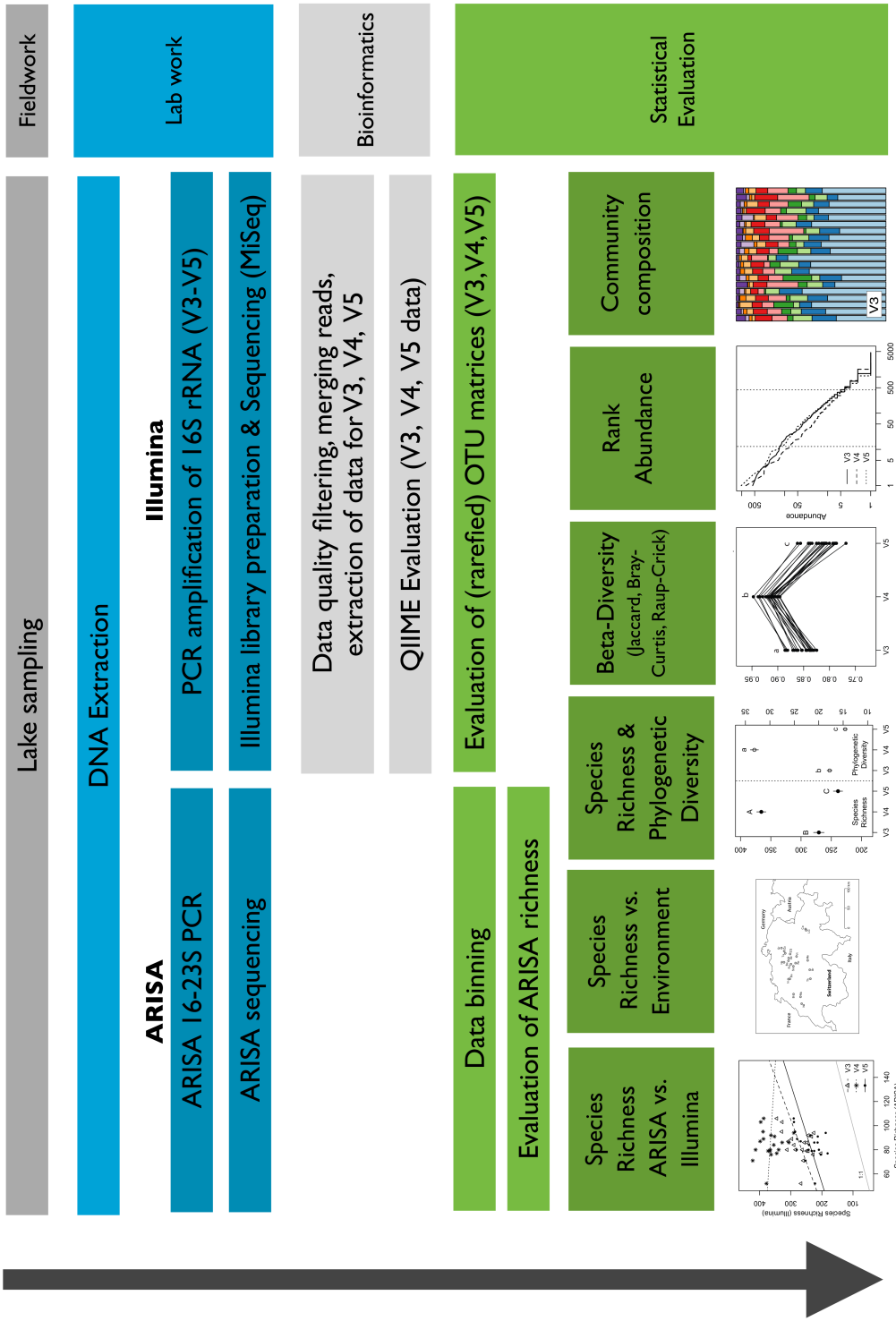

Figure 2.1: ***Conceptual figure of the study design.*** *We have sampled 20 Swiss lakes, performed bioinformatical analyses and applied several ecological concepts on evaluating the microbial communities both with a fingerprinting method (ARISA), as well as by next generation sequencing (Illumina) from three variable regions of the 16S rRNA gene.*

MATERIALS AND METHODS

Sampling and DNA extraction

We sampled 20 Swiss lakes (Figure S1, Table S1) during the stratified period in the summer of 2011 (July to October). The lakes span a broad range of environmental characteristics, such as surface area, elevation, nutrient level, and dissolved organic carbon (DOC) concentrations. All lakes were sampled at their deepest point, using water samples integrated over the first five meters of the water column. Between 60-240 mL of lake water were filtered onto 0.2 μm polyethersulfone filters (Supor 200 Membrane Disc Filters) at the same day of sampling, and filters were instantly frozen in liquid nitrogen and preserved at -80°C until further processing. Microbial DNA was extracted from preserved filters by enzymatic digestion and cetyltrimethyl ammonium bromide (CTAB) extraction (Llirós et al., 2008). The same DNA samples were used both for Automated Ribosomal Intergenic Spacer Analysis (ARISA) and NGS amplicon sequencing using Illumina technology.

Amplicon sequencing

Sample preparations and sequencing

Using a high-fidelity polymerase (Phusion High-Fidelity PCR, New England Biolabs), we amplified the microbial 16S rRNA gene between the variable regions V3, V4, and V5 using a single primer set of custom-designed degenerate primers (forward primer: 327-ACACGGYCCARACTCCTAC-345, reverse primer: 969-TTGCWTCGAATTAAWCCAC-951). The primers were placed at conserved sites identified by Wang *et al.* (Wang and Qian, 2009) and designed to reduce primer-dimers and hairpin structures, and to reduce amplification of algal chloroplasts. To keep the PCR amplification bias low, we performed three low cycle PCR reactions (15 cycles) for each sample and subsequently pooled the PCR products. Pooled PCR products were then cleaned using AMPure XP beads (Beckman Coulter). Illumina library preparations of the amplified and cleaned PCR products were performed using the Nextera XT DNA Sample Preparation Kit (Illumina). The kit requires very low amounts of starting material (1ng) and uses dual-indexing, which allows the pooling of up to 96 samples in a single sequencing run. Paired-end (2x250nt) sequencing was performed on an Illumina MiSeq at the Genetic Diversity Centre (GDC) in Zurich.

Data processing

The raw Illumina reads were filtered and de-multiplexed using the Illumina MiSeq Reporter system software version 2.3. Overlapping reads were merged using SeqPrep (Schmieder and Edwards, 2011), and possible mismatches between the overlapping fragments of the forward and reverse reads were corrected according to the base call with the higher sequencer-assigned quality score. Non-overlapping reads were kept separate. In a next step, the reads were quality-cleaned (minimum mean quality of 25) and size selected (minimum read length of 100nt) using PrinSeq Lite version 0.20.3 (Schmieder and Edwards, 2011). The quality filtering step also includes the clipping of ambiguous nucleotides from the ends and the removal of read with internal ambiguous nucleotides. Initial de-noising was performed with a 99% similarity clustering using USEARCH version 7.0.1001 (Edgar, 2010). As false priming cannot be completely excluded due to the degeneration of primers during PCR amplification of the 16S rRNA gene, de-noised reads were binned with the usearch option and the 16S reference database, both provided by QIIME (version 1.7.0, (Caporaso et al., 2010)). Reads without overlap were concatenated using Ns to facilitate read trimming. The binning parameters were determined from blasting subsets of the dataset against the NCBI 16S database to keep the error rate below 1%. *De novo* and reference chimera detection were performed with the UCHIME algorithm (Edgar, 2010). After these quality filtering steps, we retained a total of 112862 reads (merged and paired-end) from all lakes (average reads per lake sample: 5643; SD: 1155; Table S1). The cleaned reads varied in size and coverage and were trimmed into subsets of fragments covering different parts of the targeted 16S rRNA gene region (V3, V4, V5, V3-V4, V4-V5). To trim the dataset into subset datasets we have used conserved 11-mer regions in proximity of each of the variable regions. The Gold 16S reference database was used to determine conserved 11-mer regions across species. The reads were screened based on the determined 11-mers and reads that did not carry the 11-mer were collected and the reverse complements of the those reads was screened again. In a next step, all reads that contained the 11-mer were aligned and trimmed to a specific length in order to cover the same variable region. 11-mer positions are included in Table S2. Read lengths differed between subset-datasets and covered 120 nucleotides (nt) for the V3 and V4 dataset, 100 nt for V5 dataset, 360 nt for V3-V4 dataset and 311 nt for the V4-V5 dataset. Read counts for the subset datasets were in the range of hundreds to thousands of reads per lake sample (691-3085 reads) for V3, V4, and V5 and hundreds of reads per lake sample (184-432 reads) for the double-region datasets V3-V4 and V4-V5 (Table S1). As the number of reads for sites covering two variable regions (V3-V4, V4-V5) was comparatively low compared to the number of reads in the single

region datasets (Table S1), we decided to perform most of the analyses using only the single region datasets. The Illumina sequences have been submitted to the Sequence Read Archive (SRA) and can be found under the project accession number SRP047505.

Data evaluation and statistical analysis

We used QIIME version 1.7.0 (Caporaso et al., 2010) for assigning operational taxonomic units (OTUs) at a sequence similarity of 97%. Briefly, we performed de novo OTU picking using usearch61 (Edgar, 2010; Edgar et al., 2011) and picked a representative set of sequences. Taxonomy was assigned (Wang et al., 2007) using the most recent Greengenes database taxonomy (as of May 2013 (McDonald et al., 2012)). To calculate phylogenetic diversity, OTUs were aligned and filtered, and a rooted tree was produced using the default fast tree option. QIIME analyses were performed separately for each region, as this allowed for de novo OTU picking and subsequent comparisons between the datasets of the different regions. After QIIME analyses, all subsequent analyses were performed in R (version 3.0.2, (R Core Team, 2013)). The R package 'Phyloseq' (McMurdie and Holmes, 2013) was used to rarefy OTU tables to an even sampling depth. For analyses comparing datasets from single regions (V3, V4, and V5), datasets were rarefied to 650 reads, while for analyses of double region datasets (V3-V4 and V4-V5), datasets were rarefied to 150 reads per lake. The rarefaction was conducted to account for differences the number of reads in the datasets from the different regions, and most of the subsequent analyses were performed on the rarefied datasets. As the aim of our study was to compare the datasets of the three variable regions, we did not remove chloroplast sequences, which might affect diversity measures. Species richness (SR) was calculated as the number of unique OTUs, and phylogenetic diversity (PD) was calculated as the sum of phylogenetic branch lengths (Cadotte et al., 2008). To minimize the influence of rarefaction on SR and PD, these measures were computed 1000 times, each time using a different rarefied OTU table, and then averaged. We used Analysis of variance (ANOVA) and a post-hoc Tukey HSD test to evaluate if SR and PD vary significantly between variable regions and major axis (MA) regressions (Legendre and Legendre, 1998) for comparing lake-specific SR and PD data between the different regions. Linear regressions were used to compare SR estimates from Illumina sequencing to ARISA estimates of SR of the 20 lakes and to test for relationships with environmental parameters. We quantified β-diversity of the V3, V4 and V5 region datasets using three different metrics: Jaccard (presence absence data), Bray-Curtis (abundance data) and Raup Crick (RC) (Chase et al., 2011). The RC dissimilarity index is less sensitive to differences in SR among sites than the other dissimilarity metrics as it uses

the total species pool of each dataset (γ-diversity) to calculate a null model distribution for each combination of samples (N=10000 iterations), and the null distribution is then compared to the real number of shared species between samples to test wether lakes share more or less species than expected by chance. RC values range from -1 to 1, where a value of between -1 and -0.975 means that lakes are significantly more similar than expected by chance (*i.e.* lakes share more species than expected by the null model) and a value between +0.975 and +1 indicates that lakes are significantly more dissimilar than expected by the the null model (*i.e.* lakes share fewer species than expected by the null model). RC estimates between -0.975 and +0.975 indicate that there are no significant differences between the null model expectations and the observed number of shared species between lakes. Pairwise comparisons of RC between for the same combinations of lakes for each of the variable regions were plotted against each other to determine how the conclusions about microbial community similarity between lakes depend on the choice of variable region. Rank-abundance distributions were calculated in two ways. First, for a global comparison of abundance data, we combined the datasets for all 20 lakes and created rank-abundance tables for each of the single region datasets (V3, V4, V5). Second, for a pairwise comparison between lakes, the abundance data was ranked separately for each lake and region. We used the Kolmogorov-Smirnov (KS) test to test for significant differences in the shape of rank-abundance distributions between variable regions. In addition to describing bacterial diversity based on OTU abundances, we also investigated community composition at the class level from sequences clustered at 97% sequence similarity. We used paired t-tests to compare the relative abundance of the ten most common classes between communities defined by the V3, V4 and V5 datasets.

ARISA Fingerprinting

For ARISA fingerprints of the microbial communities, the ribosomal intergenic spacer (ITS) region between the microbial 16S and 23S genes was amplified using a fluorescein (6-FAM)-labeled universal forward primer 1406f-6FAM and the bacteria-specific reverse primer 23Sr (Yannarell and Triplett, 2005). Binning of the ARISA peaks was performed in R 3.0.2 (R Core Team, 2013) using a window size of 1.5 and a shift value of 0.3 (Bürgmann et al., 2011).

Influence of sequence similarity threshold

A 97% sequence similarity threshold (SST) is often used during OTU picking to cluster similar sequences at species level (Schloss and Handelsman, 2005). We tested how different SST values (99, 95, 90, and 85% sequence similarity) affect SR, PD, β-diversity as well

as rank-abundance distributions. For SR and PD calculations, we again performed 1000 repeated rarefactions and averaged the results to decrease the bias of random rarefaction.

Reference dataset analysis

To test wether our observed differences among regions are generalizable beyond our lake survey, we ran our pipeline (Fig. 2.1) with trimmed V3, V4 and V5 sequences from the Greengenes database (as of May 2013 (McDonald et al., 2012)), which were trimmed in the same way as our lake survey samples. In order to make the comparison between the Greengenes dataset and our lake survey data, we randomly selected sequences in the same quantity as in our lake dataset (Table S3). Subsequently, we performed the same QIIME analysis as described above and calculated SR and PD for the Greengenes database dataset, after rarefaction to the same level as the lake survey data (650 reads per lake sample).

RESULTS

Species Richness and Phylogenetic Diversity

Absolute estimates of Illumina SR and PD varied significantly between the three variable regions for both rarefied (SR: F=68.79 ($p<0.001$), PD: F=264.70 ($p<0.001$); Fig. 2.2A) and raw data (SR: F=64.83 ($p<0.001$), PD: F=188.80 ($p<0.001$); Figure S2). To account for differences in read counts of the different lake datasets, which were generated by differences in sequencing depth, all of the following results are based on rarefied data only. The dataset based on the amplicons covering the V4 region resulted in significantly higher SR estimates (median: 366 unique OTUs) compared to the V3 (median: 262) and the V5 (median: 235) region. Corresponding with this, PD was also highest for the V4 region and significantly lower for both the V3 and the V5 region datasets (Fig. 2.2A).

When comparing SR estimates of individual lakes between the different variable regions (Fig. 2.2B; MA statistics: Table 2.1) we found that SR generated from the different variable regions were correlated. Even though absolute SR was higher for the V4 region datasets, the slopes of the relationship were neither different from one another, nor were they different from a slope of 1. When comparing the same patterns for PD, we found that the three regions resolve PD differentially (MA statistics: Table 2.1, Figure S3). Together, these results suggest that V3 and V5 region datasets produce more similar patterns of diversity than V4 region dataset.

SR and PD were significantly positively correlated for all three regions (Fig. 2.2C; MA statistics: Table 2.1), and the slopes of these relationships did not differ significantly between the different dataset. R^2 estimates, however, were quite variable between datasets. This result indicates that SR and PD increase simultaneously, but also that the fit of the relationship between SR and PD strongly depends on the variable region (Table 2.1).We found a positive relationship when comparing SR estimates of the V4 region to SR estimates of the two datasets spanning over two variable regions (V3-V4 and V4-V5; Table 2.1, Figure S4). The V4 region dataset showed higher estimates of SR compared to the extended regions, but the slopes of the relationship were not different from 1. Evaluating the relationships between SR, respectively PD, and environmental parameters, we found either weak or no relationships (Table S4). The only significant ($p<0.05$) relationships of environmental parameters and SR were detected were a negative correlation of SR of the V3 region dataset with PO_4 concentrations ($p=0.02$) and a positive correlation of SR of the V3 dataset with Chlorophyll a concentrations ($p=0.01$). PD of the V4 region was negatively correlated with PO_4 concentrations ($p=0.04$) and positively correlated with Chlorophyll a concentrations ($p=0.02$). Given these infrequent and weak relationships, it is unclear how

the choice of variable region might alter our understanding of drivers of species diversity along environmental gradients.

Table 2.1: ***Major axis (MA) regression results***

Figure	Rarefied (reads)	Parameter comparison	x-axis	y-axis	R^2	slope value	2.5% slope	97.5% slope	posthoc test
2B	Yes (650)	SR	V3	V4	0.351	0.771	0.337	1.518	a
2B	Yes (650)	SR	V3	V5	0.869	0.861	0.708	1.042	a
-	Yes (650)	SR	V4	V5	0.555	1.022	0.645	1.627	a
S2	Yes (650)	PD	V3	V4	0.583	1.872	1.273	3.063	a
S2	Yes (650)	PD	V3	V5	0.671	0.801	0.553	1.127	b
S2	Yes (650)	PD	V4	V5	0.799	2.099	1.664	2.758	a
2C	Yes (650)	SR vs. PD	V3 SR	V3 PD	0.568	22.855	15.954	40.228	a
2C	Yes (650)	SR vs. PD	V4 SR	V4 PD	0.400	14.197	8.822	36.001	a
2C	Yes (650)	SR vs. PD	V5 SR	V5 PD	0.951	18.466	16.600	20.803	a
-	No	SR	V3	V4	0.195	4.959	2.399	337.240	a
-	No	SR	V3	V5	0.926	0.783	0.679	0.898	b
-	No	SR	V4	V5	0.251	0.170	0.027	0.322	c
-	No	PD	V3	V4	0.435	3.318	2.073	7.300	a
-	No	PD	V3	V5	0.731	0.666	0.481	0.889	b
-	No	PD	V4	V5	0.515	4.565	3.056	8.652	a
-	No	SR vs. PD	V3 SR	V3 PD	0.624	26.520	19.156	43.051	a
-	No	SR vs. PD	V4 SR	V4 PD	0.824	24.030	19.554	31.156	a
-	No	SR vs. PD	V5 SR	V5 PD	0.960	23.996	21.783	26.708	a
S4	Yes (150)	SR	V4 SR	V3-V4 SR	0.305	1.393	0.664	3.753	a
S4	Yes (150)	SR	V4 SR	V3-V4 SR	0.212	1.870	0.795	12.044	a

MA was calculated in R using the lmodel2() function (Legendre, 2013). We used the 2.5% and 97.5% slope estimates to evaluate significant relationships between variables.

β-Diversity estimates

The bacterial lake communities appeared more dissimilar from each other when characterized by the V4 region, as indicated by Jaccard, Bray-Curtis, as well as Raup-Crick dissimilarities. Median Jaccard dissimilarities were significantly different between the three variable regions ($F=163.6$, $p<0.001$) and highest for the V4 dataset (Fig. 2.3A). Bray-Curtis dissimilarities, which make use of abundance distributions, show a similar pattern, as pairwise dissimilarities between lakes were higher for the V4 dataset and lower for the V5 dataset as compared to the V3 dataset (Fig. 2.3B). However, as both Jaccard and Bray-Curtis dissimilarities can be biased by differences in species richness among sites and regions, we also compared Raup-Crick estimates between the regions (Fig. 2.4). The RC comparison revealed that both for the V3 and V5 region datasets, bacterial communities were on average more similar to each other than expected by random chance (Fig. 2.4A). On the

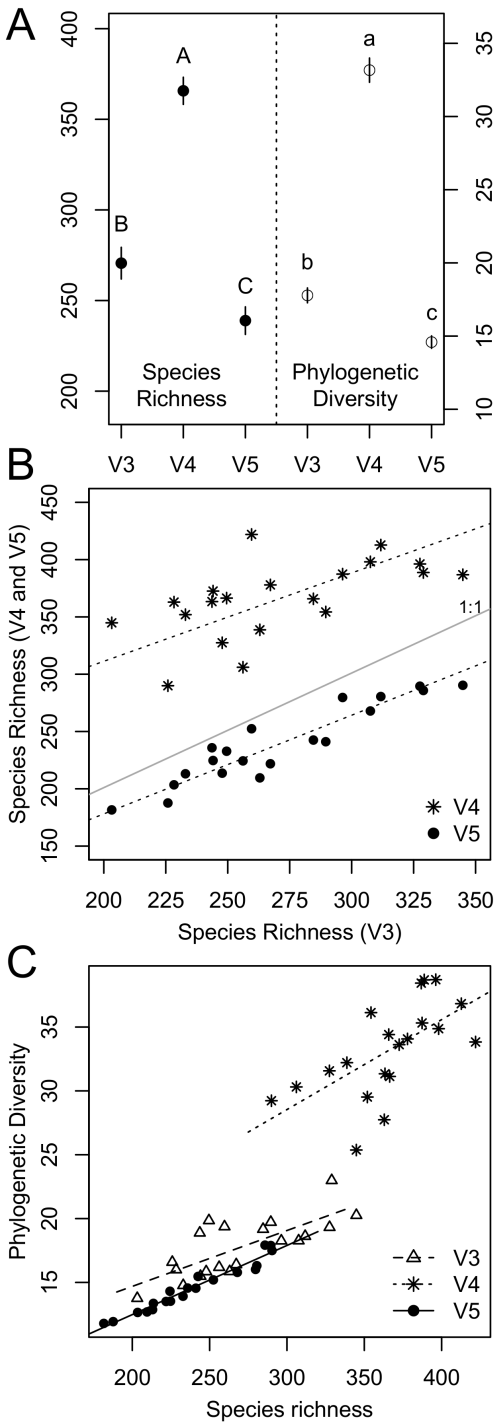

Figure 2.2: ***Number of observed species (SR; left side) and phylogenetic diversity (PD; right side) of the rarefied dataset from Illumina OTU data of the lake samples.***
A: SR and PD estimates for the three different regions. Points show the mean SR/PD of all lake samples and lines the standard error of the mean.
B: SR of individual lakes from the V3 region plotted against SR of the same lake from the V4, respectively the V5 region dataset. The solid central line shows the 1-to-1 line, dashed lines show the Major Axis (MA) regressions of the two comparisons.
C: SR (x-axis) plotted against PD (y-axis) for each of the three regions, where each dot represents one lake sample. The different symbols indicate the three different regions. Lines show the MA regression lines for each variable region dataset.

other hand, there was a high proportion of pairwise RC comparisons in which lakes shared fewer species than expected by the null model for the V4 region (Fig. 2.4B). These patterns appear to be correlated for the V3 and V5 region, as, when plotted against each other, most points fall into the lower left corner (Fig. 2.4A). This suggests that these two regions yield similar conclusions about community dissimilarity. However, when we compare RC for V3 and V4, there is more uncertainty in our conclusions. For example, more points fall at the edges of the plot (Fig. 2.4B), indicating that one of the variable regions suggests the communities are not different from random expectations, while the other suggests the communities are either more or less dissimilar than expected by chance. Overall, pairwise comparisons of RC estimates indicate that the V3 and V5 region yield similar conclusions in a high proportion of pairwise lake comparisons (Fig. 2.4D) and would lead to the conclusion that communities are more similar to each other than expected by random chance. Pairwise RC estimates of the microbial communities using the V4 region, however, less often come to the same conclusion as the other two variable regions and indicate that lakes are more dissimilar than expected by chance. These results indicate that higher SR estimates by the V4 region dataset also affects pairwise comparisons between communities and results in fewer shared species, as compared to the number of shared species found when comparing reads of the V3 or V5 region.

Rank-abundance distributions

The rank-abundance distributions of the three different variable regions also indicate significant differences between the bacterial communities characterized by the V4 dataset as compared to the V3 and V5 datasets. We detected significant differences in the shape of the rank-abundance distributions of the three variable regions, both when comparing individual lakes and when averaging over all lakes. Rank-abundance distributions averaged over all lakes were significantly different between the V4 and both the V3 and V5 region, but not between the V3 and V5 region datasets (Fig. 2.5A). Comparing rank-abundance distributions of individual lakes (Fig. 2.5B), we found no significant differences between the distributions of the V3 and the V5 datasets for any of the lakes, while for 55% of the lakes, significant differences between the distributions of the V3 and V4 datasets were detected, and for 80% of the lakes, the V4 rank-abundance distribution was significantly different from the distribution of V5 dataset (Fig. 2.5C).

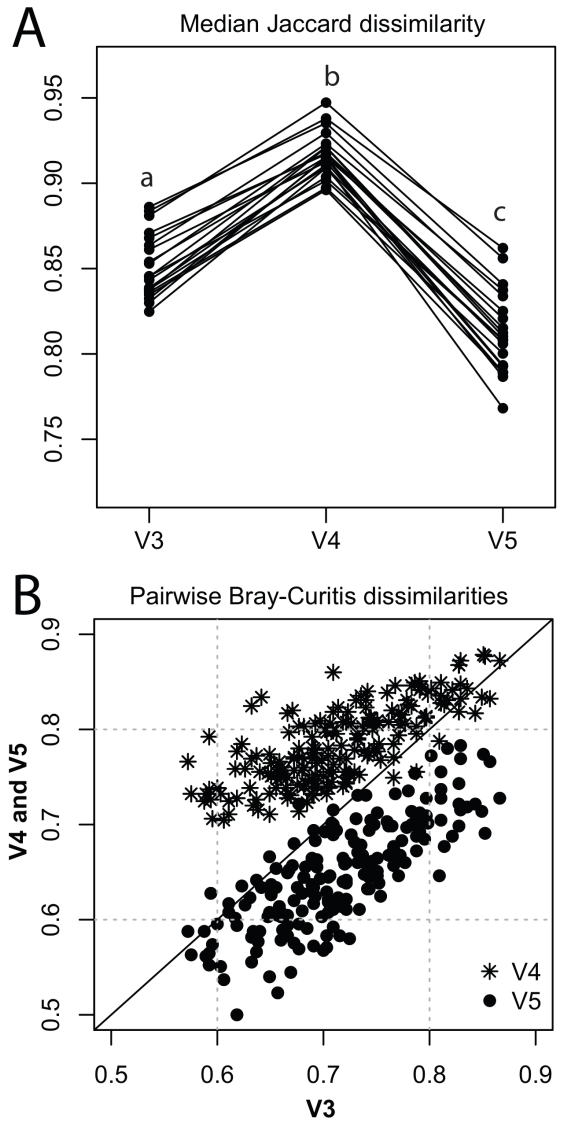

Figure 2.3: ***Comparison of Jaccard and Bray-Curtis dissimilarities between the variable 16S rRNA regions from the lake survey dataset.***
A: Median Jaccard dissimilarities of rarefied data for the three different variable regions. Each dot represents the median Jaccard dissimilarity from the pairwise comparisons of one lake to the other 19 lakes for one variable region. Lines connect the median dissimilarities of the same lake for the three different regions.
B: Bray-Curtis pairwise dissimilarities of V3 plotted against V4 (stars) and V5 (circles) pairwise dissimilarities of the same pairwise combination of lakes. Central line shows 1:1 line.

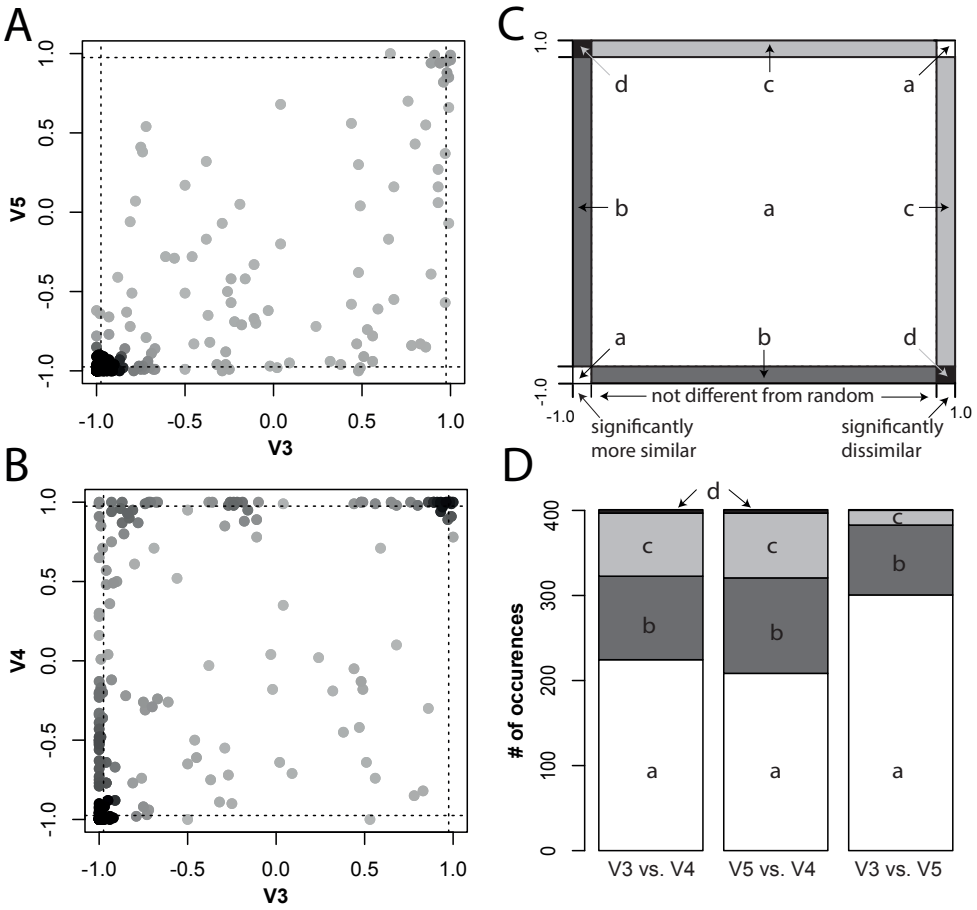

Figure 2.4: ***Raup-Crick (RC) comparisons between the three variable 16S rRNA regions from the lake survey dataset.***

A: Modified RC probability comparison of V3 and V4 (for rarefied data). Each dot represents the RC value of one pairwise dissimilarity comparison of the V3 region plotted against the same pairwise dissimilarity comparison of the V4 region. Values between -1 and -0.975 indicate that communities are significantly less dissimilar, and values between +0.975 and +1 that communities are significantly more dissimilar than expected by chance. Values between -0.975 and + 0.975 indicate that communities are not different from random expectation. Dashed lines show boundaries of significance (-0.975 and +0.975), where points falling between -1 and -0.975, respectively +0.975 and +1 indicate significant deviations from the null-model distribution. Dark areas in the plot represent high densities of points.

B: Same as A, but for V3 plotted against V4 values.

C: Conceptual figure illustrating the four different possible combinations when two RC-matrices are compared. a (white area): both regions come to the same conclusion about the dissimilarity among communities, b (dark grey): one of the regions estimates β-diversity of one lake pair to be significantly more similar than expected by chance while the other region estimates the β-diversity of the same lake pair to be not different from a random null-model distribution, c (light grey): one of the regions estimates β-diversity of one lake pair to be significantly more dissimilar than expected by chance while the other region estimates the β-diversity of the same lake pair to be not different from a random null-model distribution, d (black): cases where pairwise lake comparison of one region estimate β-diversity to be significantly more similar than expected by random chance, while the other region estimates β-diversity to be significantly more dissimilar than expected by chance.

D: Barplot showing the number of cases where the compared regions come to the same (a) or different (b, c, d) conclusions about β-diversity. Coding is illustrated in panel C.

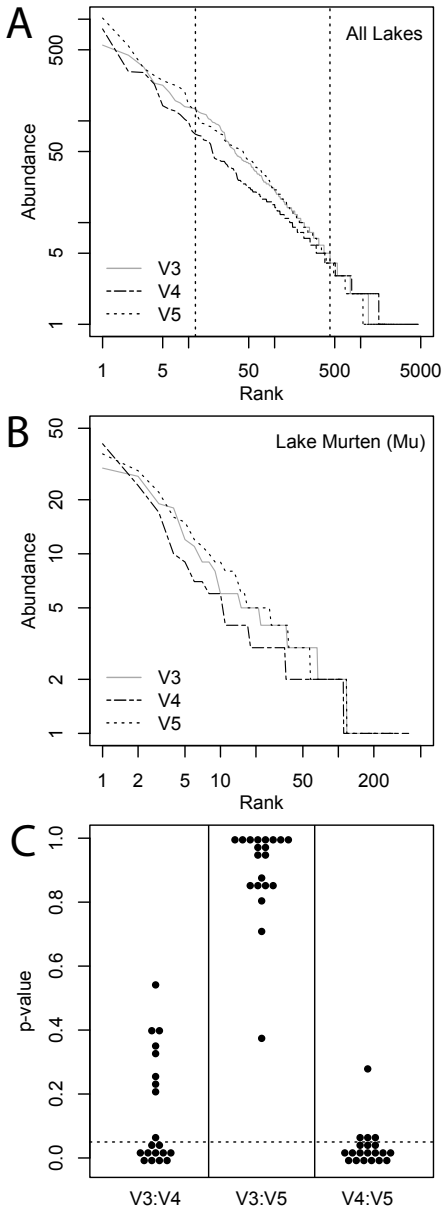

Figure 2.5: ***Rank-abundance evaluation of the variable 16S rRNA regions from the lake survey dataset.*** *A: Rank-abundance plot of the complete dataset for each of the three variable regions, where abundance data was added up for all of the 20 lakes, plotted on log-log scale. Vertical dashed lines show the range of the rank-abundance plot (ranks 12 to 440) for which we found a significant difference between the rank-abundance distributions of V4 to V3 and V5. For the same region, the V3 and V5 rank-abundance distributions did not differ significantly from each other (significant Kolomogorov-Smirnov (KS) test : $p<0.05$) .*
B: Example rank-abundance plot of the rarefied data for one lake (Murtensee), plotted on log-log scale. X-axis: OTU rank, y-axis: OTU abundance.
C: Result of KS-test using rank-abundance data of the individual lakes. X-axis: compared regions, y-axis: p-value distribution of KS test, dashed line plotted at p-value of 0.05. Each dot represents the comparison of rank-abundance curves from two regions of the same lake. P-values below 0.05 indicate a significant difference between the the rank-abundance distributions, whereas p-values above 0.05 indicate that there are no significant differences between two rank-abundance distributions.

Species richness comparisons of ARISA and Illumina sequencing data

Overall, the SR estimates based on the ARISA and the Illumina data did not show a significant ($p<0.05$) correlation for any of the three variable regions (Fig. 2.6, Table S5), but SR of V3 and V4 from Illumina sequencing were marginally positively correlated with ARISA SR estimates (V3: F=3.99, p=0.06, slope=1.42; V5: F=3.89, p=0.06, slope=1.22), while no such correlation for V4 Illumina SR estimates and ARISA estimates was found (V4: F=0.14, p=0.72, slope=-0.25). The slopes of the individual 16S rRNA regions did not differ significantly from one another (F=1.59, p=0.21). Again, however, the V3 and V5 datasets appear more similar to each other as compared to the V4 dataset.

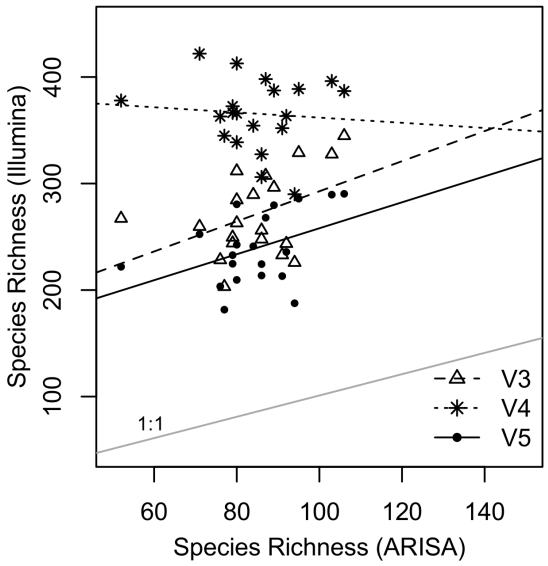

Figure 2.6: ***Species richness (SR) estimates from ARISA Fingerprinting plotted against SR estimates from Illumina sequencing.*** *Each symbol represent the SR estimates of one lake for the two different methods clustered at a SST of 97%. Different symbols represent Illumina estimates from the three different regions. Lines show major linear regressions for each variable region (regression slopes: Table S5).*

Influence of sequence similarity threshold (SST)

We furthermore analyzed how the SST, which is used during the sequence analysis to cluster similar sequences, affects the above described indices and patterns (Figure S5). As expected, SR and PD decreased for all regions when the SST was lowered, but SR and PD decreased at unequal rates. At levels between 90-95% SST, SR of the V4 region reached similar levels as SR of the V3 and V5 region at a SST of 97% (Figure S5A). SR estimates of V4 became very similar to SR estimates of V3 when the SST was lowered, while V5 SR remained lowest. PD, on the other hand, decreased at a much lower rate when the SST was reduced (Figure S5B). Even at a SST of 85%, PD of the V4 dataset was still approximately

2-fold higher than PD of the V3 and V5 dataset. In either case, the widening gap between the V4 region dataset and the V3 and V5 region datasets with increasing SST indicates that the differences between these regions are related to an increased diversity at higher clustering thresholds. As expected, Jaccard and Bray-Curtis mean pairwise dissimilarities also decreased, showing that the communities are more similar at a lower SST due to less stringent clustering parameters. Using presence-absence data (Jaccard dissimilarity), relative differences between mean pairwise dissimilarities of the three variable regions remained equal (Figure S5C), while when using abundance data (Bray-Curtis dissimilarity), differences between the datasets from the three variable regions became less pronounced and V3 and V4 dissimilarities converged (Figure S5D). Lowering the SST decreased the number of rare species and flattened rank-abundance distributions, resulting in steeper slopes of the rank-abundance curves for all three regions (Figure S5E). With a lowered SST, the V4 rank-abundance distribution became less different from the V3 distributions, but stayed significantly different from the V5 rank-abundance distributions for 30% of the lakes even at a SST of 85% (Kolgorov-Smirnov Test: $p<0.05$; Figure S6). Linear model comparisons of SR estimates from Illumina and ARISA overall revealed several cases where Illumina estimates of SR of the V3, as well as the V5 region, were marginally (p-value between 0.07 and 0.05) correlated with ARISA SR estimates (Figure S5F, Table S5). SR estimates of the V4 region, however, never showed significant correlations with ARISA estimates of SR, irrespective of the SST value. Overall, the SST analysis revealed that differences between the three variable regions remain even when the sequences are clustered at different similarity thresholds.

Taxonomic evaluation

Analyzing the community composition based on bacterial classes of the V3, V4 and V5 region datasets revealed significant differences in relative abundances of the ten most abundant classes (Fig. 2.7, Table S6). Clearly, the relative abundances of the V3 and V5 datasets are more similar in their composition, while both of them differ markedly from the V4 dataset (Fig. 2.7), and this observation is well supported by paired t-tests (Table S6).

Comparison with reference data

By analyzing the Greengenes dataset using the same parameters that were applied to the lake survey data, we detected that the V4 region consistently resolved PD differently than the V3 and V5 region datasets, but did not always show a higher SR (Figure S7A). Furthermore, the coupling of SR and PD was different for the reference data as compared

to the lake survey data (Figure S7B). The slopes of the relationship varied significantly between variable regions (F=25.88, p<0.001). This result indicates that even when using a random selection of reference sequences, the V4 region resolves PD significantly higher than the V3 and V5 region.

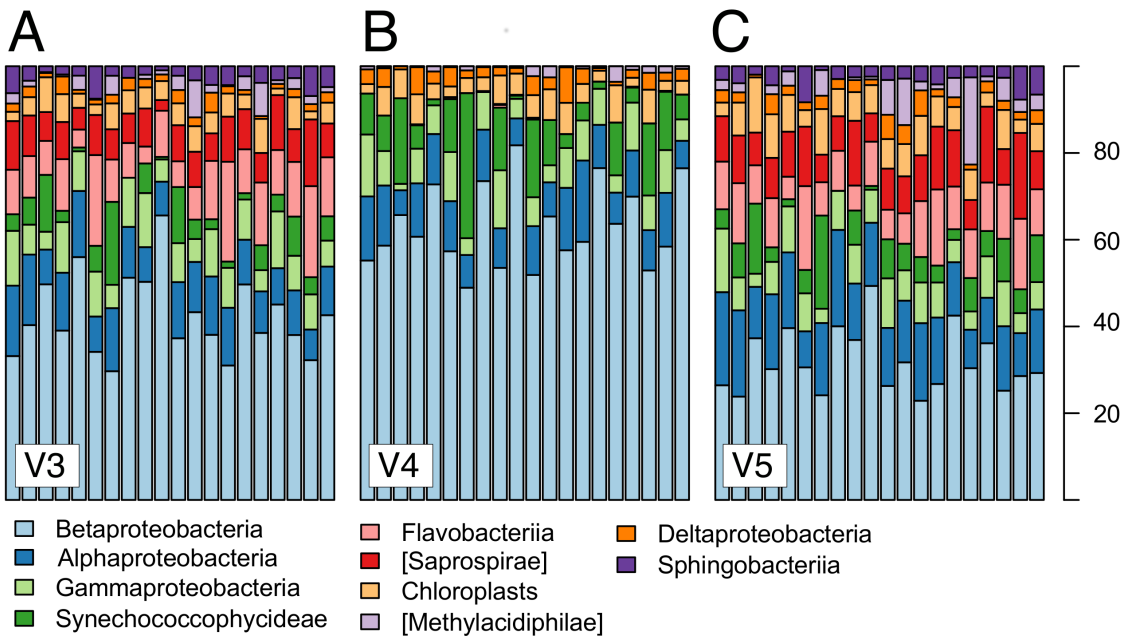

Figure 2.7: ***Barchart of the most abundant bacterial classes.*** *Relative abundances of the ten most abundant bacterial classes across the V3, V4 and V5 datasets. Each bar represents the relative class distribution in one lake and each group of bars represents the relative abundances for one of the tree variable regions (V3, V4, V5). Bars are ordered from left to right by alphabetical order (see Figure S1 and Table S3 for more information about the lakes). Appendant results of paired t-test statistics are shown in Table S5. Square brackets indicate candidate class names.*

DISCUSSION

In this study, we amplified the V3 to V5 regions of the microbial 16S rRNA from 20 natural lake water samples using a single bacteria-specific primer set, and after Illumina sequencing, trimmed the data into three datasets corresponding to three variable 16S rRNA regions (V3, V4, V5). We then estimated bacterial diversity and were able to show that the choice of variable region strongly influences the estimation of diversity based on SR and PD, and, as such, may significantly alter the ecological conclusions for a given study.

Most studies that investigated the influence of 16S rRNA region on diversity estimates have focussed on measures of α-diversity (*e.g.* (Wang and Qian, 2009; Youssef et al., 2009; Kim et al., 2011; Soergel et al., 2012; He et al., 2013)), whereas here, we additionally investigated the effect of 16S rRNA region on PD, community composition, rank-abundance distributions as well as β-diversity. One novel aspect of our approach is the use of PCR products produced by a single primer pair instead of using separate primer pairs for each of the variable 16S rRNA regions. This method minimizes the influence of different primer pairs on the composition of the PCR products, which can affect species composition and SR estimates (Rainey et al., 1994). Furthermore, we used the same bioinformatic pipeline for analyzing an environmental dataset originating from natural lake samples, as well as an *in-silico* dataset extracted from the Greengenes reference database. Comparing our dataset of environmental samples to a reference dataset allowed us to investigate the generality of differences between the variable regions of the 16S rRNA gene. Comparisons to other available datasets, however, would be necessary to show if this is a general pattern across reference data. We are aware that various choices made during sequence analysis, such as the selection of the reference database, the taxon calling, and the sequence clustering can influence our results, but we tried to minimize these results by carefully choosing our analysis pathway, by comparing our lake survey data to reference data, and by analyzing how the sequence clustering threshold influences various of the chosen measures of diversity (Fig. 2.1).

The results from our lake survey dataset suggest that different variable regions of the 16S rRNA gene resolve SR and PD differently. Both SR and PD were significantly higher for the V4 region dataset, but we think that these parallel patterns for absolute estimates of SR and PD do not only arise from the fact that higher SR was measured for the V4 dataset, as PD estimates are not directly linked to the number of species in a system (Faith, 1992). Instead, the results suggest that the three variable regions differ in how species composition and identities are resolved and contain different types of phylogenetic information. This finding was underlined by the fact that we found significant differences

between the relative abundances of bacterial classes (Fig. 2.7). The significantly higher estimates of PD for the V4 region were also found in the Greengenes reference dataset and were robust to changes in the sequence similarity threshold (SST) during sequence clustering (Figure S5), suggesting that the patterns we obtained are inherent to the 16S regions themselves and not specific to our samples or to the OTU clustering threshold. Kim *et al.* (Kim et al., 2011) already suggested that the threshold for defining a molecular species must be adapted for variable regions of the 16S rRNA gene, and that it may be necessary to change SST depending on which of the nine 16S rRNA regions is chosen. Our lake survey results are in accordance with previous studies that have also found higher SR for the V4 region compared to the V3 and V5 regions (Youssef et al., 2009; Vinje et al., 2014). Vinje *et al.* (Vinje et al., 2014) revealed that the V4 region contains in proportion at least twice as many informative sites compared to V3 and V5 to discriminate taxa, but they also noted that half of the discriminative sites were found outside of variable regions. Targeting the amplification of fragments where the number of discriminative sites is optimized would allow robust downstream analyses such as taxonomic assignment, phylogeny and species richness estimates. As a consequence, it is promising that improvements on read length are advancing for Illumina sequencing, as this will furthermore improve downstream analyses. Although the absolute number of identified species depended strongly on the region, estimates of SR derived from different 16S regions were highly correlated (Fig. 2.2B). This is encouraging as it suggests that studies choosing different target regions may be comparable on a relative scale. However, this was not the case for PD, which seems to be strongly influenced by the choice of variable region. Furthermore, SR and PD were not correlated equally between the three regions.

Estimates of β-diversity (*i.e.* differences in diversity between the lake samples) were also particularly sensitive to the choice of variable region (Fig. 2.3 and 2.4). Pairwise β-diversity dissimilarities based on Jaccard and Bray-Curtis were higher for the V4 dataset and lower for the V5 dataset as compared to the V3 dataset (Fig. 2.3). By using the Raup-Crick (RC) dissimilarity matrix in addition to Jaccard and Bray-Curtis dissimilarities, we found that the differences in dissimilarities between the three regions are not only driven by the absolute number of species. RC matrices of the V3 and V5 region appear to be more similar to each other, as pairwise distance matrices show strong overlap. As a result, the V3 and V5 regions would yield similar conclusions about patterns of β-diversity, but using the V4 region could lead to dramatically different conclusions. In many pairwise comparisons between lakes, communities that were more similar than random expectation when using the V3 or V5 region were actually more dissimilar than expected when using

the V4 region dataset (Fig. 2.4D). Hence, the ecological conclusion is reversed based on the choice of region. One potential explanation for such results is that the V4 region resolves reads at different taxonomic levels than both the V3 and V5 regions, which leads to comparably less overlap between the communities and greater dissimilarities when comparing lake pairs. Interestingly, this may also happen when taking longer regions of the 16S rRNA into account. Longer regions should provide a better phylogenetic placement of an individual read, but they can still mask sample-to-sample differences depending on the similarity cutoff used for species definitions. Hence, comparing patterns of β-diversity among samples or studies (such as performed *e.g.* by Shade *et al.* (Shade et al., 2013)) will be sensitive to the choice of region of the 16S rRNA.

We also found that rank-abundance distributions can be significantly different depending on which variable region is analyzed (Fig. 2.5). It is well known that rank-abundance distributions can be highly influenced by PCR artifacts (Pinto and Raskin, 2012) and sequencing errors (Kunin et al., 2010), but as far as we are aware, the influence of the variable region of the 16S rRNA has not been investigated. The rank-abundance distributions of individual lakes using the V3 and V5 datasets were never significantly different from each other, while they were both significantly different to the distribution of the V4 region dataset for the majority of lakes. Rank-abundance curves are influenced by the way species abundances are distributed between the different taxa, and so the difference of the V4 rank-abundance curves is likely due to higher species richness in the V4 datasets. By decreasing the SST, we were able to show that the steepness of the rank-abundance curve increases, which indicates that species with low frequencies are lost as OTUs are clustered at less stringent SSTs.

While Pilloni et al. (2012) demonstrated a strong correlation between NGS (454) and Fingerprinting (T-RFLP) data, we did not find the same pattern for the comparison between Illumina and ARISA. ARISA estimates SR by measuring the variability within the intergenic spacer region between the 16S and the 23S rRNA genes, whereas most NGS surveys target parts of the 16S rRNA to estimate SR. Generally, NGS is considered to be the more accurate technique for measuring microbial diversity, as there is an ever growing number of reference sequences to which NGS data can directly be compared. However, to our knowledge, the assumption that Illumina is a more appropriate method to estimate SR has not been tested intensively and few studies have directly compared the outcome of NGS richness to richness estimates from Fingerprinting techniques. A recent study (Gobet et al., 2013) has compared ARISA and 454 Sequencing results and found strong correlations between richness estimates of ARISA and 454 sequencing, however, it is unknown how

this relationship is influenced by the selected region of the 16S rRNA. Future work should investigate more in depth whether NGS sequencing and classical Fingerprinting techniques provide similar information about microbial diversity and evaluate how the variable region might affect the results.

Overall, our results suggest that the choice of variable region of the 16S rRNA might be important for many ecological studies, particularly in the context of biogeography (Morlon et al., 2011), metacommunity theory (Leibold et al., 2010; Pillar and Duarte, 2010) or (human) microbiome studies (O'Dwyer et al., 2012; Grice and Segre, 2011; Turnbaugh et al., 2009), where information from diversity indices and rank-abundance distributions are common tools for comparing microbial communities. Currently, there is a lot of variation in the 16S rRNA regions used by different projects. Furthermore, the lack of a relationship between ARISA results and the three variable regions from Illumina sequencing also suggests that caution is warranted for comparing conclusions among studies which have used different techniques. Large scale projects, such as the Human Microbiome Project (HMP) or the Earth Microbiome project (EMP) try to make their data comparable by mostly sequencing the same part of the 16S rRNA, but many smaller studies use various parts of the 16S rRNA and thus make data comparisons between studies difficult or even impossible. We can not make general recommendations about which regions to use for NGS sequencing, but we have demonstrated, using three different variable 16S rRNA regions, that there are inherent differences between the regions of the 16S rRNA, which researchers should be aware of. This could motivate further research in order to find better techniques or approaches for estimating bacterial diversity, which we hope will lead to an improved understanding of bacterial communities.

ACKNOWLEDGMENTS

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APPENDIX

Supplementary Figures

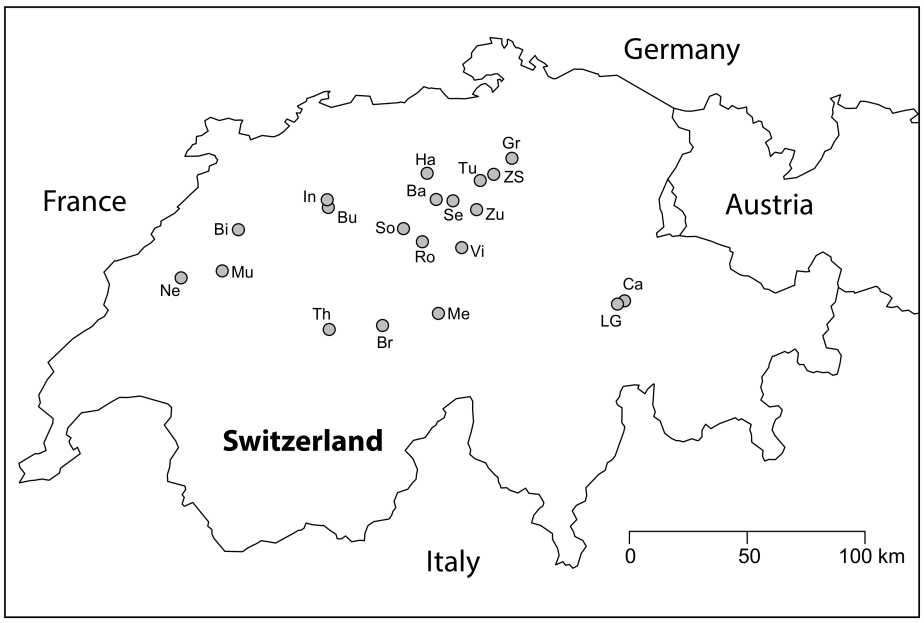

Figure S1: ***Map of the sampling locations of the 20 Swiss lakes included in the lake survey as well as lake name abbreviations (see Table S1).***

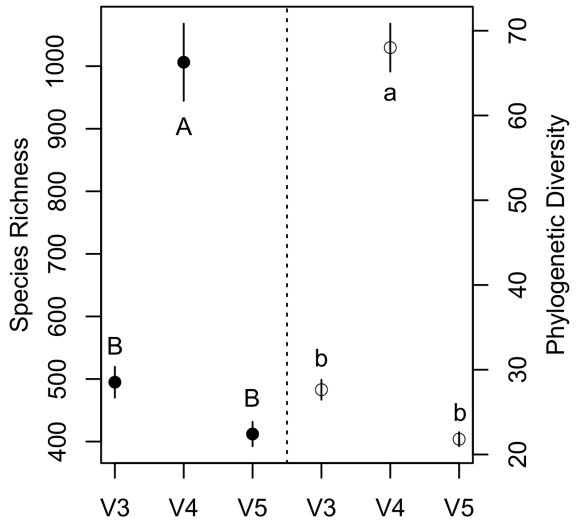

Figure S2: ***Species richness (SR) and phylogenetic diversity (PD) of the Illumina OTU data for the three different regions prior to rarefaction.*** *Points show the mean and lines the standard error of the mean.*

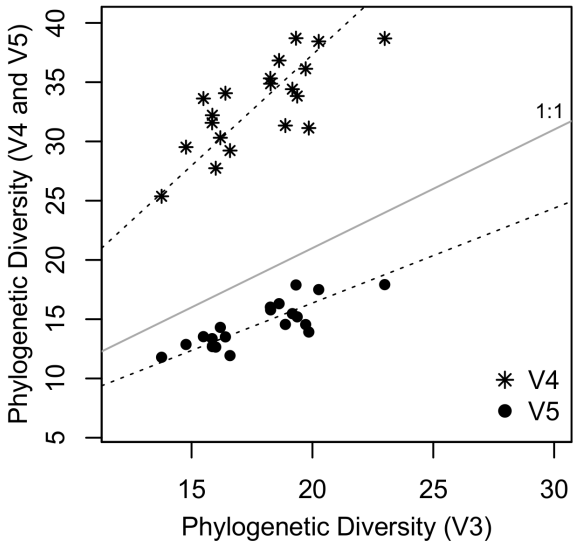

Figure S3: ***Rarefied PD of individual lakes from the V3 region plotted against PD of the same lake from V4, respectively V5.*** *Central line shows 1:1 line, dashed lines show the Major Axis (MA) model regression slopes of the two comparisons.*

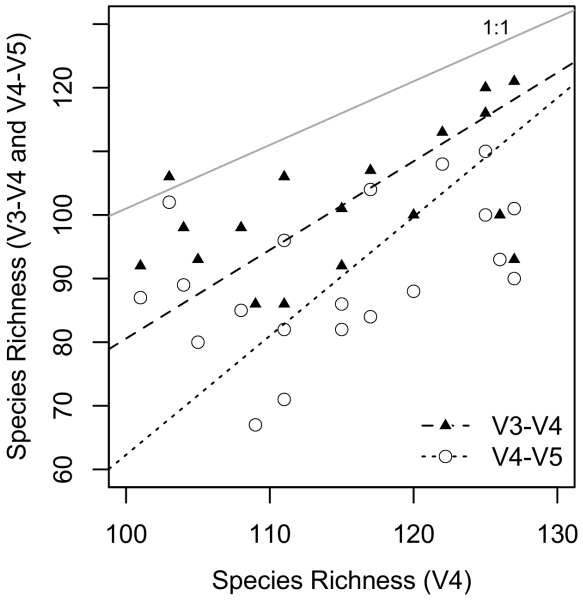

Figure S4: ***SR of individual lakes from the V4 region plotted against SR of the same lake from the V3-V4, respectively the V4-V5 region dataset using data rarefied to 150 OTUs per sample.*** *Central line shows 1:1 line, dashed lines show the MA model regression slopes of the comparisons.*

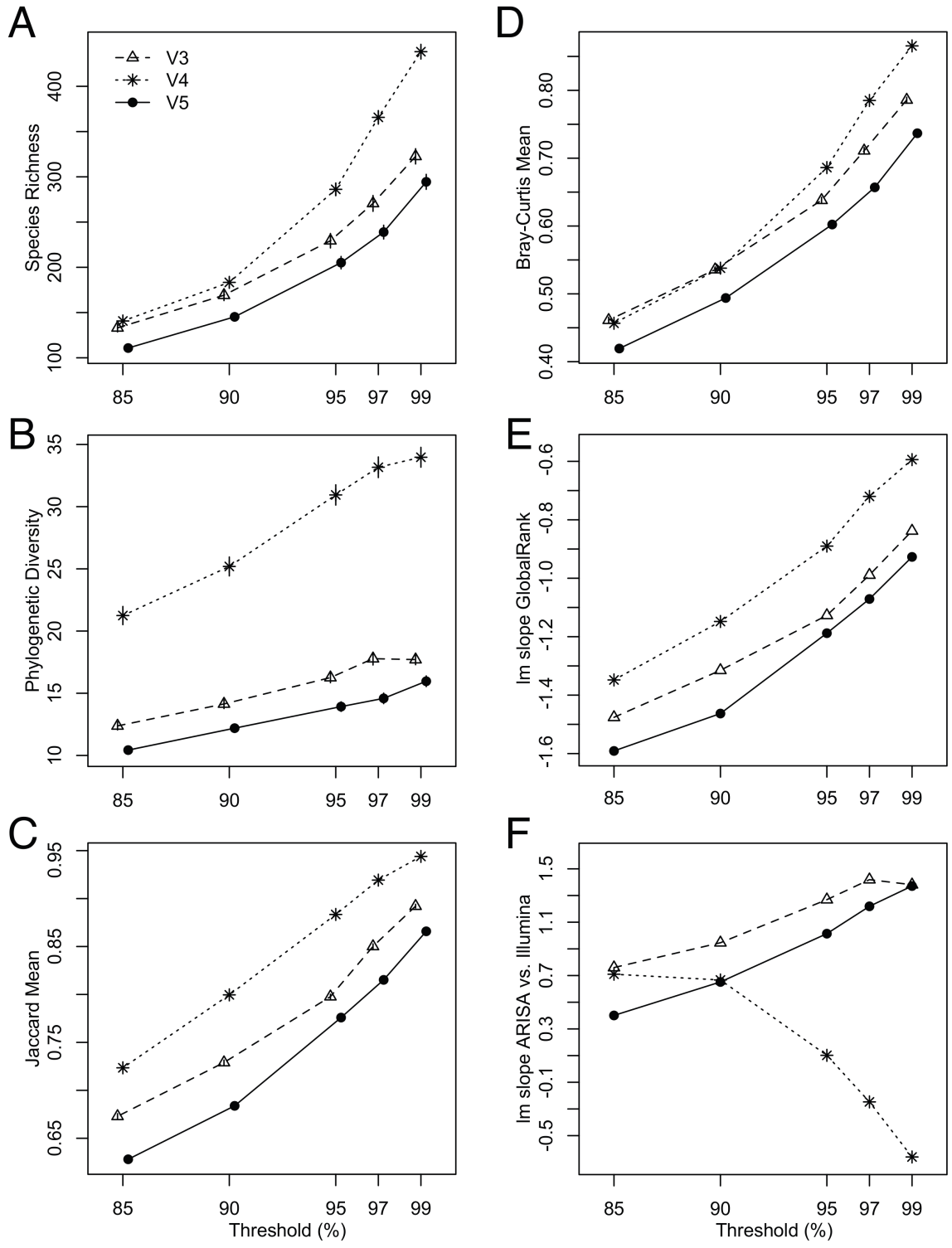

Figure S5: ***Results from applying different sequence similarity threshold levels (SST) during OTU clustering (85, 90, 95, 97, and 99% sequence similarity).*** *A: changes in SR, B: changes in PD, C: changes in Jaccard dissimilarities, D: changes in Bray-Curtis dissimilarities, E: changes in global rank-abundance slopes, F: changes in the linear model slopes between ARISA Fingerprints and Illumina sequencing. The graph shows the mean and standard error (SE) for each region and SST.*

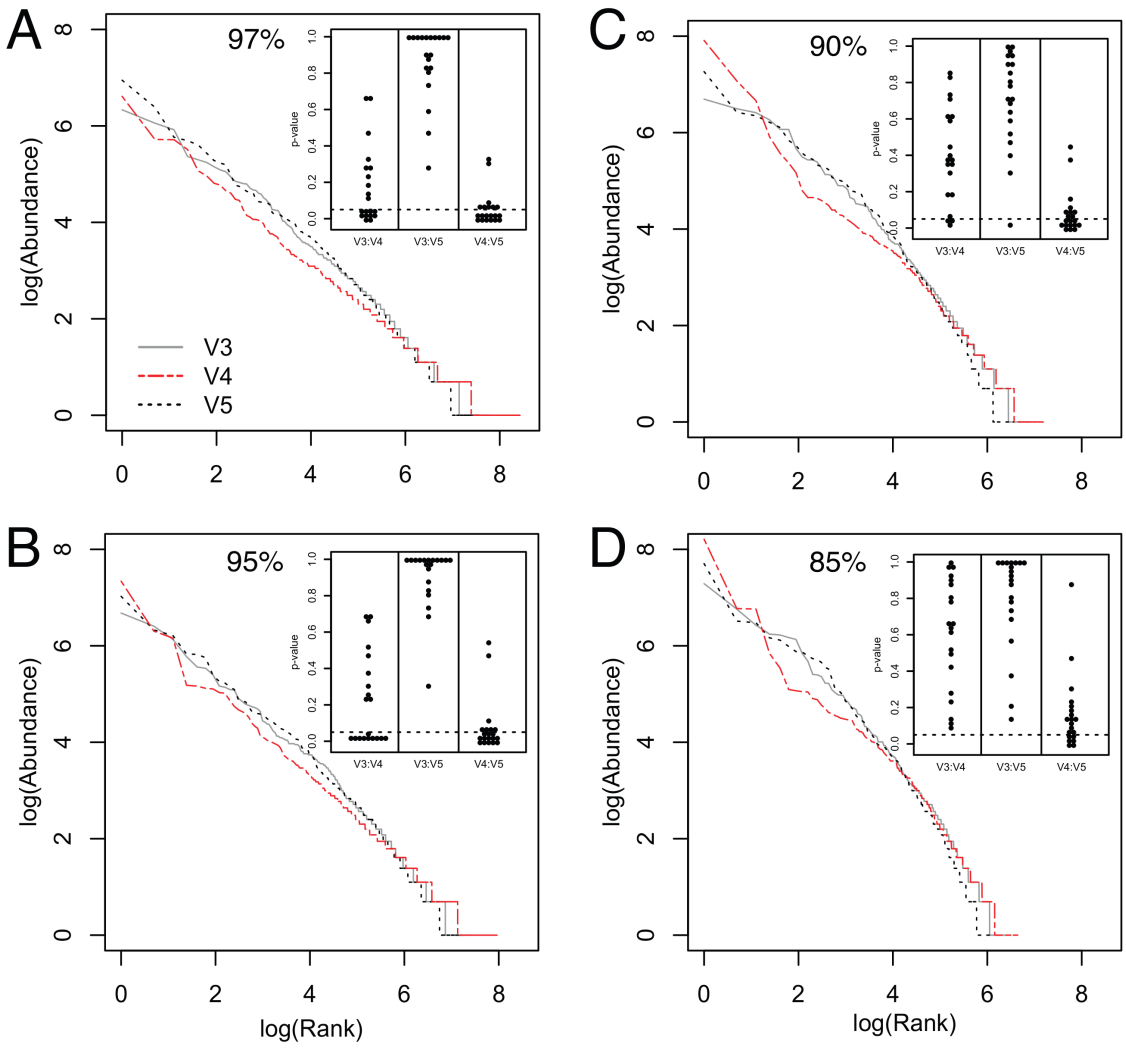

Figure S6: ***Changes in rank-abundance distributions of the three variable region datasets clustered at different sequence similarity threshold levels (85, 90, 95, 97, and 99% sequence similarity).*** *Large plots: Rank plotted against abundance on a log-log scale. Small plots show the changes in significant differences between the three variable regions (see Fig. 2.5C for a more detailed description of the inlay plots).*

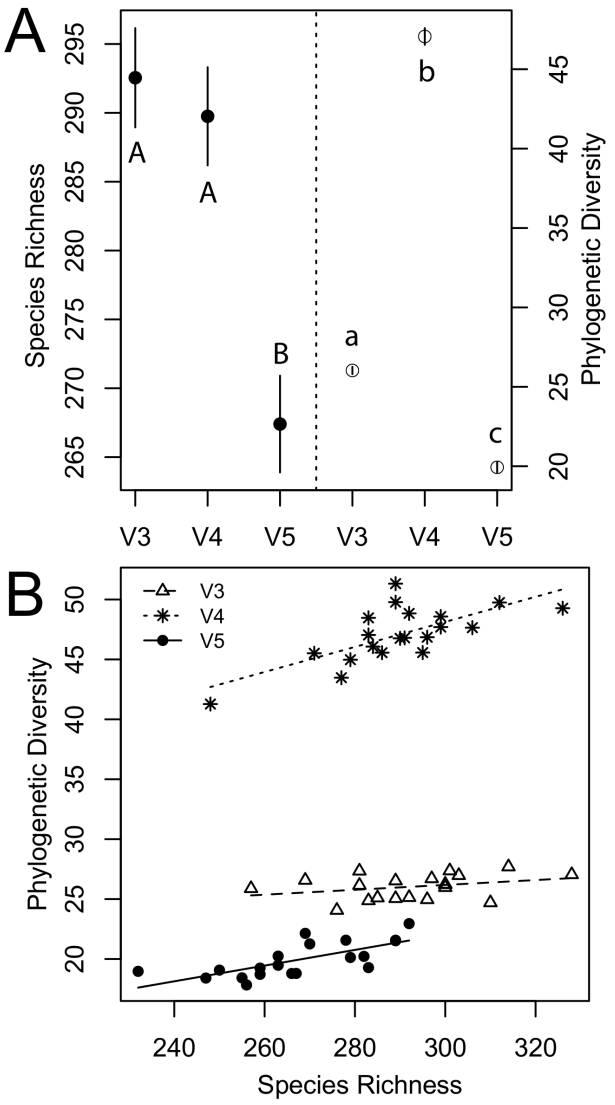

Figure S7: ***Analysis of SR and PD of the Greengenes reference dataset.*** *A: Rarefied SR and PD calculated from a subset of the Greengenes database using the same parameters used as for the lake survey data. Points show the mean and lines the standard error of the mean. PD was significantly higher in the V4 dataset ($F=1406$, $p<0.001$) compared to the V3 and V5 datasets. The SR of the V4 region dataset was only significantly different form the V5, but not from the V3 dataset ($F=14.99$, $p<0.001$). B: SR plotted against PD for each of the three variable regions where each dot represents one lake sample. Lines show the MA regression model for each dataset. R^2 values: V3 = 0.10, V4 = 0.49, V5 = 0.50.*

Supplementary Tables

Table S1: *Number of quality filtered reads of the different lakes and variable regions of the 16S rRNA (untrimmed and trimmed reads).*

		Number of reads after quality filtering					
Lake name	Abbreviaton	untrimmed	V3	V4	V5	V3-V4	V4-V5
Baldeggersee	Ba	4809	1211	1561	1065	389	301
Bielersee	Bi	5584	1141	2052	1003	359	315
Brienzersee	Br	7134	1069	2711	926	197	250
Burgäschisee	Bu	5537	1199	1865	1109	365	332
Caumasee	Ca	4344	901	1559	683	184	212
Greifensee	Gr	6302	1207	1614	1384	245	232
Halwilersee	Ha	6337	992	2034	943	192	209
Inkwilersee	In	4256	918	1449	920	243	238
Lag Grand	LG	6116	1011	2162	1405	219	285
Melchsee	Me	7959	1445	3058	1242	240	277
Murtensee	Mu	8461	1634	2872	1565	422	432
Neuenburgersee	Ne	5221	990	1730	974	250	233
Rotsee	Ro	5253	1154	1684	1082	360	330
Sempachersee	Se	3508	754	962	691	237	195
Soppensee	So	5074	959	1757	954	209	229
Thunersee	Th	5455	941	1798	901	268	268
Türlersee	Tu	5124	1081	1594	1059	281	257
Vierwaldstättersee	Vi	5262	1074	1869	969	361	333
Zürichsee	ZS	5569	1113	1288	1184	222	189
Zugersee	Zu	5557	995	1804	844	218	197
Total # of reads		112917	21798	37432	20909	5461	5315
Mean # of reads (SD)		5643 (1155)	1089	1871	1045	273	266

Table S2: *Positions of 11-mers used for subsetting the quality filtered Illumina reads as well as the number of nucleotides (nt) of the trimmed datasets. Positions are given relative to E. coli 16S rRNA positions.*

16S region	11-mer position	reading direction	# of nt
V3	518-508	reverse	120
V4	558-568	forward	120
V5	779-789	forward	100
V3-V4	415-425	forward	460
V4-V5	789-779	reverse	310

Table S3: *Environmental parameters of the lakes that were sampled for this study.*

Lake	Sampling date	Sampling location [Lat, Long]	maximum Depth [m]	Elevation [m]	DOC [mg C/L]	NO_3 [mg/L]	PO_4 [μg/L]	Temp [°C]	pH	Oxygen [mg/L]	Chl a [mg/L]	Secchi depth [m]
Baldeggersee (Ba)	10-10-2011	47.0509 N 7.1026 E	66	463	4.0	0.8	0.5	15.82	7.97	9.63	32.13	1.5
Bielersee (Bi)	14-09-2011	46.4340 N 7.5757 E	74	429	1.7	0.6	1.4	20.09	8.12	9.35	3.12	4.5
Brienzersee (Br)	12-07-2011	47.1008 N 7.4004 E	261	564	1.7	0.3	4.4	17.83	8.27	10.00	0.96	3.0
Burgaschisee (Bu)	05-10-2011	46.4911 N 9.1745 E	31	465	7.6	0.7	1.3	19.38	8.35	9.74	6.06	4.0
Caumasee (Ca)	19-07-2011	47.2110 N 8.4037 E	30	997	1.2	0.3	1.0	16.90	8.58	8.81	1.64	7.0
Greifensee (Gr)	15-07-2011	47.1749 N 8.1239 E	32	435	4.4	1.5	1.9	21.90	8.35	11.41	9.98	2.5
Halwilersee (Ha)	13-07-2011	47.1152 N 7.3943 E	47	449	8.5	0.6	4.2	23.06	8.30	9.83	1.74	6.0
Inkwilersee (In)	05-10-2011	46.4828 N 9.1525 E	6	461	7.5	0.3	2.1	17.75	7.21	4.21	37.46	1.5
Lag Grond (LG)	19-07-2011	46.4622 N 8.1622 E	5	1016	2.8	0.3	1.0	17.41	7.80	6.26	11.15	1.5
Melchsee (Me)	12-07-2011	46.5555 N 7.0506 E	18	1891	2.9	0.3	4.4	17.58	8.00	8.71	2.82	4.0
Murtensee (Mu)	15-09-2011	46.5421 N 6.5132 E	45	429	3.8	1.4	1.7	20.73	8.20	9.46	10.50	2.0
Neuenburgersee (Ne)	14-09-2011	47.0227 N 8.1106 E	153	429	2.6	0.7	1.2	21.10	7.97	9.40	2.26	7.0
Rotsee (Ro)	06-10-2011	47.1138 N 8.2110 E	16	419	1.8	0.3	2.3	18.71	8.61	12.76	8.37	3.0
Sempachersee (Se)	17-10-2011	47.0838 N 8.0907 E	87	505	3.8	0.3	1.0	15.31	8.31	8.55	4.42	4.5
Soppensee (So)	18-07-2011	47.0523 N 8.0451 E	28	596	6.4	0.9	1.2	21.00	8.49	11.42	6.15	3.5
Thunersee (Th)	11-07-2011	46.4246 N 7.4022 E	217	558	1.1	0.4	3.2	18.27	8.39	9.66	0.71	6.5
Türlersee (Tu)	20-07-2011	47.1612 N 8.3010 E	22	643	3.8	0.3	1.0	19.85	8.45	11.77	12.14	3.0
Vierwaldstättersee (Vi)	14-10-2011	47.0108 N 8.2403 E	214	434	1.2	0.4	1.0	14.75	8.15	9.50	2.62	6.2
Zugersee (Zu)	14-07-2011	47.0939 N 8.2858 E	198	413	6.9	0.3	6.0	22.42	8.11	9.75	4.06	3.0
Zürichsee (ZS)	22-07-2011	47.1734 N 8.3437 E	136	407	1.6	0.5	1.0	19.75	8.25	10.23	4.46	3.0

Table S4: *Linear model statistics of species richness (SR; ARISA and Illumina sequencing) and phylogenetic diversity (PD) versus environmental parameter.*

Parameter	ARISA slope (p-value)	V3 SR s (p)	V4 SR s (p)	V5 SR s (p)	SR F-value (p)	V3 PD s (p)	V4 PD s (p)	V5 PD s (p)	PD F-value (p)
MaxDepth	0.02 (0.51)	-7.18 (0.37)	-1.85 (0.79)	-1.51 (0.83)	0.20 (0.82)	-0.13 (0.78)	-1.02 (0.17)	-0.18 (0.65)	0.88 (0.42)
Elevation	-0.01 (0.14)	-21.97 (0.35)	18.39 (0.36)	-8.19 (0.69)	0.97 (0.39)	-0.51 (0.71)	-0.31 (0.89)	-0.42 (0.71)	0.00 (1.00)
DOC	-0.54 (0.65)	0.45 (0.98)	-13.75 (0.24)	-11.29 (0.35)	0.38 (0.69)	-0.55 (0.50)	-0.22 (0.87)	-0.43 (0.53)	0.03 (0.97)
NO_3	-2.79 (0.72)	27.54 (0.10)	21.87 (0.13)	20.75 (0.16)	0.06 (0.94)	0.88 (0.38)	2.45 (0.12)	0.89 (0.28)	0.64 (0.53)
PO_4	-0.83 (0.66)	-28.79 (0.03)	-7.92 (0.51)	-22.46 (0.05)	0.85 (0.43)	-0.40 (0.62)	-2.59 (0.04)	-1.16 (0.08)	1.56 (0.22)
Temp	-1.22 (0.31)	-73.22 (0.32)	-24.78 (0.70)	-87.46 (0.16)	0.26 (0.77)	-1.14 (0.79)	-6.27 (0.37)	-4.52 (0.20)	0.27 (0.76)
pH	-1.55 (0.87)	66.21 (0.78)	160.57 (0.42)	140.00 (0.49)	0.06 (0.95)	10.44 (0.44)	12.76 (0.57)	11.23 (0.32)	0.01 (1.00)
DO	0.10 (0.95)	34.11 (0.38)	39.69 (0.23)	39.54 (0.24)	0.01 (0.99)	3.84 (0.08)	3.29 (0.37)	2.62 (0.16)	0.05 (0.95)
Chla	0.26 (0.37)	20.54 (0.01)	-0.58 (0.94)	9.39 (0.22)	2.04 (0.14)	0.50 (0.33)	1.80 (0.02)	0.56 (0.18)	1.75 (0.18)
Secchi	-0.85 (0.58)	-24.48 (0.18)	7.31 (0.64)	-4.81 (0.77)	0.97 (0.39)	-0.27 (0.81)	-1.88 (0.28)	-0.33 (0.71)	0.53 (0.59)

Table S5: *Linear model statistics of species richness (SR) estimates from ARISA and Illumina sequencing at different sequence similarity threshold (SST) clustering values.*

16S region	SST	slope	R^2	F-value	p-value
V3	85	0.76	0.20	4.59	0.05
V3	90	0.95	0.18	3.85	0.07
V3	95	1.27	0.18	3.81	0.07
V3	**97**	1.42	0.18	3.99	0.06
V3	99	1.38	0.19	4.09	0.06
V4	85	0.71	0.12	2.54	0.13
V4	90	0.67	0.08	1.66	0.21
V4	95	0.10	0.00	0.03	0.87
V4	**97**	-0.25	0.01	0.14	0.72
V4	99	-0.66	0.05	0.86	0.37
V5	85	0.40	0.09	1.85	0.19
V5	90	0.65	0.13	2.68	0.12
V5	95	1.01	0.15	3.21	0.09
V5	**97**	1.22	0.18	3.89	0.06
V5	99	1.37	0.19	4.24	0.05

Table S6: *Paired t-test results of the ten most abundant bacterial classes.*

Bacterial class*	global	**V3 / V4**		**V3 / V5**		**V4 / V5**	
	Abund. [%]	T-statistic	p-value	T-statistic	p-value	T-statistic	p-value
Betaproteobacteria	33.16	-12.58	<0.01	10.81	<0.01	19.19	<0.01
Alphaproteobacteria	8.53	1.14	0.27	-3.48	<0.01	-3.20	<0.01
Gammaproteobacteria	5.66	2.99	<0.01	3.23	<0.01	1.19	0.25
Synechococcophycideae	5.33	-4.54	<0.01	-1.63	0.12	3.39	<0.01
Flavobacteriia	5.16	10.08	<0.01	2.82	0.01	-13.24	<0.01
[Saprospirae]*	4.42	13.65	<0.01	-2.98	<0.01	-12.16	<0.01
Chloroplasts	3.90	-0.42	0.68	-4.97	<0.01	-3.10	<0.01
Deltaproteobacteria	1.78	-3.62	<0.01	-2.72	0.01	1.68	0.11
[Methylacidiphilae]*	1.64	3.66	<0.01	-2.14	0.05	-3.24	<0.01
Sphingobacteriia	1.59	8.68	<0.01	0.75	0.47	-7.50	<0.01

**Note:* Data was calculated for the ten most abundant classes across the complete dataset. Square brackets indicate candidate class names.

3

CHAPTER II: META-COMMUNITY DYNAMICS

CONSEQUENCES OF GRAZING PRESSURE AND DILUTION ON BACTERIAL AND PHYTOPLANKTON COMMUNITY ASSEMBLY

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ABSTRACT

Consumers can have strong biotic and abiotic effects on food web and ecosystem dynamics at multiple trophic levels. Their effects have been well studied in various ecosystems and for multiple trophic levels, but not much is known about how consumers can affect bacterial communities, which are responsible for many ecosystem functions and dynamics. We have used a replicated outdoor metacommunity mesocosm experiment in which we manipulated the presence of a keystone grazer (*Daphnia*), crossed with a dilution manipulation, to investigate effects on diversity (of phytoplankton and bacteria) and ecosystem functioning. The *Daphnia* manipulation had strong effects on local and regional diversity of both the phytoplankton and the bacterial community, while dilution affected community dissimilarity within metacommunities. We found strong effects of *Daphnia* on the bacterial community composition, rank abundance distributions, and ecosystem functioning (respiration rates).

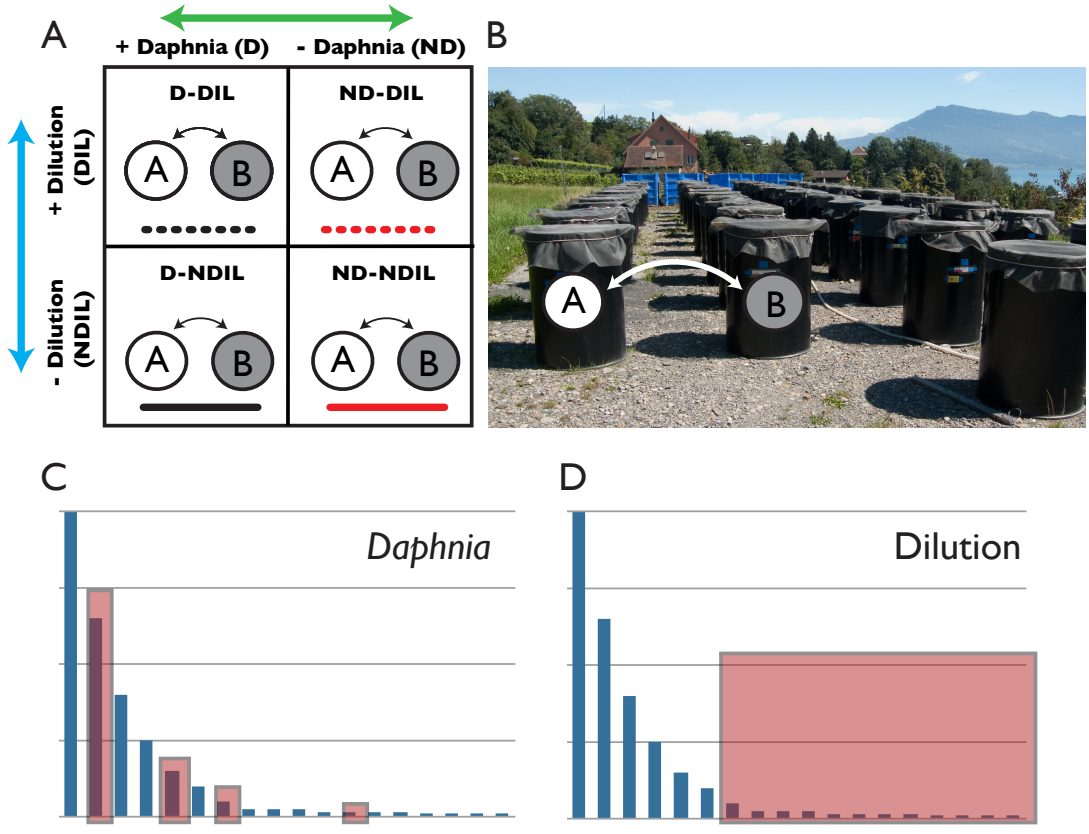

Figure 3.1: ***Experimental design (left) and setting (right) of the mesocosm experiment. Circles (A) represent 300L tanks, which were distributed randomly at the field site in Kastanienbaum, Switzerland (B). Graphs in panels C+D illustrate different hypotheses of how Daphnia and dilution might affect community assembly.***

INTRODUCTION

It is well established that consumers can profoundly modify community and ecosystem dynamics (Paine, 1966; Duffy, 2002; Schmitz et al., 2000). Consumers can have biotic effects (consumptive and non-consumptive) that influence prey biomass, composition, and diversity, as well as abiotic effects that alter the cycling of nutrients (Elser and Urabe, 1999; McIntyre et al., 2008) and energy flux (Jones et al., 1997) in ecosystems. The effects of consumers often extend well beyond their prey communities, and can propagate both laterally and vertically throughout food webs (Duffy et al., 2007), influencing both biomass (Shurin et al., 2002) and diversity (Duffy, 2002) at multiple trophic levels (e.g. meta-communities and -ecosystems), where they can influence coexistence, community composition and assembly (Chase et al., 2009; Verreydt et al., 2012) and ecosystem flux among patches (Gravel et al., 2010a).

While there is strong tradition for studying the propagation of consumer effects throughout ecosystems (Hillebrand et al., 2009), there is growing evidence that consumers can indirectly control key biogeochemical processes, such as carbon flux (Atwood et al., 2013; Schmitz et al., 2014) and nutrient recycling (Ngai and Srivastava, 2006). For example, experimentally manipulating predators can significantly alter CO_2 fluxes across a diverse range of aquatic ecosystems (ponds, streams, and bromeliads; Atwood et al. (2013)). Such biogeochemical effects of consumers imply strong top down control on microbially mediated ecosystem dynamics (e.g. sulfate reduction, iron oxidation, methane oxidation, nitrification and anaerobic ammonia oxidation), that are under direct control by the composition and activity of microbial communities (Falkowski et al., 2008). While there is a growing appreciation for the importance of bacterial diversity for regulating ecosystem functioning (Bell et al., 2005; Battin et al., 2003; Besemer et al., 2009), much less is known about how consumers influence biogeochemical processes through effects on microbial diversity.

Studying the effects of keystone grazer species (e.g. *Daphnia*) in spatially structures aquatic ecosystems is a useful starting point for exploring the effects of consumers on microbial communities and biogeochemical processes. *Daphnia* have strong biotic and abiotic effects on aquatic ecosystems, including effects on water transparency (Mazumder and Lean, 1994), nutrient cycling (Elser and Urabe, 1999), atmospheric carbon exchange (Schindler, 1997), phytoplankton abundance, productivity, and diversity (Sarnelle, 2005; Harvey et al., 1935), and bacterial abundance, composition, and productivity (Zöllner et al., 2003; Sarnelle, 2007; Jack and Gilbert, 1994; Langenheder and Jürgens, 2001; Jürgens and Matz, 2002). However, much of this work was done before the emergence of next-generation

sequencing (NGS) methods that can resolve bacterial community composition in much more detail. Early fingerprinting methods of microbial diversity (e.g. DGGE and ARISA) revealed strong effects of environmental variation on the spatial structure of bacterial communities (Van der Gucht et al., 2007), and some evidence for consumer effects on bacterial communities (Declerck et al., 2012; Jürgens et al., 1994; Jürgens, 1994; Cottingham et al., 1997; Jürgens and Matz, 2002). However, next-generation sequencing of bacterial communities can reveal a much greater taxonomic resolution that can be used to study fundamental questions about the dynamics of community assembly, while also providing information about phylogenetic relationships among species in a community. In a very recent paper, Berga et al. (2014) found that *Daphnia* strongly influenced the composition of bacteria in a metacommunity and influenced respiration rates. Such results support the notion that consumers can drive compositional shifts in the microbial communities that can alter the biogeochemistry of ecosystems.

Here, we investigate how the diversity (of algae and bacteria) and functioning of aquatic metacommunities (Leibold et al., 2004) are jointly influenced by *Daphnia* grazing (presence and absence), as well as by the initial diversity and abundance of the the bacterial community, which we manipulated by diluting the initial bacterial inoculum. We performed a large scale mesocosm experiment using 24 replicated freshwater metacommunities, each comprised of two 300L mesocosms connected by low levels of dispersal (weekly manual dispersal). We manipulated the presence and absence of *Daphnia* to test for effects on the spatial structure of diversity for both the phytoplankton and bacteria community. We expected both communities to be strongly affected by *Daphnia*, via a combination of direct (Degans et al., 2002) and indirect effects (Zubkov and Tarran, 2008), but we were also interested in how *Daphnia* affects bacterial community composition and phylogenetic structure. Taxonomic groups of bacteria likely exhibit different tradeoffs associated with resource allocation, survival, and growth, and, as a result, changes in the biotic and abiotic environment caused by *Daphnia* could drive compositional changes in bacterial communities (Jürgens and Matz, 2002) and affect elemental cycling in ecosystems (Jiang and Krumins, 2006). We diluted the initial inoculum of bacteria with the aim of manipulating the diversity of natural microbial communities (Fig. 3.1D), acknowledging that this manipulation also affected other parts of the inoculum community. Our rational was that the dilution treatment would preferentially remove rare species from the community, and provide a useful contrast to *Daphnia* grazing, which might preferentially affect the most common species in the community, but may also have indirect effects on rare species (Fig. 3.1C). Such a dilution of the inoculum could also influence the balance of stochastic versus deterministic

factors governing how predators affect community assembly (Chase et al., 2009; Hillebrand et al., 2009) . By using high throughput sequencing of the microbial community, we could track changes in relative abundance of microbial community constituents, and test for interactive effects of *Daphnia* and dilution on diversity and community structure.

MATERIALS AND METHODS

Experimental design and setup

The mesocosm experiment ran for 12 weeks in late the summer/early autumn of 2011 and was conducted in 300L tanks (mesocosms) with two tanks making up a metacommunity (Fig. 3.1). Each experimental metacommunity consisted of two tanks, between which small amounts of water (50mL) were dispersed on a weekly schedule. The experiment was designed in a randomized block design with four different treatments, which were replicated six times, adding up to a total of 48 mesocosms. We used a factorial design with two levels of *Daphnia* abundance (+ *Daphnia*, - *Daphnia*) crossed with two levels of dilution (+ Dilution, - Dilution; Fig. 3.1A). Mesocosms were filled with approximately 250L of tap water, which originate from the nearby lake Lucerne. Each block received lake water from two randomly selected lakes from a Swiss lake survey, which was either prefiltered through 5μm filters to remove zooplankton and large phytoplankton (ZF water), or prefiltered in the same way and then additionally filtered through 0.2 μm to remove bacterial cells (BF water). Undiluted (- Dilution) mesocosms received 10L of ZF water from one of the lakes to seed the bacterial community and additionally 10L of BF water from the other lake to keep the environmental within a metacommunity the same. Diluted (+ Dilution) mesocosm received 10L of BF water from one of the lakes, 1mL of ZF water from the same lake to seed a 1-million-fold diluted bacterial community, as well as 10L of BF water from the other lake to preserve environmental conditions within the metacommunity. Mesocosms from the + *Daphnia* treatment received 30 individuals of a *Daphnia galeata* clone (G100). One metacommunity thus consisted of two mescosoms with the bacterial community from two different lakes which were connected by regular dispersal, whereas half of the metacommunities contained *Daphnia*. All mesocosm additionally received the same amount of phytoplankton inoculum from lake Lucerne.

Mesocosm sampling

Over the course of 12 weeks, various biological, physical, chemical and ecosystem functioning parameters were sampled and measured on a regular basis (Table 3.1). We performed weekly samplings of algal biomass (Chlorophyll a), dissolved organic carbon (DOC) concentration and composition, as well as bacterial cell densities and cell size distribution. As we were interested in how the treatments affected the bacterial and phytoplankton community after several weeks, we conducted an intensive sampling at the end, where we also measured several environmental parameters and two ecosystem functions (Table 3.1).

Table 3.1: ***Sampling schedule of the mesocosm experiment.***

		sampling week											
Metrics	**Parameter**	1	2	3	4	5	6	7	8	9	10	11	12
Biological	Chlorophyll a	x	x	x	x	x	x	x	x	x	x	x	x
	Bacterial cell density[1]	x	x	x	x	x	x	x	x	x	x	x	x
	Bacterial cell size distribution[1]	x	x	x	x	x	x	x	x	x	x	x	x
	Phytoplankton density[1]	x	x	x	x	x	x	x	x	x	x	x	x
	Phytoplankton counts[2]												x
Physical/ Chemical	DOC concentration	x	x	x	x	x	x	x	x	x	x	x	x
	DOC spectral slope	x	x	x	x	x	x	x	x	x	x	x	x
	DOC absorption a320	x	x	x	x	x	x	x	x	x	x	x	x
	Dissolved oxygen	x						x					x
	pH	x						x					x
	Temperature	x						x					x
	Conductivity	x						x					x
	PAR light extinction												x
Ecosystem Functions	Enzyme (phosphatase) activity												x
	Respiration												x
Biodiversity	Bacterial diversity[3]												x
	Phytoplankton diversity[2]												x

Analysis techniques: 1: Flowcytometry, 2: Stereo-microscopic counting, 3: Illumina sequencing

Sample processing and analysis

Chlorophyll a concentrations were determined spectrophotometrically at 665nm from GF/F (Whatman) filtered water. The filtrate was acidified and stored at 4°C for measuring DOC concentration on a Shimadzu TOC-V CPH, as well as for recording a DOC absorption spectrum over the wavelengths of 190 to 800 nm. For flowcytometric measurements of bacterial cell density and cell size distribution, 40 mL of tank water were fixed with a filter-sterilized solution of paraformaldehyde and glutaraldehyde (0.01% and 0.1% final concentrations, pH 7; (Nizzetto et al., 2012)) and stored at 4°C until flowcytometric measurements were carried out on a BD Accuri™ C6 Flowcytometer.

Phytoplankton was sampled from the water column, preserved with Lugol's solution and manually counted and identified by stereo microscopy. Dissolved oxygen, pH, temperature and conductivity were measured in situ at approximately 0.5 m water depth using portable water sensors. Enzyme (phosphatase) activity was quantified following a protocol by German et al. (2011). Respiration rates were assessed in the lab using custom-built temperature controlled glass vials and fiber-optic oxygen mini-sensors (FIBOX 3, PreSens), in which oxygen concentrations were measured every 30 seconds at 16°C over the course of six hours.

The bacterial community was analyzed using Illumina sequencing. Briefly, mesocosm water was filtered onto 0.2μm polyethersulfone filters (Supor 200 Membrane Disc Filters), which were instantly frozen in liquid nitrogen and preserved at -80 °C until further processing. DNA was extracted by enzymatic digestion and cetyltrimethyl ammonium bromide (CTAB) extraction using a modified protocol of Lliros et al. (2008). Ilumina sequencing and sequence analysis was performed as described previously (Chapter I). We have used the V3 region of the 16S rRNA for the analysis of bacterial diversity. Sequences have been submitted to GenBank and can be found under accession number PRJNA264620.

Data analysis

We analyzed the results from the end of the experiment using linear mixed effect model statistics. All data was analyzed using the statistical software R (R Development Core Team, 2014). Sequencing data was analyzed using the bioinformatics pipeline QIIME (Kuczynski et al., 2011) and has afterwards been processed in R, as described in (PAPER 1). Unless specified differently, all analyzed data of the bacterial communities has been rarefied to 200 OTUs (Operational Taxonomic Units) per mesocosm due to large variations in read counts of the individual mesocosms. When metacommunities were analyzed, mesocosms were aggregated by metacommunity and OTUs were summed up.

Profile analysis (Fidell and Tabachnick, 2006) of the time-series data was performed for bacterial abundance, bacterial size distribution and chlorophyll concentrations, which were measured weekly during the course of the experiment. The analysis was conducted on the 4 cross-treatments as well as on the *Daphnia* and the dilution contrast. Additionally, we used the same LME statistic as described above to test for significant effects of *Daphnia*, dilution and the interaction of the two treatments for each week of the time-series data separately.

Estimates of α, β and γ-diversity, phylogenetic diversity (PD) and metacommunity UniFrac distances were computed based on averages of 1000 rarefied OTU matrices. Species

richness (SR; α-diversity = local diversity) was calculated as the number of unique OTUs per tank and then averaged across metacommunities. β-diversity was estimated for each metacommunity using Jaccard and Bray-Curtis dissimilarities, and γ-diversity (regional diversity) was calculated as the number of unique OTUs per metacommunity. Phylogenetic diversity (PD) was quantified for each metacommunity as the sum of phylogenetic branch length (Cadotte et al., 2008).

UniFrac distances, a measure of phylogenetic β-diversity (Lozupone and Knight, 2005), were computed in multiple ways. First, weighted and unweighted UniFrac distances were calculated for each metacommunity separately. Second, to illustrate if there is a treatment effect in UniFrac clustering, we computed an unweighted UniFrac matrix using the non-rarefied OTU table, which was transformed to relative abundances and merged by metacommunity. We then performed hierarchical clustering using Ward's minimum variance and plotted the result, accompanied by approximately unbiased (AU) bootstrap p-values (Shimodaira, 2002). To test for treatment effects on UniFrac distances, we computed weighted and unweighted UniFrac distance matrices, and, using 1000 random permutations, calculated how often the sum of the random permutations was smaller than the within treatment result (treatments here are *Daphnia*, Dilution and the interaction). The test was repeated 1000 times after which test results were averaged. Together with the hierarchical clustering of the unweighted UniFrac distance matrix, we plotted the a bar chart of the ten most abundant bacterial classes and ordered the bar chart by hierarchical clustering results to illustrate which bacterial classes might be causing the hierarchical clustering pattern. For this, we used the same non-rarefied OTU table, which had been used before to compute the UniFrac matrix. In order to test for treatment effects on the rank abundance distribution of bacteria (Fig. 3.1C+D), we calculated rank abundance slopes for metacommunity-aggregated OTU tables 1000 times and analyzed the averages using the above described LME statistics. Furthermore, we determined the average ranks of the 300 most abundant OTUs by calculating the average rank of each OTU with and without *Daphnia* and with and without Dilution. Average rank changes were calculated by subtracting the average OTU rank with *Daphnia*/ dilution from the average OTU rank without *Daphnia*/ dilution.

We have used the rank changes of the 300 most abundant OTUs and tested wether these showed any evidence of phylogenetic signal. Using Blomberg's K, we calculated the phylogenetic signal of rank change as result of *Daphnia* presence/ absence and dilution/ no dilution. We repeated this analysis 100 times to avoid rarefaction artifacts and we then calculated the proportion of significant p-values ($p<0.05$). We performed this analysis

using the phylogenetic tree of the 300 most abundant OTUs as well as a phylogenetic tree of the 300 most abundant OTUs aggregated to class level. To visualize rank-abundances and rank changes across the phylogenetic tree we plotted the class level tree of the 300 most abundant OTUs of one single rarefaction, together with relative abundance and rank change information.

RESULTS

Ecosystem dynamics through time

Over the course of the experiment, we found significant effects of *Daphnia* and dilution on bacterial abundance, bacterial size distribution and algal biomass (Flatness test; Table 3.2, Fig. 3.2). Chlorophyll a concentrations were significantly higher in metacommunities containing *Daphnia* during the course of the whole experiment (Levels test; Table 3.2, Fig. 3.2C), while the trajectory of bacterial abundance and bacterial cell size distributions were significantly different among treatments (Parallelism test; Table 3.2, Fig. 3.2A+B). ANOVA at different time points revealed significant effects of *Daphnia*, dilution, and interactive effects (Fig. 3.2). For example, bacterial density and bacterial cell size distribution both showed significant *Daphnia* effects during two of the twelve weeks. Bacterial density showed significant interactive effects in week #2 and bacterial cell size distribution exhibited significant effects of dilution in week #8 (Fig. 3.2A+B). Algal biomass was significantly lower in the presence of *Daphnia* but was also affected by dilution and interactive effects during three of the twelve weeks (Fig. 3.2C). Overall, bacterial densities did not exhibit large changes as response to treatment, but we saw a trend for increased proportions of small bacterial cells in metacommunities with *Daphnia* (Fig. 3.2B).

Table 3.2: ***Results of profile analysis on bacterial abundance (BaN), bacterial size class distribution (BactSize) and Chlorophyll a (Chl-a).***

Test	**Comparison**	Test statistic	BaN	BactSize	Chl-a
Time (Flatness)	Treatment	$F_{10,11}$	**6.29 (<0.01)**	**8.58 (<0.01)**	**10.03 (<0.01)**
	Daphnia	$F_{12,11}$	**6.07 (<0.01)**	**8.73(<0.01)**	**7.99 (<0.01)**
	Dilution	$F_{12,11}$	**7.08 (<0.01)**	**6.82 (<0.01)**	**7.37 (<0.01)**
Treatment (Levels)	Treatment	$F_{3,20}$	1.37 (0.28)	0.77 (0.53)	**9.42 (<0.01)**
	Daphnia	$F_{1,22}$	0.81 (0.38)	2.48 (0.13)	**18.76 (<0.01)**
	Dilution	$F_{1,22}$	0.00 (0.99)	0.01 (0.92)	0.69 (0.41)
Time x Treatment (Parallelism)	Treatment	Wilk's $\lambda_{3,20}$	**0.04 (0.05)**	**0.03 (0.02)**	0.07 (0.23)
	Daphnia	Wilk's $\lambda_{1,22}$	**0.27 (0.04)**	**0.17 (<0.01)**	0.41 (0.23)
	Dilution	Wilk's $\lambda_{1,22}$	0.33 (0.10)	0.35 (0.13)	0.40 (0.20)

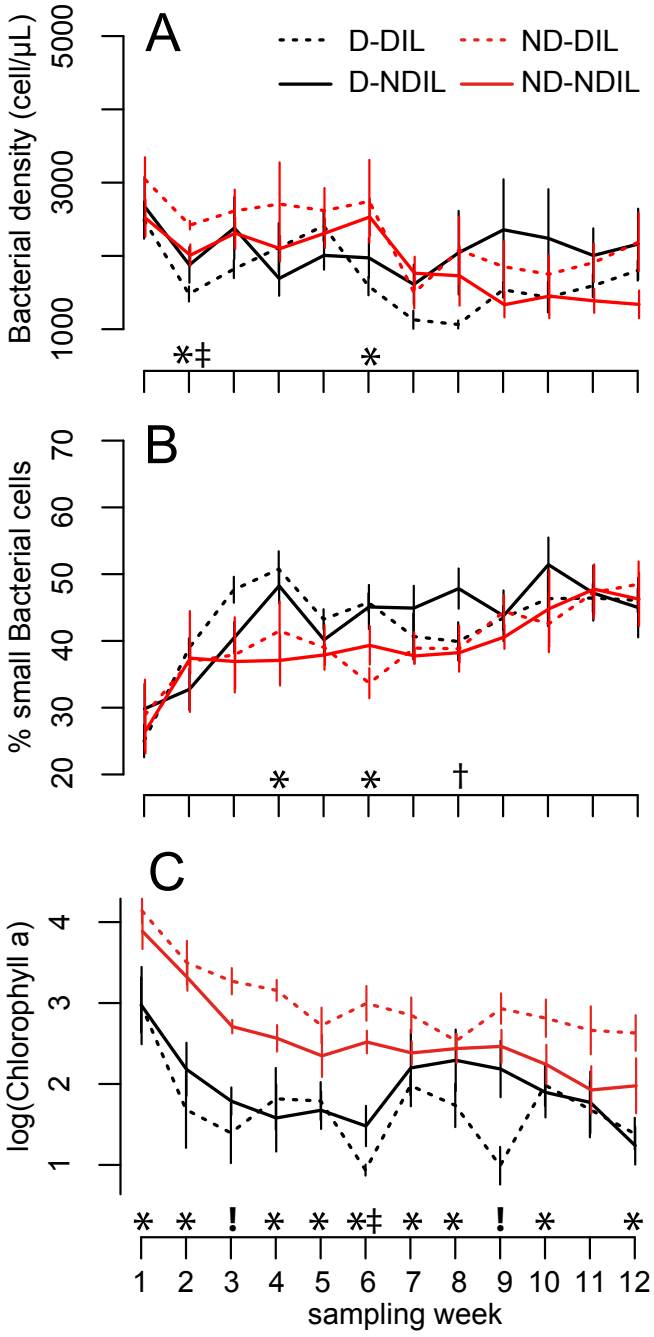

Figure 3.2: ***Time series plot of bacterial density (A), proportion of small bacterial cells (B) and chlorophyll a (C).*** *Symbols indicate significant result of linear mixed effect model for the 12 weeks of the experiment. ∗: significant effect of Daphnia treatment, †: significant effect of Dilution treatment, ‡: significant interaction, !: Daphnia, Dilution and Interaction significant.*

Ecosystem properties at the end of the experiment

At the end of the experiment, we found significantly lower phytoplankton densities in metacommunities with *Daphnia*, as determined by flowcytometry, microscopic counts, and chlorophyll a concentrations. However, no significant changes in bacterial densities were found, nor did we find significant differences in bacterial size classes. We furthermore found significantly higher dissolved oxygen concentrations and respiration rates, and a trend for decreased pH in the presence of *Daphnia*. No significant effects of *Daphnia* on dissolved organic carbon (DOC) concentrations or composition, temperature and conductivity were detected. The dilution treatment did not significantly alter environmental parameters at the last sampling time point (Table 3.2).

Diversity

We found that the presence of *Daphnia* significantly reduced local (α) and regional (γ) diversity of both the bacterial (BC) and the phytoplankton (PC) community (Fig. 3.3A+D, Table 3.3). In contrast to the effects of fish on plankton, (Chase et al., 2009) bacterial and phytoplankton metacommunities did not become significantly more similar to each other in the presence of *Daphnia* (Jaccard: BC: p=0.28, PC: p=0.77; Bray-Curtis: BC: p=0.07, PC: p=0.99; Fig. 3.3C+F, Table 3.3). Although we did not find significant differences in α- and γ-diversity of the bacterial community between diluted and undiluted tanks at the end of the experiment, β-diversity measures showed that tanks within a metacommunity were significantly more similar to each other if they received a diluted source community (Fig. 3.3B+C, Table 3.3). In contrast, the phytoplankton community increased in α- and γ-diversity, and diluted tanks showed a trend for decreased community similarity (p=0.08).

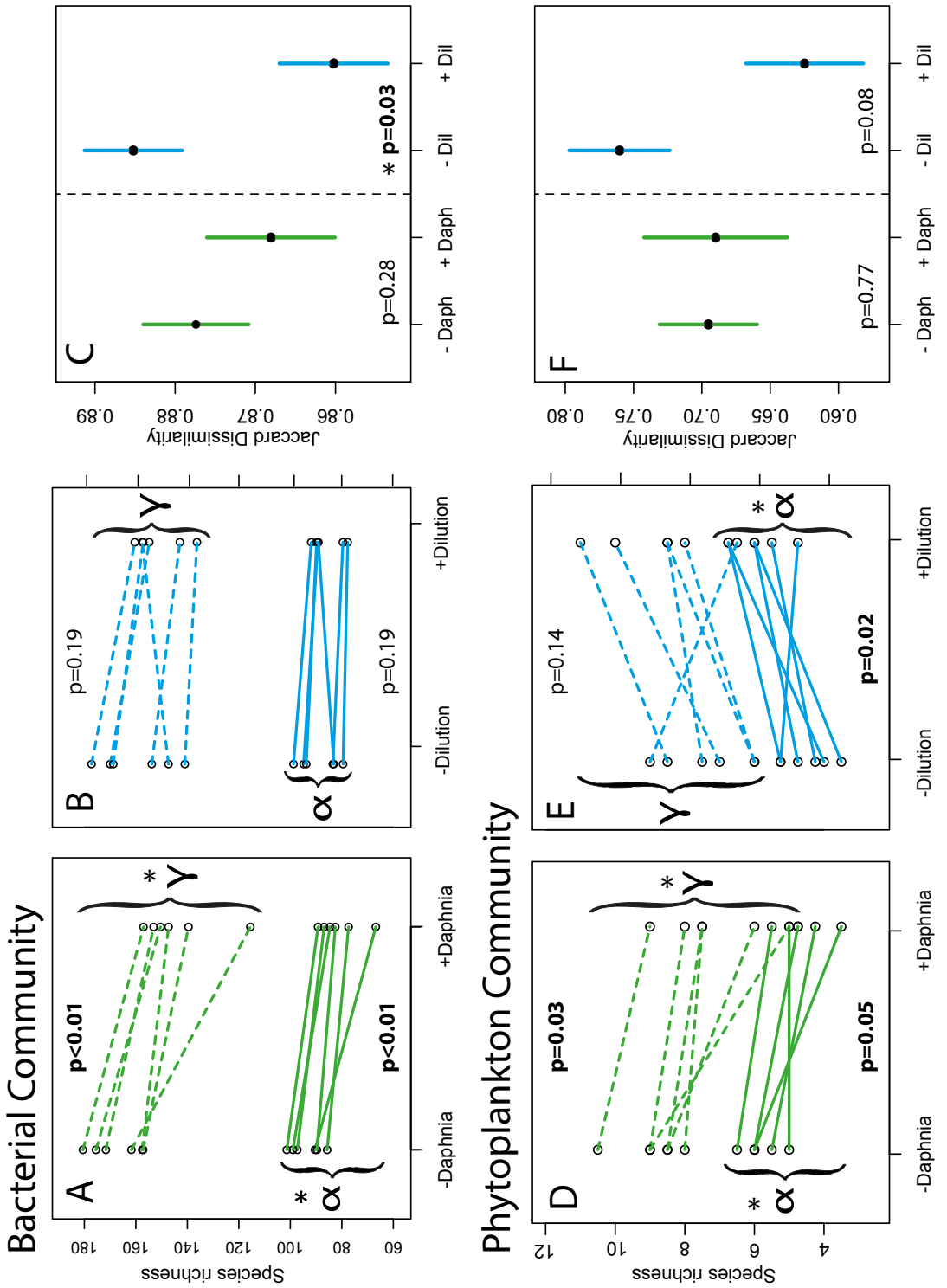

Figure 3.3: ***Diversity results for bacterial (A-C) and phytoplankton community (D-F).*** *Colors indicate treatment contrast (see Fig.1). Solid lines (A,B,D,E): α-diversity, dashed lines (A,B,D,E): γ-diversity, C+F: β-diversity (Jaccard Dissimilarity). p-values indicate significance as determined by linear mixed effect model (Table 1). A,B,D,E: Changes in α and γ-diversity as an effect of Daphnia and Dilution treatment. Each dot represents block average. C+D: Changes in β diversity with Daphnia and Dilution treatment, showing the mean as well as the SE (black dot) and the standard error (solid lines).*

Phylogenetic Diversity

Although *Daphnia* had weak effects on abundance, *Daphnia* had strong effects on the phylogenetic composition of the bacterial metacommunities. The average metacommunity PD (Fig. 3.4A) was significantly lower in the presence of *Daphnia* ($p<0.01$), but not significantly different between diluted and undiluted tanks ($p=0.21$). UniFrac distances of mesocosm within a metacommunity (Fig. 3.4B) were not significantly different as response to *Daphnia* (unweighted UniFrac: $p=0.72$, weighted UniFrac: $p=0.73$), but showed a trend to be lower in diluted metacommunities (unweighted UniFrac: $p=0.08$, weighted UniFrac: $p=0.15$). Using a UniFrac distance matrix, however, we found a clear separation and significant differences between metacommunities with and without *Daphnia*, but no separation between diluted and undiluted metacommunities. A permutation test revealed a significant influence of the *Daphnia* treatment, but no significant dilution effect nor interaction. This results was the same both for the (repeated) rarefied OTU data as well as for the non-rarefied OTU data (Table S3).

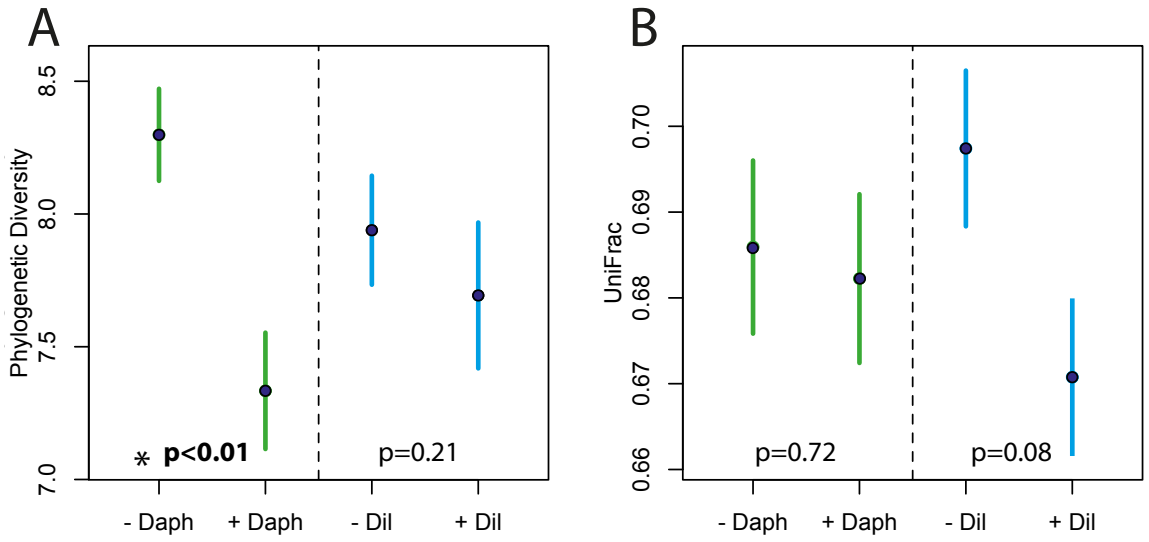

Figure 3.4: ***Changes in Phylogenetic diversity (A) and UniFrac distance (B) as an effect of Daphnia and Dilution treatment.*** *Colors and statistics: see Fig. 3.*

Community composition

We found strong treatment effects on composition of the ten most abundant classes of the bacterial community, which together represented approximately 82% of all OTUs (Fig. 3.5B, Table 3.3). We found an interactive effect of *Daphnia* and dilution on *Betaproteobacteria*, the most abundant class of bacteria, which comprised almost 40% of all OTUs. The abundance of two of the top ten classes was significantly affected by *Daphnia* (significant increase

in *Alphaproteobacteria* and significant decrease in *Betaproteobacteria*). Dilution significantly increased the abundance of *Saprospirae* and decreased the abundance of *Flavobacteriia* and *Chloroplasts*. Furthermore, the top ten bacterial classes comprised a significantly higher proportion of bacterial OTUs in metacommunities with *Daphnia* (D: 85%, ND: 80%; $p<0.01$).

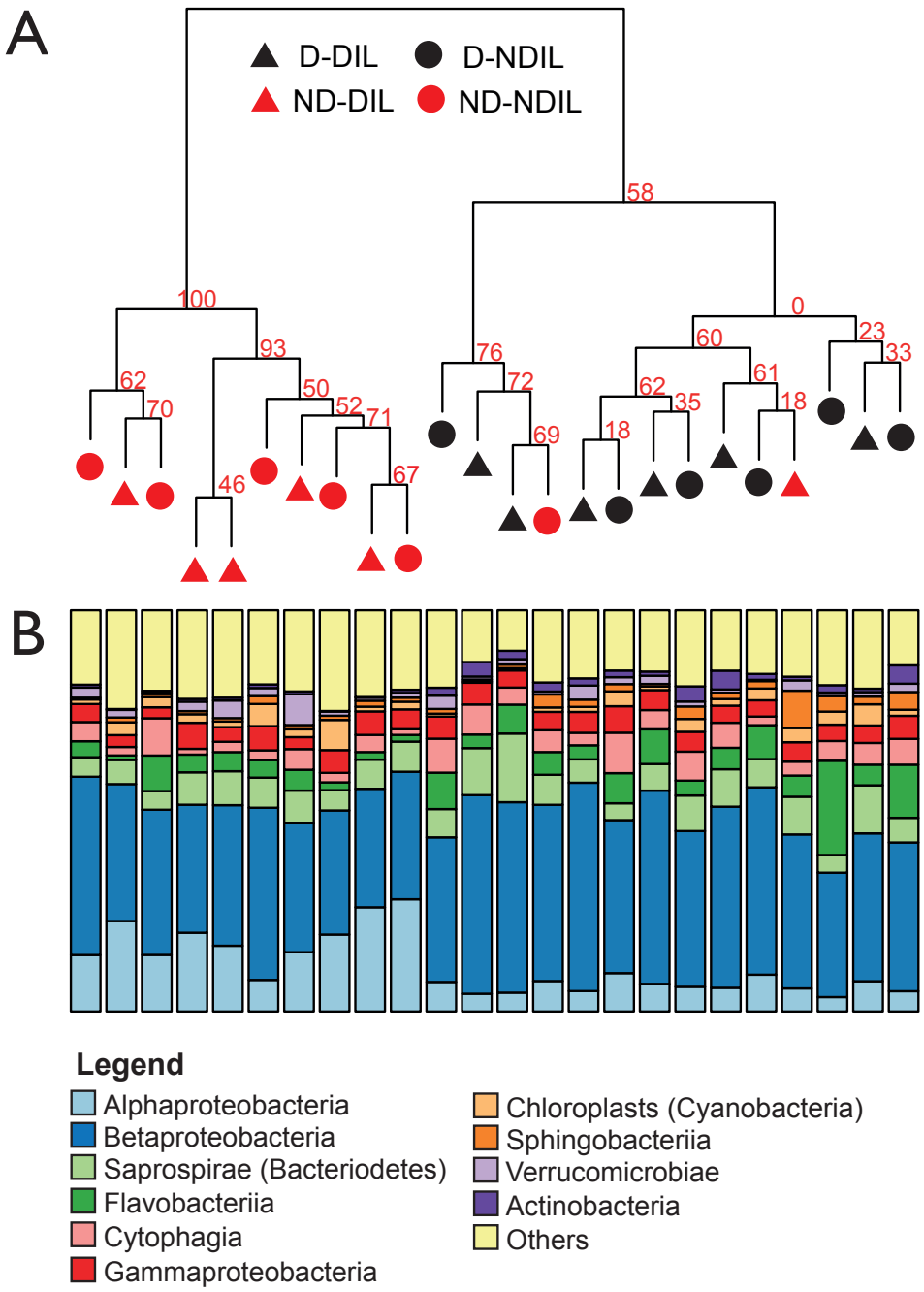

Figure 3.5: ***Hierarchical clustering of UniFrac distances of the 24 metacommunities (Top) as well as bacterial composition of the corresponding metacommunities at the Class level.*** *Colors and symbols of the top part indicate the 4 different treatments (see Fig. 1), red numbers: AU (approximately unbiased) p-values (Shimodaira, 2002).*

Rank abundance

The bacterial rank-abundance distributions (Fig. 3.1C+D), which are a combined measure of species richness and evenness, differed significantly between treatments. Rank-abundance slopes of metacommunities with *Daphnia* were significantly (p=0.01) steeper than in meta-communities without *Daphnia* (Fig. 3.6A+B, Table 3.3), indicating a more even distribution of OTUs as well as more OTUs with low abundances in the absence of *Daphnia* and higher abundances of a few dominant OTUs in the presence of *Daphnia*. Dilution did not have significant effects on rank-abundance slopes (p=0.20). We also investigated the extent of average rank changes of individual OTUs as a result of *Daphnia* and dilution treatment and found that rank changes were much stronger in the presence of *Daphnia*, i.e. that the abundances of individual OTUs were changing more strongly in metacommunities containing *Daphnia* (Fig. 3.6C+D). Generally, low-rank (i.e. highly abundant) OTUs often remained low-rank OTUs, while high-rank (i.e. low abundant) OTUs were facing large rank changes (Fig. 3.6C). This was true both when looking at the *Daphnia* and the dilution contrast (Fig. 3.6C), but rank changes were much more pronounced for the *Daphnia* contrast (Fig. 3.6D).

Phylogenetic Signal

We found a clear phylogenetic signal of rank changes as a result of *Daphnia* presence/absence, but not of dilution (Fig. 3.7, Table S4), both when analyzing the phylogenetic tree of the 300 most abundant OTUs as well as when aggregating the data to the class level. This indicates that closely related species have a similar response (either increase or decrease in abundance) to the presence of *Daphnia*.

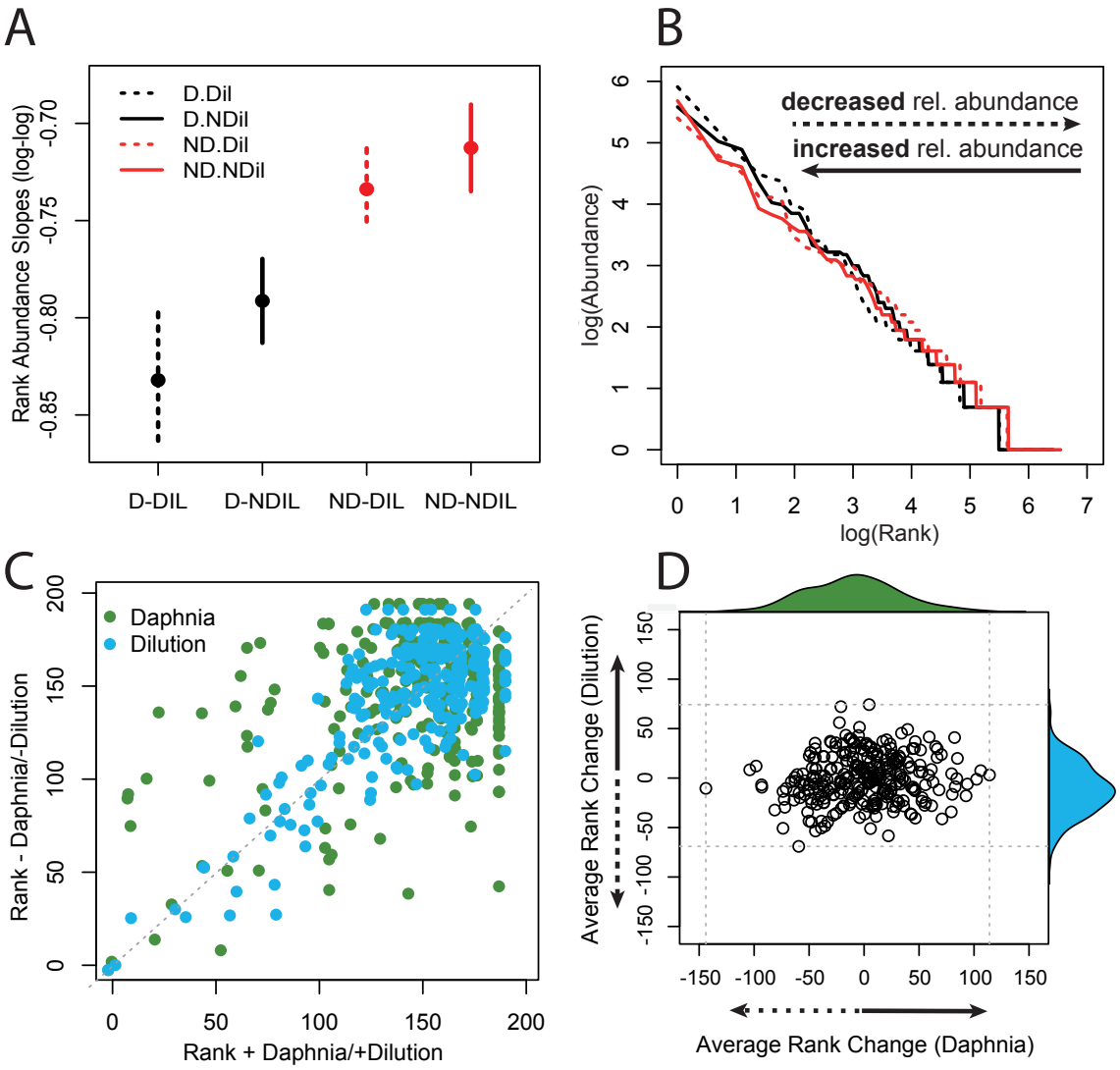

Figure 3.6: ***Rank Abundance plots.*** *A: Changes in rank abundance slopes (log-log) with treatment (treatment codes: see Fig. 1), indicating mean and standard error. B: Average rank abundance plot of the four treatments. C: Rank plot of individual OTUs using the Top 300 OTUs across the rarefied dataset. x-axis: Average rank with Daphnia/ Dilution, y-axis: average rank without Daphnia/ Dilution. Green dots: Daphnia contrast, blue dots: Dilution contrast. D: Average rank change of individual OTUs of the Top 300 OTUs as an effect of Daphnia treatment (x-axis) and Dilution treatment (y-axis). Each dot shows the rank change of an individual OTU, histograms show the distribution of the points as an effect of treatment.*

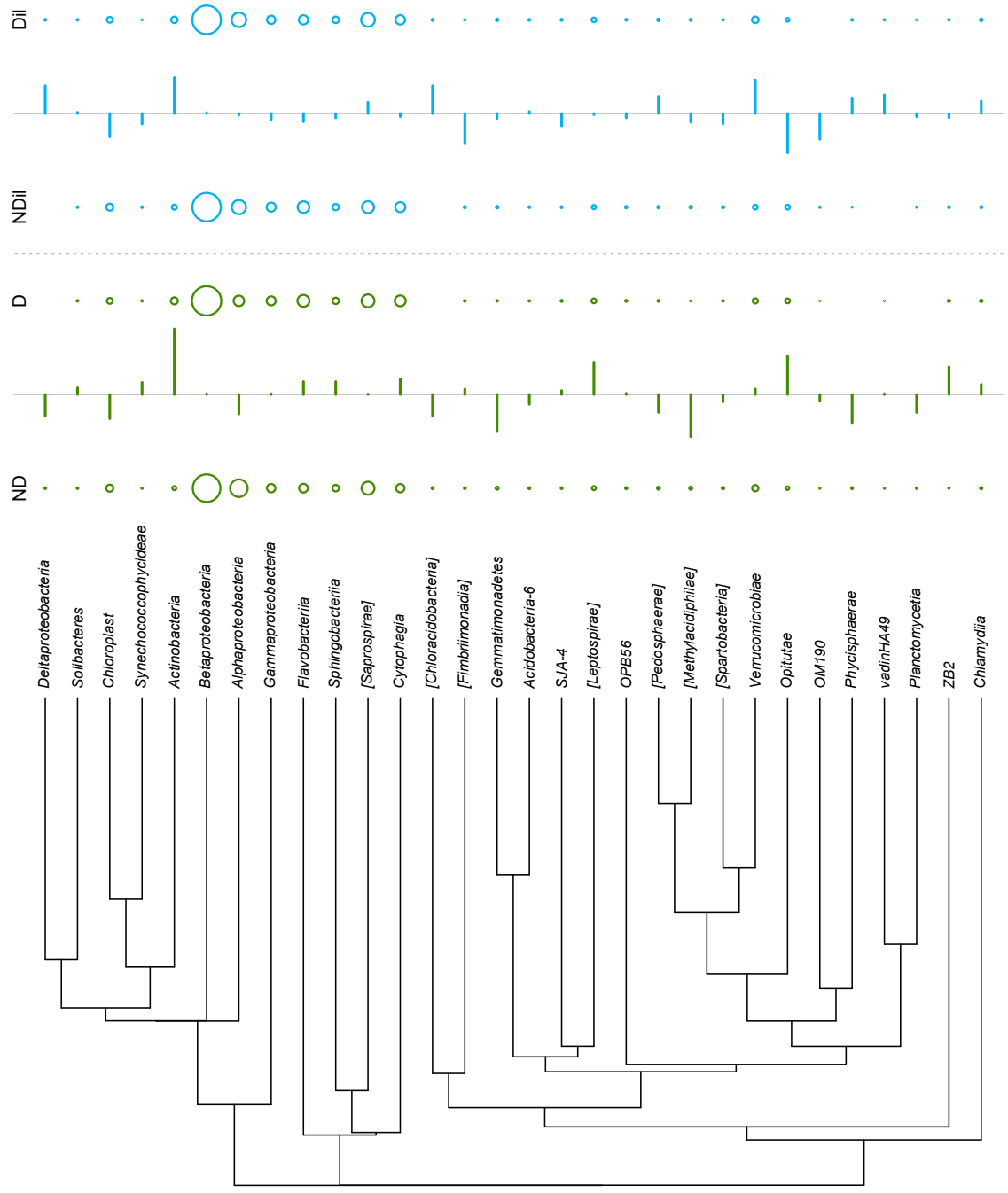

Figure 3.7: ***Phylogenetic tree of the Top 300 OTUs of the rarefied dataset, aggregated to Class level, indicating average abundance and rank change of the Daphnia (green) and Dilution contrast (blue).*** *Green dots: Average abundance of the phylogenetic groups in metacommunities without Daphnia (ND) and with Daphnia (D). Green lines: rank change of the phylogenetic groups between D/ND metacommunities. Blue dots and lines: Average abundance and rank change in the undiluted (NDil) as well as the diluted (Dil) metacommunities.*

Table 3.3: ***Summary of ANOVA results from the last sampling (week 12) of the metacommunity experiment.*** *F and p results for the effect of Daphnia, dilution, and interactive effects. Significant values are highlighted wild bold letters.*

		F and p values of ANOVA analysis		
Metrics	**Parameter**	***Daphnia***	**Dilution**	**Interaction**
Bacterial Community	α-diversity (Species richness)	**14.80 (<0.01)**	1.90 (0.19)	1.02 (0.33)
	β-diversity (Jaccard)	1.23 (0.28)	**5.70 (0.03)**	0.24 (0.63)
	β-diversity (Bray-Curtis)	3.77 (0.07)	**4.43 (0.05)**	0.21 (0.66)
	γ-diversity	**13.59 (<0.01)**	1.90 (0.19)	1.02 (0.33)
	Phylogenetic Diversity	**13.08 (<0.01)**	1.74 (0.21)	0.57 (0.46)
	Rank abundance slopes (log-log)	**10.44 (0.01)**	1.80 (0.20)	0.21 (0.66)
	UniFrac distance	0.14 (0.72)	3.43 (0.08)	0.04 (0.85)
	weighted UniFrac distance	0.12 (0.73)	2.33 (0.15)	0.00 (0.95)
	Bacterial density	0.72 (0.41)	0.62 (0.44)	3.51 (0.08)
	Bacterial size	0.28 (0.60)	0.06 (0.82)	0.03 (0.86)
Top 10 Bacterial Classes	Betaproteobacteria [39%]	**30.67 (<0.01)**	**14.17 (<0.01)**	**16.94 (<0.01)**
	Alphaproteobacteria [11%]	**18.79 (<0.01)**	0.47 (0.50)	1.04 (0.32)
	Saprospirae (Bacteriodetes) [8%]	1.55 (0.23)	**4.90 (0.04)**	0.35 (0.56)
	Flavobacteriia [6%]	1.26 (0.28)	**8.29 (0.01)**	3.04 (0.10)
	Cytophagia [5%]	3.59 (0.08)	0.14 (0.71)	0.07 (0.80)
	Gammaproteobacteria [5%]	0.72 (0.41)	0.09 (0.77)	0.04 (0.85)
	Chloroplasts (Cyanobacteria) [2%]	2.35 (0.15)	**4.93 (0.04)**	0.83 (0.38)
	Sphingobacteriia [2%]	0.58 (0.46)	1.74 (0.21)	2.16 (0.16)
	Verrucomicrobiae [2%]	3.44 (0.08)	0.02 (0.89)	2.42 (0.14)
	Actinobacteria [2%]	**18.25 (<0.01)**	1.36 (0.26)	1.72 (0.21)
	Others [18%]	**18.73 (<0.01)**	2.56 (0.13)	2.70 (0.12)
Phytoplankton Communtiy	α-diversity (Species richness)	**5.53 (0.03)**	**7.10 (0.02)**	0.01 (0.91)
	β-diversity (Jaccard)	0.09 (0.77)	3.42 (0.08)	0.11 (0.75)
	β-diversity (Bray-Curtis)	0.00 (0.99)	0.12 (0.74)	0.16 (0.69)
	γ-diversity	**4.61 (0.05)**	2.44 (0.14)	0.02 (0.89)
	Rank abundance slopes (log-log)	1.71 (0.21)	0.40 (0.54)	1.89 (0.19)
	Phytoplankton density (FCM)	**6.49 (0.02)**	0.02 (0.89)	0.40 (0.54)
	Phytoplankton counts	**10.37 (0.01)**	0.09 (0.77)	1.45 (0.25)
	Chlorophyll-a	**10.43 (0.01)**	0.02 (0.89)	1.41 (0.25)
Physical/ Chemical	DOC	0.05 (0.82)	0.21 (0.65)	0.13 (0.73)
	DOC Spectral slope	2.91 (0.11)	0.07 (0.79)	0.04 (0.85)
	DOC Absorption a320	1.06 (0.32)	0.41 (0.53)	0.06 (0.81)
	Dissolved oxygen	**9.40 (0.01)**	0.57 (0.46)	0.89 (0.36)
	pH	3.98 (0.06)	0.25 (0.62)	0.13 (0.73)
	Temperature	3.56 (0.08)	1.47 (0.24)	3.28 (0.09)
	Conductivity	0.85 (0.37)	0.63 (0.44)	0.36 (0.56)
Ecosystem Functions	Enzyme (phosphatase) activity	1.36 (0.26)	0.16 (0.70)	0.00 (0.99)
	Respiration	**10.73 (0.01)**	2.82 (0.11)	**6.01 (0.03)**

DISCUSSION

Our experiment is one of the first to explore the interactive effects of *Daphnia* grazing and dilution on the diversity of bacterial and phytoplankton meta-communities. Using a replicated setup of large outdoor mesocosms, we have shown that bacterial and phytoplankton community assembly is strongly affected by *Daphnia* grazing, and, though to a lesser extent, by the initial abundance of bacterial cells (Table 3.3).

A previous study by Chase et al. (2009) found that fish predation reduced the diversity of macroinvertebrates, zooplankton and amphibians at both local (α) and regional (γ) scales, and increased community similarity (lower β-diversity). They concluded that deterministic processes become more important for community assembly in the presence of a fish predator, probably because species that persisted with fish were a similar size (Chase et al., 2009). Our study has taken a similar approach, but instead of studying the effects of fish predation on macroinvertebrates, zooplankton and amphibians, we have studied effects of predation at lower trophic levels, namely how *Daphnia* grazing affects community assembly of bacteria and phytoplankton. In bacteria, we know little about the distribution of traits among species that affect growth and survival in the presence of *Daphnia*. In addition to measures of diversity, we also used molecular methods to characterize the bacterial community composition and phylogenetic structure. Similar to the results from Chase et al. (2009), we also found a significant decrease in α- and γ-diversity of both the bacterial and the phytoplankton community in the presence of *Daphnia* (Fig. 2.3). *Daphnia* did not affect β-diversity of both the bacterial and the phytoplankton community, i.e. that communities did not become more similar to each other in the presence of *Daphnia*.

Although the diversity of both bacterial and phytoplankton communities decreased in the presence of *Daphnia*, these patterns are likely to arise from very different mechanisms. *Daphnia* are considered non-selective filter feeders (Hartmann and Kunkel, 1991), and are well known to feed on phytoplankton, but the ingestion of phytoplankton by *Daphnia* depends on the mesh size of *Daphnia* (Gophen and Geller, 1984) as well as the size of the phytoplankton species (Böing et al., 1998). We have shown, that over the course of the 12-week experiment, chlorophyll a concentrations were significantly lower in the presence of *Daphnia* (Fig. 3.2C) and we assume that the strong grazing pressure reduced in α- and γ-diversity within the phytoplankton community. Our finding that β-diversity remained constant irrespective of *Daphnia* regime further strengthens the assumption that the shaping force of *Daphnia* on the phytoplankton community was not species selective, but rather size selective. By decreasing overall levels of phytoplankton diversity, stochastic effects are likely to be the main driver of community composition in the *Daphnia*-containing

metacommunities, while deterministic mechanisms, such as resource availability, inter- and intraspecific competition, and niche differentiation, are more likely to be the main drivers of phytoplankton community composition in the absence of *Daphnia*. However, differences in species composition, such as shifts to inedible phytoplankton taxa in the presence of *Daphnia* grazing (Mccauley and Briand, 1979) might be hidden in the species composition, which was not investigated in this study. Both α- and γ-diversity of the phytoplankton community increased with dilution treatment, while community dissimilarity (β-diversity) decreased. The increase in local and regional diversity might be explained by priority effects (Chase, 2003), where the phytoplankton community was able to establish larger diversity and occupy empty niches when the initial abundance of bacterial cells was reduced. Decreased β-diversity indicates that deterministic processes, such as environmental properties and niche properties (Chase, 2003), might be important drivers of phytoplankton community composition when initial abundance of bacterial cells is low, while stochastic processes might have been the main drivers of phytoplankton community assembly at higher bacterial densities due to higher competition pressure for nutrients and niche space with the bacterial community (Cottingham et al., 1997).

While we already know quite a bit about the mechanisms how *Daphnia* shape phytoplankton communities, less is known about the mechanisms how *Daphnia* predation shapes bacterial communities. We were able to show that bacterial cell densities, other than phytoplankton densities, remained fairly constant throughout the experiment (Fig. 3.2A) and no significant differences between treatments were found at the end of the experiment. Jürgens and Matz (2002) have shown, that small bacterial cells dominate in the presence of *Daphnia* due to reduced densities of phagotrophic protists, such as heterotrophic nanoflagellates (HNF) and ciliates, which feed on bacterial cells. When *Daphnia* grazing is reduced, bacterial composition often shifts towards larger morphologies, which are resistant towards protist grazing. In our study, we also found a clear trend towards smaller bacterial cell sizes in the presence of *Daphnia* during the course of the experiment (Fig. 3.2B), which confirms the notion that small bacterial cells are more likely to be found in the presence of *Daphnia* (Jürgens and Matz, 2002). Bacterial densities might remain constant due to a combination of direct and indirect effects, where the presence of the keystone predator indirectly releases grazing pressure of protists on the bacterial community. However, *Daphnia* also can feed directly on large bacterial cells (Kamjunke and Zehrer, 1999). In the presence of *Daphnia*, α- and γ-diversity, as well as phylogenetic diversity, were significantly reduced. We also found a clear differentiation between metacommunities with and without *Daphnia* when comparing UniFrac distances (Fig. 3.5), as well as significant differences

in community composition of the bacterial community, where some classes showed significant differences in abundance between metacommunities with and without *Daphnia* predation (e.g. *Alphaproteobacteria, Betaproteobacteria* and *Actinobacteria*). Summarizing bacterial community at the class level masks a lot of variation among bacterial OTUs, but finding clear patterns even at the class level is a strong evidence that the presence of *Daphnia* poses a strong and general shaping force on the bacterial community.

Changes in bacterial community composition might be due to bacterial size and differences in grazing resistance of bacterial taxa (Pernthaler, 2005), as well as trophic interactions, which has previously been shown to lead to changes in bacterial size and community composition (Jürgens and Matz, 2002). *Alphaproteobacteria*, for example, are known to form filaments to resist ingestion by HNF (Jürgens et al., 1999), which increases in the absence of *Daphnia*, and they were also found to be significantly more abundant in the absence of *Daphnia* during our study. *Betaproteobacteria*, which were significantly more abundant in the presence of *Daphnia*, have previously been shown to be negatively affected by HNF grazing (Salcher et al., 2010). We think that both the presence and the absence of *Daphnia* lead to distinctly different food-web structures, where microbial communities are controlled either through direct and indirect effects of *Daphnia*, or direct and indirect effects of other organisms, such as HNF, in the absence of *Daphnia*, which both lead to distinct bacterial communities.

Similar as for the phytoplankton community, dilution of the initial bacterial inoculum decreased dissimilarity in the microbial community, which indicates that the bacterial community was affected by the initial abundance of bacterial cells, despite showing similar species richness at the end of the experiment. Species richness, which did not show significant differences as a result to dilution treatment, was likely not affected by dilution as metacommunities were open and could receive bacterial cells from the surrounding environment (e.g. through rain, air and other incoming organisms). Increased similarity in the diluted metacommunities might stem from priority effects (Alford and Wilbur, 1985), where early arriving bacterial taxa could occupy niches and adapt to the local conditions more easily. In diluted metacommunities, bacteria were facing less competition for resources and niche spaces due to lower concentrations of bacterial cells and might therefore have assembled more stochastically. Undiluted metacommunities, on the other hand, were more dissimilar from each other, suggesting that stochastic processes were more important when bacterial densities were higher. Significant shifts several bacterial taxa as response dilution treatment furthermore indicate that the bacterial community did show a distinct response to the dilution manipulation.

One of the novel aspects of our study is the analysis of phylogenetic signal of the bacterial community in response to *Daphnia* grazing as well as dilution, where we found a clear signal of rank change as result of *Daphnia* presence/absence at both the OTU and the class level (Fig. 3.7, Table S4). Genetically closely related bacterial OTUs, respectively classes, are likely to respond to the presence/absence of *Daphnia* in a similar way due to similar functional capacities, such as described above for *Alphaproteobacteria* and *Betaproteobacteria*. This indicates that ecological traits, which lead to differences in OTU abundance as response to the presence or absence of *Daphnia*, are phylogenetically conserved (Koeppel and Wu, 2013). Other studies have used phylogenies to explore how species relatedness can predict responses to environmental change or stress (Helmus et al., 2007). Our results suggest that bacterial phylogenetic relationships might also help us explore different mechanisms of community assembly.

The *Daphnia* manipulation also affected rank-abundance distributions of the bacterial community (Fig. 3.6). By decreasing diversity of the bacterial community, *Daphnia* also changed the dominance structure, leading to fewer but more dominant OTUs, whereas more rare OTUs could persist in the absence of *Daphnia*. High grazing pressure of *Daphnia* might explain the removal of rare OTUs, which are stochastically removed due decreased population sizes. Interestingly, *Daphnia* also affected the average rank distribution of bacterial OTUs, leading to large rank changes of individual OTUs when their rank was compared in metacommunities with or without *Daphnia*. This again indicates that *Daphnia* pose strong selection pressure on the microbial community and strongly affects dominance of individual OTUs. Due to various direct and indirect effects of *Daphnia* and HNF grazing, which have been described above, large shifts in bacterial community composition, especially among the rare OTUs, were observed, while the most abundant OTUs remained abundant.

Daphnia significantly increased respiration rates of the bacterial community, indicating higher activity of the bacterial community in the presence of *Daphnia* and significant changes in community functions. One explanation for increased respiration in the presence of *Daphnia* might changes in community structure of the bacterial community, which might promote fast-growing bacterial taxa. *Betaproteobacteria*, for example, which were the most abundant group of bacteria in all metacommunities and significantly more abundant in the presence of *Daphnia* are known to be fast-growing (Simek et al., 2006) and might drive differences in community respiration. *Daphnia*, however, did not significantly change phosphates activity. Phosphatase is an important enzyme to utilize phosphorus, which is an essential nutrient, and it is therefore likely that this function is carried out by various

members of the phytoplankton. Previous work has found that such generalized ecosystem functions are not as sensitive to compositional changes as are enzyme activities driven by more specialized bacteria (Comte and del Giorgio, 2010).

CONCLUSION

Overall, using a replicated metacommunity mesocosm experiment, we were able to show that the presence/absence of *Daphnia* strongly affects bacterial and phytoplankton diversity as well as bacterial community structure and composition. By using high-throughput sequencing, we were able to resolve the microbial community in far more detail than traditional fingerprinting techniques, which allowed us to analyze the bacterial community composition, dominance structure and phylogenetic structure, which were strongly affected by *Daphnia*. Dilution of the bacterial source community had weaker effects on the bacterial and the phytoplankton community, but increased phytoplankton species richness, which indicates that priority effects were important drivers of phytoplankton community richness as phytoplankton could occupy more nice space in the diluted metacommunities.The *Daphnia* manipulation significantly affected respiration, which indicates that ecosystem functioning may be affected by food-web structure and bacterial composition.

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APPENDIX

Supplementary Tables

Table S2: *Read count summary of the different treatments from the mesocosm experiment*

Treatment	# Tanks	# Metacommunities	Mean	SE	Min	Max
D-DIL	12	6	676	52	370	958
D-NDIL	12	6	740	82	292	1220
ND-DIL	12	6	835	92	255	1236
ND-NDIL	12	6	897	87	282	1397
global	48	24	787	41	255	1397

Table S3: *p-values of permutation test of the UniFrac distance matrices*

Distance matrix	Rarefied	*Daphnia*	Dilution	Interation
unweighted UniFrac	x	<0.01	0.12	0.10
weighted UniFrac	x	<0.01	0.24	0.11
unweighted UniFrac		<0.01	0.12	0.14
weighted UniFrac		<0.01	0.27	0.11

Table S4: *Phylogenetic Signal of Daphnia and dilution contrast using the 300 most abundant OTUs*

	Average K *Daphnia* (SD)	% significant* K values	Average K dilution (SD)	% significant* K values
Top 300 OTUs	0.842 (0.018)	83	0.783 (0.019)	3
Top 300 OTUs, Class level	0.838 (0.017)	81	0.786 (0.020)	4

*Significance threshold: 0.05

4

CHAPTER III: META-ECOSYSTEM DYNAMICS

EXPERIMENTAL EVIDENCE THAT HETEROGENEITY AND DISPERSAL AFFECT THE SPATIAL STRUCTURE AT MULTIPLE TROPHIC LEVELS IN META-ECOSYSTEMS

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ABSTRACT

Meta-community theory is a usefull concept for investigating species distributions across space, and a growing number of field and experimental surveys have measured the effects of dispersal, environmental heterogeneity, grazing, and trophic structure on meta-community dynamics. However, meta-community theory does not consider how the movement of organisms interacts with the material fluxes between patches, and this limitation prompted the development of a meta-ecosystem theory. Previous experiments have measured how ecosystems respond to manipulations of dispersal, but no empirical study has quantified the importance of material fluxes in relation to the dispersal of organisms. Using mesocosms, we have manipulated meta-ecosystem dynamics in two ways. First, we manipulated how resources are distributed across space by creating heterogenous and homogenous meta-ecosystems, and second, we applied two different dispersal regimes to test for how responses vary depending on if only material is dispersed, versus if material and organisms are dispersed. We found that both environmental properties and communities responded to nutrient loading and dispersal regime, but community responses varied between trophic groups. The nutrient loading contrasts affected both spatial heterogeneity and average ecosystem responses. Bacterial richness and community composition were strongly shaped by dispersal regime, while phytoplankton richness interactively responded to dispersal and resource loading, and community composition differed strongly with nutrient loading. The zooplankton community showed only weak responses to the manipulations, but individual taxa were affected by both resource heterogeneity, dispersal, and their interaction. In addition, nutrient identity had strong effects on community composition at all trophic levels. Overall, we could show that meta-ecosystem dynamics, i.e. the flux of both organisms, and materials within spatially structured landscapes, are important for understanding both for ecosystems and community responses to environmental change.

INTRODUCTION

Ecologists have progressively developed conceptual models of spatial community dynamics in response to the growing recognition that local communities can be strongly influenced by surrounding communities and environments at various spatial scales. Research on meta-population dynamics initially focussed on the distribution of the populations of individual species across space (Hanski and Gilpin, 1991). A decate later, meta-community theory was developed to include the spatial dynamics of multiple populations and entire communities. Both concepts exclusively focus on populations and communities that are connected via dispersal of organisms (Leibold et al., 2004) and do not include the spatial flow of energy and non-living organic matter that can also affect community structure and ecosystem functioning. Around the same time, the field of landscape ecology began focussing on how the flow of material between ecosystem patches might affect ecosystem functioning in general, and biogeochemical cycles driven particularly by organisms at low trophic levels (Massol et al., 2011). Recently, these two perspectives have been merged into meta-ecosystem theory (Loreau et al., 2002; Gravel et al., 2010a), which integrates the flow of material into meta-community theory so as to investigate how dispersing organisms and spatial fluxes of any material might interactively affect community and ecosystem dynamics. Such fluxes can include the movement of nutrients between patches either through inorganic material (direct flow) or through organic material stored in living organisms and detritus (indirect flow) (Gravel et al., 2010a), and can affect community structure both in the source and sink habitat.

Since the onset of meta-community theory, the spatial scale of environmental heterogeneity has been a central concept for understanding the diversity and functioning of ecosystems. Species diversity, for example, can be strongly influenced by variation in nutrient availability among sites, whereas the form of the relationship can depend on the spatial scale (Chase and Leibold, 2002). At the local scale species richness often peaks at intermediate productivity, while at the regional scale richness increases linearly with productivity, because productivity tends to increase community dissimilarity among local sites. The importance of spatial scale and ecosystem size becomes increasingly important when quantifying the diversity of multiple groups of organisms, as spatial scaling of species richness varies among species of different body size, dispersal mode, and trophic level (Logue et al., 2011a; Reche et al., 2005). Species sorting, either along environmental gradients, or among heterogeneous patches is commonly considered to be an important structuring force in meta-communities (Cottenie, 2005), but likely varies among communities with different amounts of niche variation among species. For example, Soininen

(2014) recently found that the degree of species sorting varies between trophic groups and ecosystem types, with the importance of species sorting being lower for freshwater and terrestrial communities, as compared to estuaries and marine sites. Heterogenous environments can clearly promote species sorting and contribute to regional (γ) diversity within communities so long as species vary in their niches (Ricklefs, 1977; Grubb, 1977; Chesson and Warner, 1981; Ai et al., 2013). Environmental heterogeneity can also increase or decrease community dissimilarity (β-diversity), depending on the relative importance of stochastic versus deterministic factors that drive local diversity (Chase and Myers, 2011). By impacting the structure of biodiversity at multiple trophic levels in meta-communities, environmental heterogeneity likely plays a key role in the dynamics of meta-ecosystems (Langenheder et al., 2010; Bulling et al., 2008; Cardinale et al., 2000).

Understanding why and how species from multiple trophic levels coexist in meta-ecosystems is a fundamental issue in ecology. A previous comparative study of plankton diversity among lakes suggests that different trophic groups of aquatic organisms (bacteria, phytoplankton, and zooplankton) are regulated independently by different environmental gradients (Longmuir et al., 2007), meaning that heterogeneity in a meta-ecosystem might have contrasting effects on different organismal groups. For example, Longmuir et al. (2007) found that zooplankton richness was negatively correlated with elevation and total organic carbon, while phytoplankton and bacteria richness were positively correlated with water transparency (measured as light extinction and secchi depth). The observed lack of relationship between richness across trophic levels suggest weak trophic control over the mechanisms that maintain biodiversity in these systems (Longmuir et al., 2007), however this has yet to be tested experimentally. Organisms at different trophic levels might also differ in their dispersal capacities among sites within a meta-ecosystem, which might change the relative importance of space and environmental differences for structuring the diversity of local patches (Beisner et al., 2006; De Bie et al., 2012). Larger organisms, for example, are much more likely to be dispersal limited and exhibit meta-community dynamics than are smaller species (Beisner et al., 2006). Dispersal limitation of equally sized organisms is also lower for organisms which possess the ability to fly as compared to passive dispersers (De Bie et al., 2012). Larger species, as well as those with resting stages, may move more or less independently from fluxes of organic materials among meta-ecosystem sites. Such variation in dispersal capacities among species and trophic groups is likely to be important for both diversity and ecosystem dynamics. A recent meta-community study by Declerck et al. (2012) has found that even small amounts of dispersal can significantly reduce β-diversity of zooplankton, bacteria and viruses, irrespective of

nutrient heterogeneity. A different study of bacterial lake communities, however, has found strong positive correlations of heterogeneity on bacterial abundance, richness, and community similarity (Jankowski et al., 2014). To elucidate the relative roles of both environmental heterogeneity and dispersal in structuring the spatial variation of diversity across trophic levels in meta-ecosystems, more research and empirical studies are needed.

Here, we present the results from an experimental study testing the joint influence of dispersal and nutrient heterogeneity on community structure of three different trophic levels (zooplankton, phytoplankton and bacteria). We conducted a mesocosms experiment in which we established meta-ecosystems (three patches of 300L each) that were either homogeneous or heterogeneous in nutrient conditions among patches, while keeping the total loading of nutrients added to each meta-ecosystem equal. The nutrient heterogeneity treatment was crossed with two different dispersal regimes, to test the importance of the flow of energy within meta-ecosystems relative to the flow of organisms and energy. In one case we dispersed the living organisms, while in the other case we killed the organisms prior to dispersal in order to disperse only the organic and inorganic material within the water. To our knowledge, no experimental tests of have yet been conducted to test for the importance of these two processes for meta-ecosystem dynamics. We hypothesized that heterogeneous environments would contain a larger variety of niche spaces and thus lead to higher species richness due to species sorting. Furthermore, we expected that species sorting would vary among species at different trophic levels, with richness of largest organisms (zooplankton) being the least strongly affected by resource heterogeneity. We also hypothesized that the dispersal of live versus dead organisms would alter both richness and composition of the local meta-ecosystem sites. One possible outcome is that killing the migrants would not allow species to disperse among local sites, and this might limit opportunities for species sorting and reduce overall diversity and potentially inhibit the development of β-diversity over time. Another possible outcome is that pulses of organic matter (and organisms) from outside the local sites could introduce new resources that would promote local diversity, leading to increases in both α- and γ-diversity. We also anticipated that the strength of such effects would vary with trophic position, as groups of organisms vary in their dispersal limitation, their niche variation among species, and their likelihood to be affected by interactions among trophic levels.

MATERIALS AND METHODS

Experimental design and setup

Using a mesocosm design (n=60), we studied the interactive effects of nutrient heterogeneity and dispersal regime within a meta-ecosystem. We created a 2x2 factorial block design (Fig. 4.1) by crossing two different nutrient loading regimes (homogenous (Ho) and heterogeneous (He) nutrient loading) with two different dispersal regimes (alive (A) and dead (D) dispersal). Different dispersal regimes were established in order to investigate how the dispersal of organic material and live organisms, in contrast to the dispersal of just organic material, affects community and ecosystem properties. The different nutrient loading regimes were established to create differences in niche spaces among patches within meta-ecosystems. Each meta-ecosystem replicate consisted of three 300L tanks (mesocosms), which were connected by bi-weekly manual dispersal. In total, the experiment thus consisted of 20 meta-ecosystems, divided into four different treatment combinations (5 replicate blocks): 1: alive dispersal and heterogeneous nutrient loading (A-He), 2: alive dispersal and homogenous nutrient loading (A-Ho), 3: dead dispersal and heterogenous nutrient loading, and 4: dead dispersal and homogenous nutrient loading (D-Ho). The mesocosm experiment ran for 20 weeks (May - September 2012) at the aquatic research institute Eawag in Kastanienbaum (Switzerland). Mesocosm were initially filled with unfiltered water from the nearby lake Lucerne.

The different nutrient conditions were established through bi-weekly additions of phosphorus (P), nitrogen (N) and dissolved organic carbon (DOC). Within homogenous meta-ecosystems, each mesocosm received the same amounts of P, N, and DOC, while in heterogeneous meta-ecosystems, we established a high nutrient (HN), a low nutrient (L), as well as a DOC mesocosm (Table 4.1, Fig. 4.1). The HN mesocosms received ten times as much N and P as both the L and the DOC mesocosms. The DOC mesocosms additionally received higher quantities of DOC leachate compared to the other tanks. While the loading of DOC also increased the loading of P and N (Table 4.1, average loadings were equal for all meta-ecosystems. DOC leachate was produced by soaking standard garden turf for several weeks and then filtering the leachate through a coarse net to remove large particles prior to the addition to the mesocosms. In sum, the homogeneous mesocosms received the same amount of nutrients and DOC as the three heterogeneous mesocosms, but here the amounts were split equally between tanks. To initiate the different nutrient conditions, each mesocosm received twice as much nutrients and DOC as added during the bi-weekly additions.

To establish different dispersal regimes, equal quantities of water (1L) were sampled every

second week from each tank within a meta-ecosystem (prior to the nutrient additions), mixed, and then re-distributed to in equal amounts (300mL) to each mesocosm. To kill the organisms within the dispersed water of the dead dispersal treatment, we autoclaved the water before re-distributing it between tanks. All tanks additionally received equal quantities of phytoplankton and zooplankton from lake Alpnach and lake Rot prior to the start of the experiment.

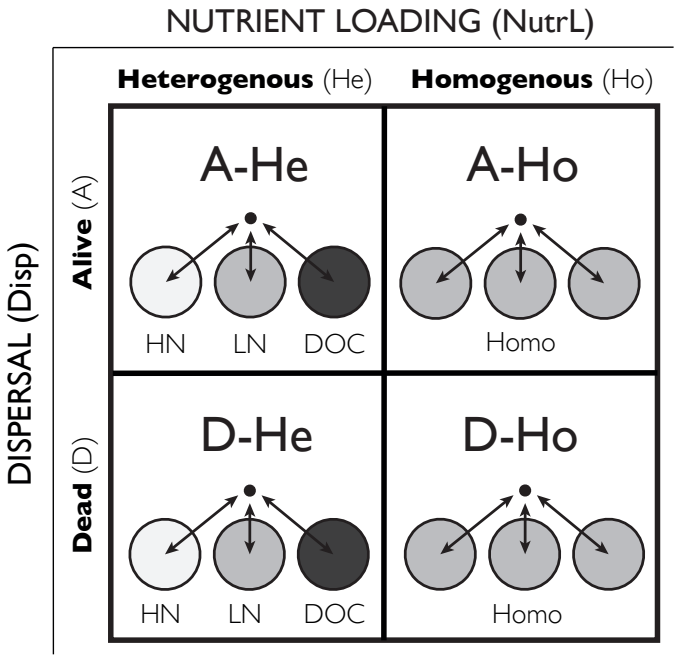

Figure 4.1: ***Experimental Design.*** *Design of the mesocosm experiment, showing the two nutrient loading regimes (heterogeneous and homogenous), the two dispersal regimes (alive and dead dispersal), as well as coding for the different treatments and contrasts.*

Mesocosm sampling

Over the course of the 20 week experiment, various biological, physical, chemical and ecosystem functioning parameters have been sampled and measured on a regular basis (Table 4.2). We performed ten bi-weekly samplings of algal biomass (Chlorophyll a), dissolved organic carbon (DOC) concentration and composition, as well as bacterial cell densities and cell size distributions. As our main interest was to investigate how the different treatments affected the bacterial, phytoplankton and zooplankton community after several weeks of nutrient additions and dispersal, we sampled these three communities, and measured several other parameters at the end of the experiment (sampling week 19, Table 4.2). The bacterial community was sampled by filtering mesocosm water onto 0.2μm polyethersulfone filters (Supor 200 Membrane Disc Filters), which were instantly frozen

Table 4.1: ***Experimental Design and nutrient loading.***
Summary table of the mesocosm design, dispersal, as well as nutrient and DOC additions to the mesocosm experiment. M: meta-ecosystem, Treat: treatment combination. (i): amounts/ concentrations in bi-weekly nutrient loadings, (ii): average amounts of nutrients within the DOC of the bi-weekly loadings, (iii): cumulative sum of nutrient loading over the 20-week experiment (both from DOC and N and P additions), (iv): Meta-ecosystem (M) sum of total nutrient loading to show, that each meta-ecosystem received the same total loading.

						(i) DOC/ Nutrient Loading			(ii) Avg. Nutr. in DOC Loading			(iii) Total Nutr. Loading			(iv)
Tank	M	Treat	Disp	NutrL	NutrID	DOC (L)	N (μg/L)	P (μg/L)	C (μg/L)	N (μg/L)	P (μg/L)	C (mg)	N (mg)	P (mg)	Sum of (iii)
1	1	A-He	Alive	Hetero	HN	0.5	800	50	103	0.82	0.03	310	2402	150	
2	1	A-He	Alive	Hetero	L	0.5	80	5	103	0.82	0.03	310	242	15	
3	1	A-He	Alive	Hetero	DOC	3.8	80	5	786	6.26	0.19	2357	259	15	6060
4	2	A-Ho	Alive	Homo	Homo	1.6	320	20	331	2.63	0.08	992	968	60	
5	2	A-Ho	Alive	Homo	Homo	1.6	320	20	331	2.63	0.08	992	968	60	
6	2	A-Ho	Alive	Homo	Homo	1.6	320	20	331	2.63	0.08	992	968	60	6060
7	3	D-He	Dead	Hetero	HN	0.5	800	50	103	0.82	0.03	310	2402	150	
8	3	D-He	Dead	Hetero	L	0.5	80	5	103	0.82	0.03	310	242	15	
9	3	D-He	Dead	Hetero	DOC	3.8	80	5	786	6.26	0.19	2357	259	15	6060
10	4	D-Ho	Dead	Homo	Homo	1.6	320	20	331	2.63	0.08	992	968	60	
11	4	D-Ho	Dead	Homo	Homo	1.6	320	20	331	2.63	0.08	992	968	60	
12	4	D-Ho	Dead	Homo	Homo	1.6	320	20	331	2.63	0.08	992	968	60	6060

in liquid nitrogen and preserved at -80 °C until further processing. Phytoplankton was sampled by sampling mesocosm water from the water column and preserved with Lugol's solution. Integrated zooplankton samples were taken from the water column, filtered through a 30 μm net, and stored frozen prior to analysis.

Table 4.2: ***Sampling schedule of the mesocosm experiment.***

			sampling week								
Metrics	**Parameter**	**Abbreviation**	1	3	5	7	9	11	13	15	17	19
Biological	Chlorophyll a (water)	Chla	x	x	x	x	x	x	x	x	x	x
	Chlorophyll a (Periphyton)	Chla P				x			x			x
	Microbial density	BaN	x	x	x	x	x	x	x	x	x	x
	Microbial cell size distribution	BactBig/BactSmall	x	x	x	x	x	x	x	x	x	x
Physical/ Chemical	DOC concentration	DOC	x	x	x	x	x	x	x	x	x	x
	DOC spectral slope	DOCaS	x	x	x	x	x	x	x	x	x	x
	DOC absorption a320	DOCa320	x	x	x	x	x	x	x	x	x	x
	Phosphate	PO_4	x				x					x
	Nitrogen	$NO_3.NO_2$										x
	Water temperature	WaterTemp		x			x	x				x
	Dissolved oxygen	DO		x				x				x
	Sedimentation rate	Sedim.				x			x			x
	pH	pH					x					x
	Conductivity	Cond					x					x
	PAR light extinction	PAR										x
Ecosystem Functions	Respiration (16°C)	Resp16	x				x					x
	Primary productivity	GPP, NPP, Resp		x				x				x
Biodiversity	Bacterial community	Bact										x
	Phytoplankton community	Phyto										x
	Zooplankton community	Zoop										x

Sample processing

Chlorophyll a (Chl-a) concentrations from the water column were determined chromatographically on a high performance liquid chromatograph (HPLC) from GF/F (Whatman) filtered water. For measuring the chlorophyll concentration of the periphyton community, we hung plastic strips into the tanks at the beginning of the experiment and cut a 50cm² after several weeks (see Table 4.2). Chlorophyll was extracted from the plastic strips using 90% ethanol and measured in the same way as the for the water samples. The filtrate from the chlorophyll filtrations was acidified and stored at 4°C for measuring DOC concentration on a Shimadzu TOC-V CPH, for recording a DOC absorption spectrum over the wavelengths of 190 to 800 nm spectrophotometrically, and for measuring the concentrations of phosphate (PO_4) and nitrogen (the sum of NO_3 and NO_4). To determine bacterial cell densities and cell size distributions flowcytometrically, 40 mL of tank water were fixed with a filter-sterilized solution of paraformaldehyde and glutaraldehyde (0.01% and 0.1%

final concentrations, pH 7; Nizzetto et al., 2012) and stored at 4°C until measurements were carried out on a BD Accuri^{TM} C6 Flowcytometer.

Conductivity, pH, temperature and conductivity were measured in situ at approximately 0.5 m water depth using portable water sensors. We estimated primary productivity be measuring the O_2 concentrations in each tank three times over the course of 24 hours (morning - evening - morning) and calculated gross and net primary productivity (GPP, NPP) and respiration (Resp) at the whole ecosystem level. We also measured respiration rates (Resp16) at controlled temperature conditions were additionally assessed in the lab using custom-build temperature controlled glass vials at 16°C and fiber-optic oxygen mini-sensors (FIBOX 3, PreSens). O_2 concentrations were measured every 30 seconds over the course of six hours and O_2 uptake rates were calculated per hour. We measured the sedimentation rate by hanging a 50mL Falcon tube into each tank and weighing the dried, deposited material after several weeks (see Table 4.2).

The bacterial community composition was analyzed using Illumina sequencing. DNA was extracted by enzymatic digestion and cetyltrimethyl ammonium bromide (CTAB) extraction using a modified protocol from Llirós et al. (2008). Ilumina sequencing and sequence analysis was performed as described previously (Chapter I). We have used the V3 region of the 16S rRNA for the analysis of bacterial diversity. Phytoplankton and zooplankton abundances were determined manually by stereo microscopy at the genus level. Phytoplankton taxa that occurred in less than three tanks were removed. The zooplankton community was divided into macro- and micro-zooplankton for statistical analysis, where the micro-zooplankton fraction consisted of rotifer species only.

Data analysis

All data was analyzed using the statistical software R (R Development Core Team, 2014). The sequencing data of the bacterial communities was analyzed using the bioinformatics pipeline QIIME (Kuczynski et al., 2011) prior to analyses in R, as described in (Chapter I). Sequences were clustered at a sequence similarity threshold of 97%, bacterial communities were rarefied to 325 OTUs (Operational Taxonomic Units) per mesocosm due to large variations in read counts. When meta-ecosystems parameters were analyzed, mesocosm were aggregated by meta-ecosystem using OTU sums.
Profile analysis (Fidell and Tabachnick, 2006) of the time-series data was performed for the meta-ecosystem mean and standard deviation of Chl-a concentrations, for various measures of DOC, bacterial abundance, and bacterial size distribution. All of these parameters were measured at each sampling point during the course of the experiment. The analysis

was conducted on the four cross-treatments (Treatment), as well as for the dispersal and the nutrient contrasts (Disp and NutrL).
We estimated the effect size by quantifying log response rations (LRR) of the nutrient (N) and dispersal manipulation (D), as well as their interaction (NxD) of the environmental data as well as the species abundance data using:

$$LRR_N=(\ln Y_{D\text{-}Ho} + \ln Y_{A\text{-}Ho})\text{-}(\ln Y_{D\text{-}He} + \ln Y_{A\text{-}He})$$
$$LRR_D=(\ln Y_{D\text{-}He} + \ln Y_{D\text{-}Ho})\text{-}(\ln Y_{A\text{-}He} + \ln Y_{A\text{-}Ho})$$
$$LRR_{NxD}=(\ln Y_{D\text{-}Ho} + \ln Y_{A\text{-}He})\text{-}(\ln Y_{A\text{-}Ho} + \ln Y_{D\text{-}He})$$

where Y is the mean response value among metacommunities. The significance of the effects for the mean and coefficient of variation of the environmental data was tested using linear mixed effect models with block as a random factor. The species abundance data was log-transformed prior to LRR calculation, and significance of the effect sizes was tested using either linear mixed effect models.
As a measure of local and regional species diversity, we calculated average α- and γ-diversity for each meta-ecosystem. Average α-diversity (richness) was calculated as the number of unique taxa/ OTUs for each mesocosm and then averaged across metacommunities, and γ-diversity as the number of unique taxa/ OTUs within each meta-ecosystem. We calculated average jaccard dissimilarity for each meta-ecosystem on the presence-absence data as a measure of β-diversity. As for the environmental data, we used linear mixed effect model statistics with block as a random factor to identity if the diversity indices were significantly affected by dispersal contrast, nutrient contrast, and the interaction of dispersal and nutrient regime. The species composition of bacteria, phytoplankton, and the two zooplankton communities was furthermore analyzed for treatment effects using distance based redundancy analysis (db-RDA) of both hellinger-transformed abundance and presence-absence data and run with random block effect. P-values were calculated for the model attributes using 10000 random permutations. The first two axes of the db-RDA have been plotted to visualize relationships between metacommunities.

RESULTS

Ecosystem dynamics through time

Even though all replicated meta-ecosystems received identical total amounts of both nutrients and DOC over the course of the 20 weeks experiment (Table 4.2), mean environmental parameters and spatial variability differed among our treatments. Over time, chlorophyll a (Chl-a) and DOC properties, as well as bacterial densities and the distribution of bacterial size classes, responded in different ways to both the spatial distribution of nutrient loading and the nature of dispersal (Fig. 4.2+4.3).

Meta-ecosystems with dispersal of living organisms had significantly higher levels of Chl-a (Fig. 4.2A, Table 4.3) and different temporal dynamics in the spatial variability of both Chl-a and DOC (Fig. 4.3A+C) among sites compared with meta-ecosystems receiving the dead dispersal treatment). Meta-ecosystems with different loading regimes had similar levels of Chl-a, but heterogeneous meta-ecosystems had significantly lower mean levels and different temporal dynamics of both DOC (Fig. 4.2D) and bacterial densities (Fig. 4.2F). The nutrient heterogeneity treatment also increased the variability of both DOC and Chl-a among meta-ecosystem sites, but the effect was much larger for DOC (Fig. 4.3B+D).

Table 4.3: ***Profile analysis results.***

		Mean		**Coefficient of variation**	
Test	**Comparison**	Levels (Treatment)	Parallelism (Time x Treatment)	Levels (Treatment)	Parallelism (Time x Treatment)
Chl-a	Treatment	**$F_{3,16}$ 5.20 (0.01)**	$F_{3,16}$ 0.14 (0.64)	$F_{3,16}$ 1.88 (0.17)	**$F_{3,16}$ 0.01 (<0.01)**
	Disp	**$F_{1,18}$ 10.85 (<0.01)**	$F_{1,18}$ 0.38 (0.19)	$F_{1,18}$ 0.55 (0.47)	**$F_{1,18}$ 0.07 (<0.01)**
	NutrL	$F_{1,18}$ 1.26 (0.28)	$F_{1,18}$ 0.48 (0.38)	**$F_{1,18}$ 5.02 (0.04)**	$F_{1,18}$ 0.30 (0.07)
DOC	Treatment	**$F_{3,16}$ 11.16 (<0.01)**	**$F_{3,16}$ 0.03 (0.04)**	**$F_{3,16}$ 53.89 (<0.01)**	$F_{3,16}$ 0.07 (0.24)
	Disp	$F_{1,18}$ 3.48 (0.08)	$F_{1,18}$ 0.62 (0.70)	$F_{1,18}$ 0.00 (0.96)	**$F_{1,18}$ 0.27 (0.05)**
	NutrL	**$F_{1,18}$ 18.83 (<0.01)**	**$F_{1,18}$ 0.22 (0.02)**	**$F_{1,18}$ 163.51 (<0.01)**	$F_{1,18}$ 0.36 (0.15)
DOCaS	Treatment	**$F_{3,16}$ 4.02 (0.03)**	**$F_{3,16}$ 0.01 (<0.01)**	**$F_{3,16}$ 23.34 (<0.01)**	$F_{3,16}$ 0.08 (0.30)
	Disp	$F_{1,18}$ 0.33 (0.57)	$F_{1,18}$ 0.32 (0.10)	$F_{1,18}$ 0.11 (0.74)	$F_{1,18}$ 0.63 (0.74)
	NutrL	**$F_{1,18}$ 12.59 (<0.01)**	**$F_{1,18}$ 0.22 (0.02)**	**$F_{1,18}$ 75.57 (<0.01)**	$F_{1,18}$ 0.34 (0.12)
DOCa320	Treatment	**$F_{3,16}$ 5.00 (0.01)**	$F_{3,16}$ 0.12 (0.53)	**$F_{3,16}$ 102.81 (<0.01)**	**$F_{3,16}$ 0.01 (<0.01)**
	Disp	$F_{1,18}$ 1.05 (0.32)	$F_{1,18}$ 0.60 (0.68)	$F_{1,18}$ 0.00 (0.96)	$F_{1,18}$ 0.60 (0.68)
	NutrL	**$F_{1,18}$ 13.27 (<0.01)**	$F_{1,18}$ 0.56 (0.56)	**$F_{1,18}$ 342.39 (<0.01)**	**$F_{1,18}$ 0.10 (<0.01)**
BaN	Treatment	**$F_{3,16}$ 5.84 (<0.01)**	$F_{3,16}$ 0.07 (0.09)	$F_{3,16}$ 1.95 (0.16)	$F_{3,16}$ 0.12 (0.32)
	Disp	$F_{1,18}$ 3.13 (0.09)	$F_{1,18}$ 0.67 (0.71)	$F_{1,18}$ 3.11 (0.09)	$F_{1,18}$ 0.66 (0.68)
	NutrL	**$F_{1,18}$ 10.35 (<0.01)**	**$F_{1,18}$ 0.25 (0.02)**	$F_{1,18}$ 0.93 (0.35)	$F_{1,18}$ 0.35 (0.08)
BactSmall	Treatment	$F_{3,16}$ 1.79 (0.19)	**$F_{3,16}$ 0.05 (0.04)**	$F_{3,16}$ 1.73 (0.20)	$F_{3,16}$ 0.27 (0.87)
	Disp	$F_{1,18}$ 1.50 (0.24)	$F_{1,18}$ 0.66 (0.68)	$F_{1,18}$ 0.64 (0.43)	$F_{1,18}$ 0.78 (0.90)
	NutrL	$F_{1,18}$ 3.59 (0.07)	**$F_{1,18}$ 0.23 (0.01)**	$F_{1,18}$ 3.88 (0.06)	$F_{1,18}$ 0.47 (0.25)

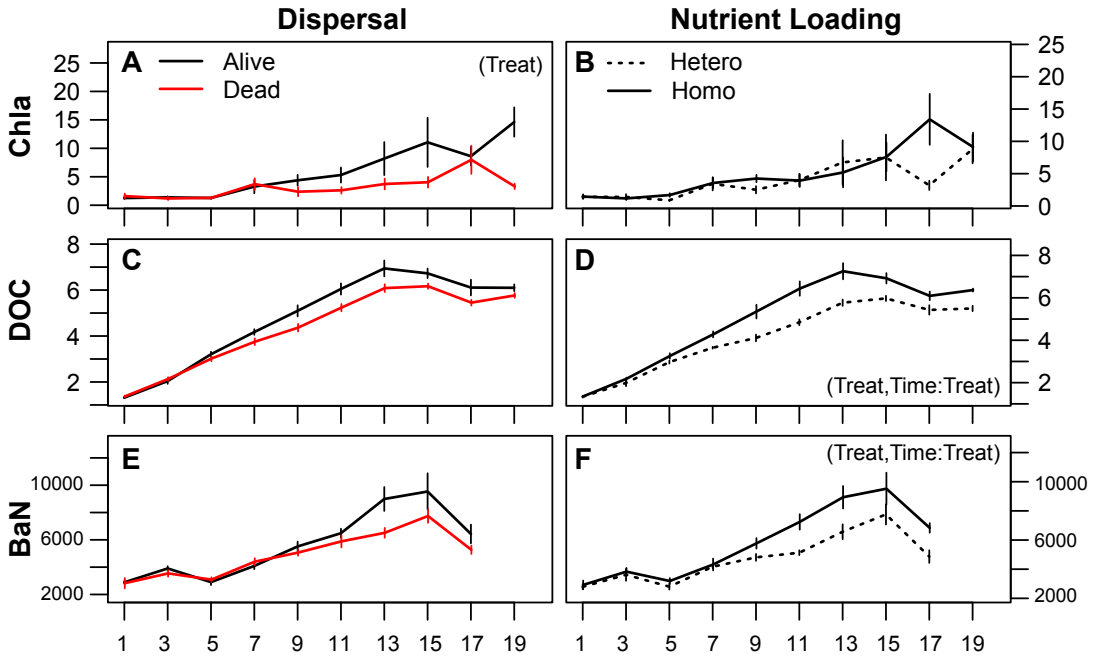

Figure 4.2: ***Time series plot of mean chlorophyll a (Chla), DOC concentrations (DOC), and bacterial numbers (BaN).*** *Mean meta-ecosystem changes over time in chlorophyll a concentrations [mg/L], DOC concentration [mg C/L] and bacterial numbers [cells/µL].*
A,C,E: data series aggregated by dispersal treatment (Alive - Dead), B,D,F: data aggregated by nutrient treatment (Homogenous - Heterogeneous).

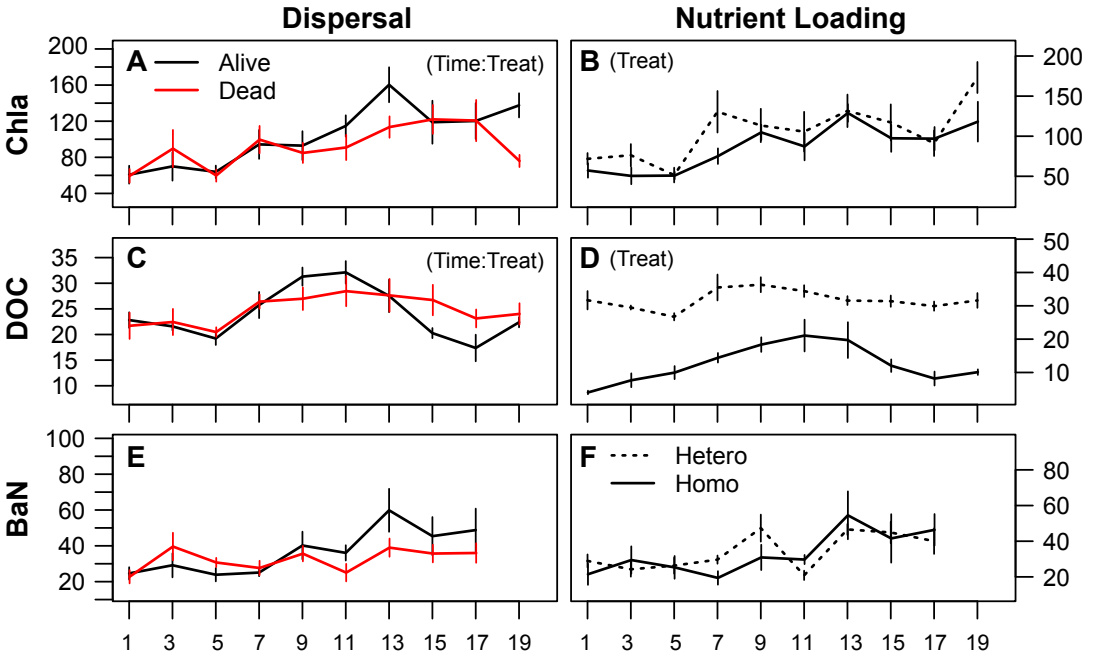

Figure 4.3: ***Time series plot of the coefficient of variation for chlorophyll a (Chla), DOC concentrations (DOC), and bacterial numbers (BaN).*** *Units and labels are the same as in Fig. 4.2.*

Ecosystem properties at the end of the experiment

Dispersal and nutrient loading regimes had significant effects on both the meta-ecosystem mean and the spatial variability (coefficient of variation) of various environmental parameters at the end of the experiment (Table 4.4, Fig. 4.4). For example, Chl-a was both more abundant (Fig. 4.4A) and more variable (Fig. 4.4B) in meta-ecosystems with live dispersal. Heterogenous nutrient loading led to increased mean nitrogen, conductivity, and the proportion of large bacteria, whereas homogenous nutrient loading resulted in higher mean pH and DOC concentrations. The heterogenous nutrient treatment increased the spatial variability of pH, sedimentation, DOC properties (DOC concentration and absorption in the UV range (DOCa320)), and of ecosystem functioning (respiration, GPP, and NPP). Interestingly, the spectral slope of DOC absorption (DOCaS) was affected by the interaction of nutrient loading and dispersal (Fig. 4.4A), suggesting that a key resource for bacterial growth, i.e. DOC, was modified by treatment combinations.

Table 4.4: ***Summary of ANOVA results from the last sampling (week 19) of the meta-ecosystem experiment.*** *F and p results for the effect of dispersal, nutrient loading, and interactive effects. Significant values are highlighted wild bold letters.*

	Mean			**Coefficient of variation**		
Parameter	Disp	NutrL	Interaction	Disp	NutrL	Interaction
DOC	3.90 (0.07)	**7.39 (0.02)**	0.91 (0.36)	0.19 (0.67)	**55.18 (<0.01)**	0.01 (0.94)
DOCaS	4.44 (0.06)	2.96 (0.11)	**7.94 (0.02)**	1.79 (0.21)	1.17 (0.30)	1.56 (0.24)
DOCa320	0.47 (0.51)	2.21 (0.16)	0.08 (0.78)	0.31 (0.59)	**50.24 (<0.01)**	0.00 (0.99)
Chla	**9.87 (0.01)**	0.36 (0.56)	0.91 (0.36)	**19.54 (<0.01)**	1.77 (0.21)	0.02 (0.89)
Chla P	0.06 (0.82)	3.89 (0.07)	0.02 (0.89)	0.58 (0.46)	0.15 (0.70)	0.55 (0.47)
BaN	2.69 (0.13)	3.69 (0.08)	0.21 (0.66)	0.16 (0.70)	3.04 (0.11)	3.67 (0.08)
BactSmall	3.80 (0.08)	**5.97 (0.03)**	0.00 (0.97)	0.01 (0.94)	0.86 (0.37)	0.64 (0.44)
PO4	1.81 (0.20)	0.81 (0.39)	0.04 (0.85)	1.80 (0.20)	4.17 (0.06)	0.89 (0.36)
NO3.NO2	2.46 (0.14)	**14.35 (<0.01)**	0.05 (0.83)	0.04 (0.84)	0.18 (0.68)	0.33 (0.58)
GPP	1.80 (0.21)	0.84 (0.38)	0.00 (0.98)	0.22 (0.64)	**12.14 (<0.01)**	2.39 (0.15)
NPP	1.94 (0.19)	0.67 (0.43)	0.00 (0.96)	0.00 (0.99)	**6.63 (0.02)**	2.22 (0.16)
Resp	1.45 (0.25)	0.92 (0.36)	0.01 (0.92)	0.52 (0.48)	**7.12 (0.02)**	1.49 (0.25)
Resp16	1.11 (0.31)	0.12 (0.74)	0.43 (0.84)	0.38 (0.55)	1.05 (0.33)	0.07 (0.80)
Sedim.	0.42 (0.53)	0.07 (0.80)	0.06 (0.81)	0.25 (0.63)	**9.22 (0.01)**	0.34 (0.57)
PAR	0.05 (0.82)	0.29 (0.60)	0.26 (0.62)	0.03 (0.86)	4.33 (0.06)	0.29 (0.60)
pH	0.01 (0.94)	**5.93 (0.03)**	0.13 (0.73)	0.29 (0.60)	**5.08 (0.04)**	1.80 (0.20)
Cond	1.02 (0.33)	**15.89 (<0.01)**	0.03 (0.86)	0.66 (0.43)	0.27 (0.61)	0.40 (0.54)
WaterTemp	1.56 (0.24)	0.01 (0.92)	1.13 (0.31)	0.12 (0.73)	0.18 (0.68)	0.01 (0.92)

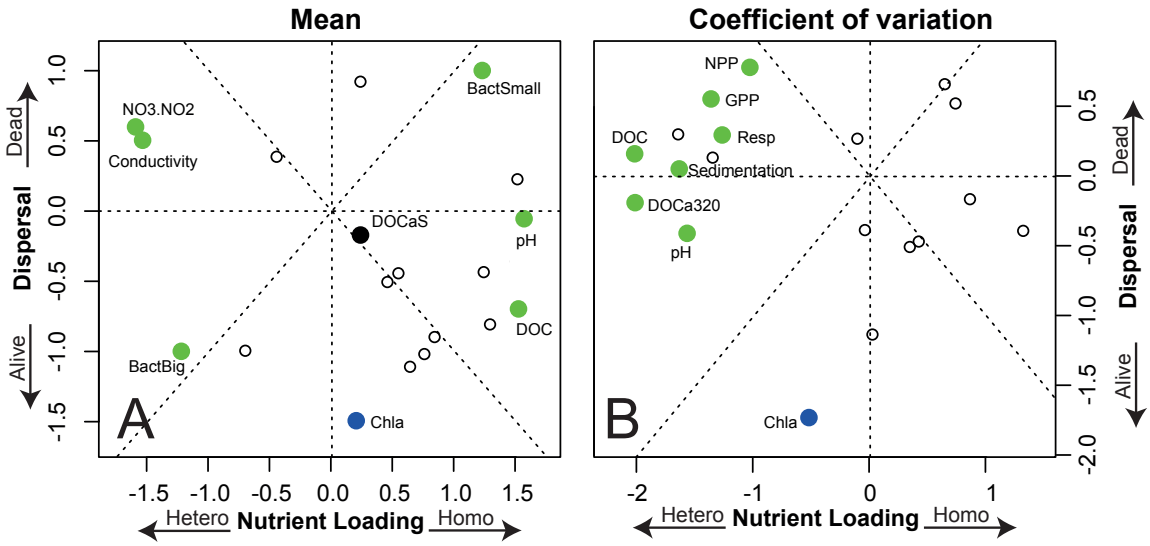

Figure 4.4: ***Effect size (log response rations LRR) and corresponding linear mixed effect results of the environmental parameters.*** *Environmental parameters with significant LME statistics displayed by colored, labeled dot. Green dot: significant nutrient effect, blue dot: significant dispersal effect, black dot: significant interaction. A: LRR/LME of meta-ecosystem mean values, B: LRR/LME of meta-ecosystem coefficient of variation.*

Diversity and community composition

The spatial structure of biodiversity was affected by dispersal, nutrient loading, and their interaction, but responses varied among trophic groups (Table 4.5, Fig. 4.5), with greatest observed effects on small organisms (bacteria and phytoplankton). Both average α- and γ-diversity of the bacterial community were higher in meta-ecosystems which received the dispersal of live organisms (Fig. 4.5A+E), but mean community dissimilarity (β-diversity) did not differ significantly among treatments (Fig. 4.5I). Phytoplankton γ-diversity and β-diversity were significantly higher in meta-ecosystems with heterogenous sites and live dispersal (Fig. 4.5F), and both average α-diversity was interactively affected by nutrient loading and dispersal regime (Fig. 4.5B). Phytoplankton communities were most dissimilar in homogenous tanks with live dispersal and in heterogenous tanks with dead dispersal (Fig. 4.5J). Differences in α-diversity between dead and alive dispersal, as well as the nutrient addition contrasts, were particularly pronounced for heterogenous meta-ecosystems (Fig. S1, Table S1). Average α- and γ-diversity of both rotifer (micro-zooplankton) and macro-zooplankton communities were not affected by dispersal and nutrient loadings (Fig. 4.5C,D,G,H), but rotifer β-diversity was interactively affected by the treatment combinations with communities being most similar in homogenous meta-ecosystems receiving the alive dispersal treatment (Fig. 4.5L).

Treatments had strong effects on the community composition of bacteria and phytoplankton (Table 4.6, Fig. 4.6, Fig. S2). The bacterial community composition was significantly affected by dispersal and nutrient loading regimes when comparing species identities, based on db-RDA (presence/absence data, Fig. 4.6A), and by nutrient loading when comparing species abundances (Fig.S2A). Nutrient loading had significant effects on the community composition of the phytoplankton community based on species identities (Fig. 4.6B). The community composition of zooplankton taxa from both size classes (macro- and micro-zooplankton) was not significantly different among meta-ecosystems (Fig. 4.6C+D, Fig. S2C+D). Nutrient identity, i.e. resource loading regime (HN, L, DOC, Homo), significantly affected the community composition at all trophic levels (Table 4.7, Fig. 4.7, Fig. S3).

Table 4.5: ***Summary of ANOVA results from the last sampling (week 19) of the meta-ecosystem experiment.*** *F and p results for the effect of dispersal (Disp), nutrient loading (NutrL), and interactive effects (Interaction). Significant values are highlighted wild bold letters.*

		F and p values of ANOVA analysis		
Metrics	**Parameter**	**Disp**	**NutrL**	**Interaction**
Bacteria	α-diversity	8.07 (**0.01**)	0.31 (0.59)	1.27 (0.28)
	γ-diversity	7.54 (**0.02**)	0.61 (0.45)	0.62 (0.45)
	β-diversity (Presence/Absence)	0.31 (0.59)	2.74 (0.12)	1.65 (0.22)
	β-diversity (Abundance)	3.36 (0.09)	2.62 (0.13)	0.55 (0.47)
Phytoplankton	α-diversity	12.45 (<**0.01**)	14.82 (<**0.01**)	5.15 (**0.04**)
	γ-diversity	6.77 (**0.02**)	13.44 (<**0.01**)	2.02 (0.18)
	β-diversity (Presence/Absence)	6.12 (**0.03**)	2.56 (0.14)	8.43 (**0.01**)
	β-diversity (Abundance)	0.17 (0.69)	0.19 (0.67)	7.05 (**0.02**)
Macro-Zooplankton	α-diversity	0.07 (0.80)	0.00 (1.00)	0.41 (0.54)
	γ-diversity	0.05 (0.83)	0.43 (0.53)	0.21 (0.65)
	β-diversity (Presence/Absence)	0.61 (0.45)	2.77 (0.12)	0.01 (0.92)
	β-diversity (Abundance)	2.44 (0.14)	2.45 (0.14)	3.30 (0.09)
Micro-Zooplankton (Rotifers)	α-diversity	1.88 (0.20)	0.26 (0.62)	0.30 (0.60)
	γ-diversity	2.89 (0.11)	2.89 (0.11)	1.79 (0.21)
	β-diversity (Presence/Absence)	0.00 (0.99)	5.52 (**0.04**)	1.85 (0.20)
	β-diversity (Abundance)	0.03 (0.86)	1.54 (0.24)	0.08 (0.79)

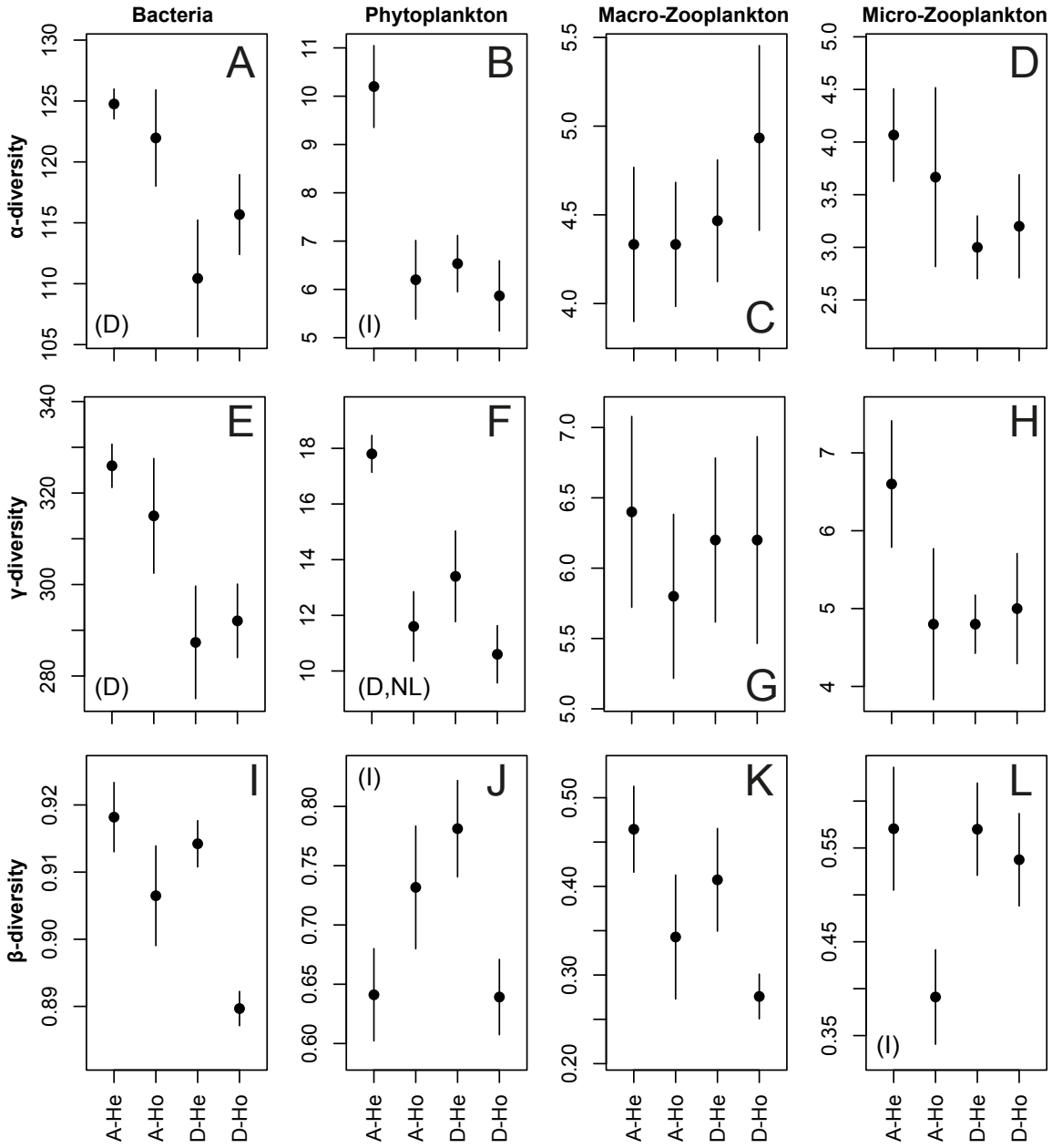

Figure 4.5: ***Diversity estimates of the bacterial, phytoplankton and zooplankton community.*** *Numbers indicate significant LME statistics. (1): significant dispersal effect, (2): significant nutrient effect, (3): significant interaction. A-D: Average meta-ecosystem α-diversity, E-H: meta-ecosystem γ-diversity, I-L: Average meta-ecosystem Jaccard dissimilarity (β-diversity).*

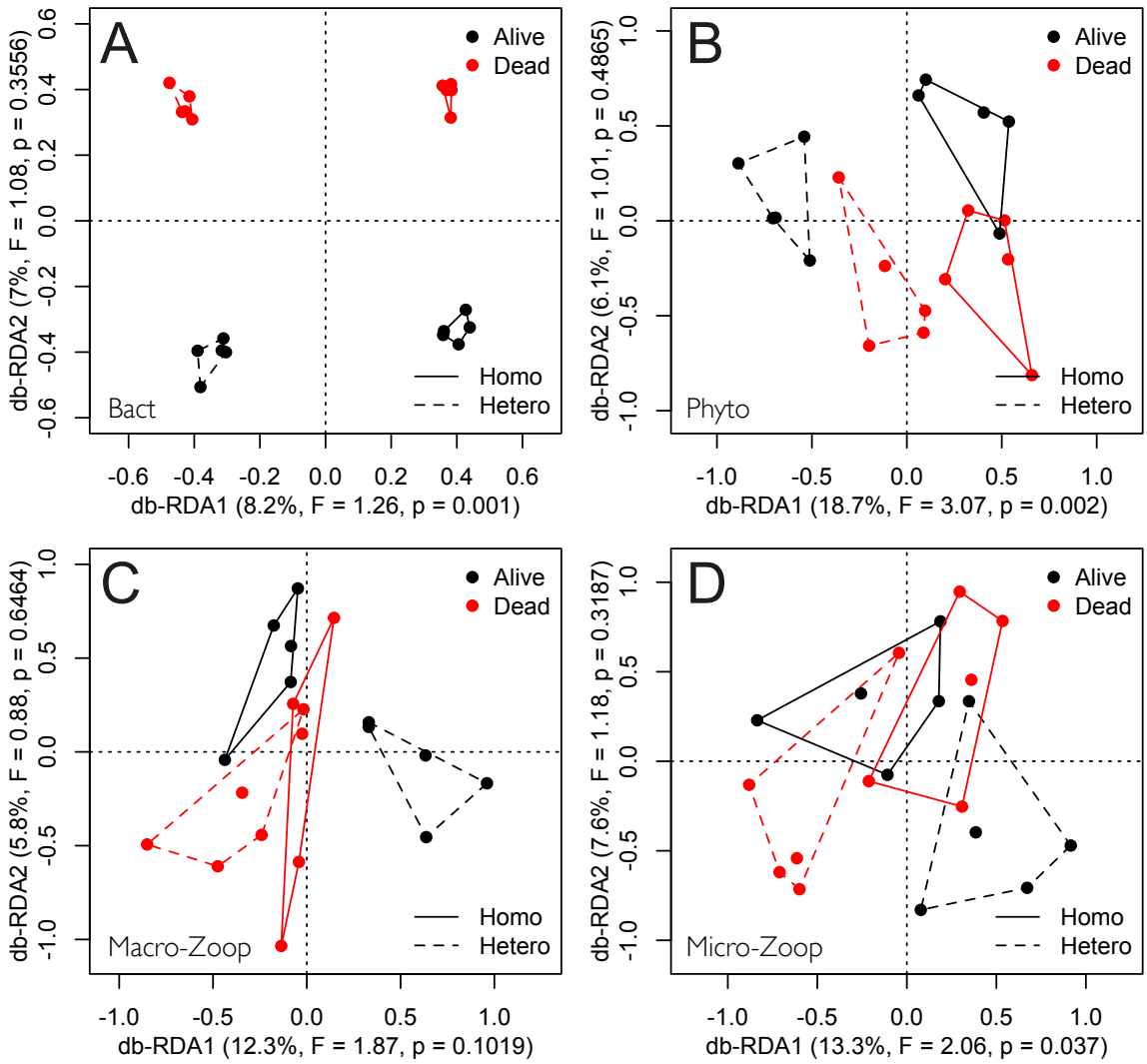

Figure 4.6: ***db-RDA of Dispersal x Nutrient Loading using presence-absence (showing the first two axes).*** *A: Bacterial community, B: Phytoplankton, C: Macro-Zooplankton, D: Micro-Zooplankton (Rotifers).*

Table 4.6: ***Results of db-RDA: Dispersal (Disp) x Nutrient Loading (NutrL).***

Test	Comparison	Abundance	Presence-Absence
Bacteria	Disp	$F_{1,12}$ 1.14 (0.07)	**$F_{1,12}$ 1.08 (0.05)**
	NutrL	**$F_{1,12}$ 1.75 (<0.01)**	**$F_{1,12}$ 1.26 (<0.01)**
	Disp:NutrL	$F_{1,12}$ 0.96 (0.60)	$F_{1,12}$ 0.98 (0.70)
Phytoplankton	Disp	$F_{1,12}$ 1.47 (0.15)	$F_{1,12}$ 1.33 (0.19)
	NutrL	$F_{1,12}$ 1.23 (0.25)	**$F_{1,12}$ 2.54 (0.01)**
	Disp:NutrL	$F_{1,12}$ 0.89 (0.52)	$F_{1,12}$ 0.55 (0.91)
Macro-Zooplankton	Disp	$F_{1,12}$ 1.39 (0.18)	$F_{1,12}$ 1.14 (0.33)
	NutrL	$F_{1,12}$ 0.83 (0.59)	$F_{1,12}$ 0.73 (0.69)
	Disp:NutrL	$F_{1,12}$ 1.55 (0.12)	$F_{1,12}$ 1.34 (0.21)
Micro-Zooplankton (Rotifers)	Disp	$F_{1,12}$ 0.54 (0.83)	$F_{1,12}$ 0.57 (0.84)
	NutrL	$F_{1,12}$ 0.78 (0.59)	$F_{1,12}$ 1.19 (0.29)
	Disp:NutrL	$F_{1,12}$ 2.12 (0.06)	$F_{1,12}$ 1.78 (0.08)

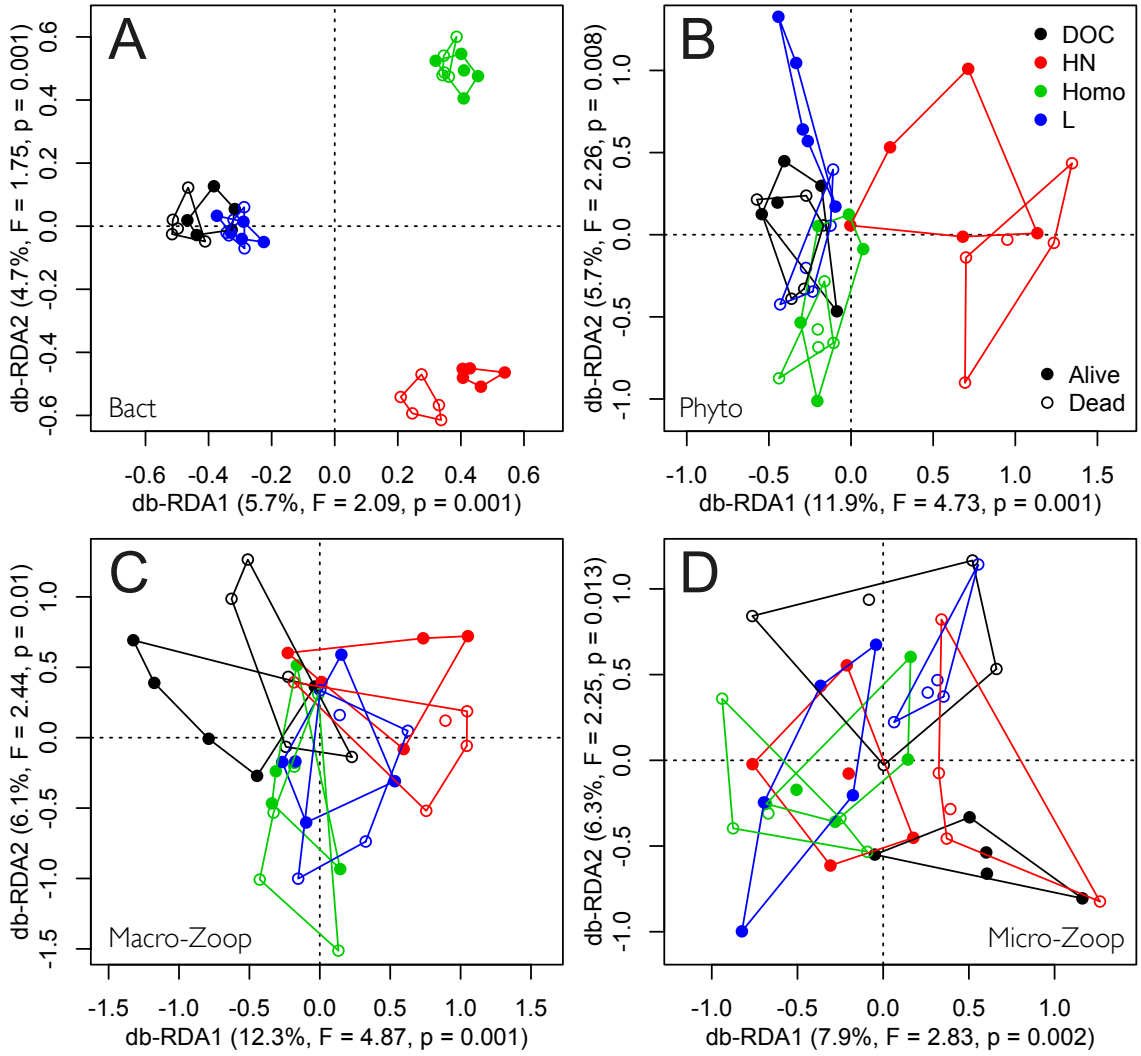

Figure 4.7: ***db-RDA of Dispersal x Nutrient identity (NutrID) using presence-absence data (showing the first two axes).*** *A: Bacterial community, B: Phytoplankton, C: Macro-Zooplankton, D: Micro-Zooplankton (Rotifers).*

Table 4.7: ***Results of db-RDA: Dispersal (Disp) x Nutrient identity (NutrID).***

Test	Comparison	Abundance	Presence-Absence
Bacteria	Disp	$F_{1,28}$ 1.12 (0.17)	$F_{1,28}$ 1.05 (0.17)
	NutrID	**$F_{3,28}$ 2.73 (<0.01)**	**$F_{3,28}$ 1.64 (<0.01)**
	Disp:NutrID	$F_{3,28}$ 0.99 (0.48)	$F_{3,28}$ 0.99 (0.59)
Phytoplankton	Disp	$F_{1,28}$ 1.38 (0.12)	**$F_{1,28}$ 1.77 (0.02)**
	NutrID	**$F_{3,28}$ 2.23 (<0.01)**	**$F_{3,28}$ 2.52 (<0.01)**
	Disp:NutrID	$F_{3,28}$ 1.00 (0.45)	$F_{3,28}$ 0.77 (0.90)
Macro-Zooplankton	Disp	**$F_{1,28}$ 2.31 (0.01)**	$F_{1,28}$ 1.37 (0.16)
	NutrID	**$F_{3,28}$ 1.90 (<0.01)**	**$F_{3,28}$ 2.41 (<0.01)**
	Disp:NutrID	$F_{3,28}$ 1.13 (0.24)	$F_{3,28}$ 1.05 (0.39)
Micro-Zooplankton (Rotifers)	Disp	$F_{1,28}$ 0.74 (0.71)	$F_{1,28}$ 0.93 (0.52)
	NutrID	**$F_{3,28}$ 2.13 (<0.01)**	$F_{3,28}$ 1.02 (0.44)
	Disp:NutrID	$F_{3,28}$ 1.26 (0.15)	$F_{3,28}$ 1.25 (0.16)

Taxon and OTU specific responses

Nutrient loading and dispersal regime both had significant effects on the abundances of individual taxa, respectively bacterial OTUs (Fig. 4.8). Of the 100 most abundant OTUs, 22 OTUs were significantly affected by either nutrient loading, dispersal, or both, as determined by linear mixed effect models (Fig. 4.8A). We found some clear patterns in the distribution of bacterial taxa with resource loading and dispersal regime. OTUs which were significantly more abundant in heterogeneous meta-ecosystems all belonged to the *Betaproteobacteria* class, which covered half of the significant OTUs (11 out of 22). OTUs which were associated with homogeneous meta-ecosystems included *Flavobaceria*, *Saprospirae*, *Chloroplasts*, *Verrucomicrobiae* and *Opitutae*. *Alphaproteobacteria* were significantly affected by dispersal regime. By determining the average meta-ecosystem rank of the Top100 OTUs (Fig. 4.9), we also found that L and DOC communities, which were both low nutrient tanks, were more similar to each other compared to the HN and Homo communities, as large rank changes were observed between L/DOC and HN/Homo, but not between L and DOC mesocosms. The low nutrient bacterial communities, as well as HN and Homo communities were also more similar at the bacterial class level (Fig. S5). For example, *Betaproteobacteria* were more abundant in the low nutrient tanks and covered over 60% of all bacterial OTUs, whereas bacterial classes were more evenly distributed across the HN and Homo tanks. The abundance of several phytoplankton taxa was affected by nutrient loading and dispersal regime (Fig. 4.8B). Nutrient loading affected the abundance of *Ankyra* and *Oocystis*, whereas *Ankyra* was more abundant in homogenous and *Oocystis* in heterogeneous meta-ecosystems. *Kirchnerriella* showed higher abundances in alive dispersal meta-ecosystems, and *Mougeotia*, *Scenedesmus*, *Elakatothrix*, *Tetraedon*, and *Coelastrum* were more abundant in heterogenous systems receiving the alive dispersal. Zooplankton taxa were also affected by dispersal and nutrient loading, but the pattern was less clear compared to the bacterial and phytoplankton community (Fig. 4.8C). *Chironomids* were found more frequently in heterogenous meta-ecosystems, while *Scapholeberis sp.* was more abundant in meta-ecosystems with dead dispersal. *Daphnia* was affected by both dispersal and nutrient loading, being more abundant in homogenous systems receiving the dead dispersal treatment. *Alona sp.*, *Polyarthra* and *Cyclopoids* were all affected by an interaction of the two treatment regimes. All of these three were more abundant in meta-ecosystems with alive dispersal, whereas *Cyclopoids* were more abundant in homogenous mesocosms, and *Alona sp.* and *Polyarthra* in heterogenous mesocosms.

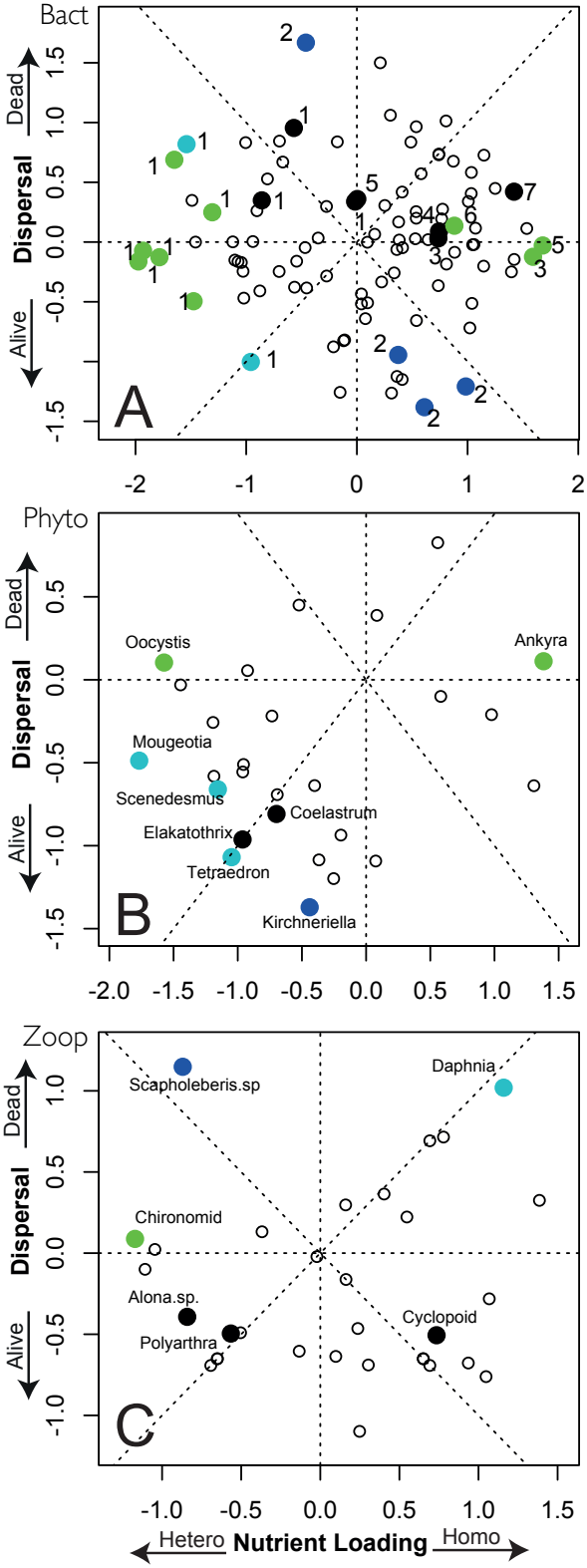

Figure 4.8: ***Effect size (log response rations LRR) and corresponding linear mixed effect results of individual taxa/ OTUs.*** *Taxa/ OTUs with significant LME statistics displayed by colored, labeled dot. Green dot: significant nutrient effect, blue dot: significant dispersal effect, turquoise dot: significant nutrient and dispersal effect, black dot: significant interaction. A: Bacterial community (100 most abundant OTUs). Numbers show class affiliation of significant OTUs : 1-Betaproteobacteria, 2-Alphaproteobacteria, 3- Flavobacteriia, 4-[Saprospirae], 5-Chloroplasts, 6-Verrucomicrobiae, 7-Opitutae; B: Phytoplankton community; C: Zooplankton community*

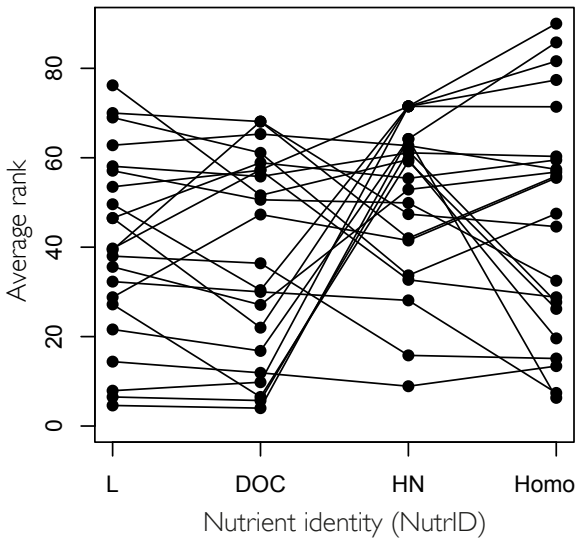

Figure 4.9: ***Rank-change of the 100 most abundant OTUs between different nutrient identities.*** *Low ranks indicate hight abundances, high ranks low abundances.*

DISCUSSION

Meta-ecosystem theory is an important extension of meta-community theory and, through the integration of material fluxes between patches, might lead to a better understanding of the spatial structure of ecosystems and organisms. Classical meta-community theory has already investigated the spatial structure of community dynamics in response to environmental heterogeneity (Declerck et al., 2012), grazing (Berga et al., 2014) or trophic structure (De Bie et al., 2012; Verreydt et al., 2012). In our mesocosm experiment, we additionally investigated the importance of meta-ecosystem fluxes in spatially structured environments, by dispersing either live organisms or killing the organisms prior to dispersal. We found that not only the fluxes of nutrients and organic material, but also the dispersal of organisms shaped community and environmental responses. The responses varied depending on environmental heterogeneity and trophic position of the dispersing organisms. Dispersal affected bacterial and phytoplankton diversity and community composition, while heterogeneity affected phytoplankton diversity and the composition of both of these communities. Although zooplankton diversity and community composition did not respond significantly to the two treatments, individual species were more commonly affiliated with certain treatment combinations.

Environmental responses

Our results suggest that the spatial structure of nutrient loading in meta-ecosystems can dramatically affect both the temporal dynamics (Fig. 4.2) and spatial variability of envi-

ronmental conditions (Fig. 4.3+4.4), with potential effects on biodiversity structure. As expected, many environmental parameters showed greater spatial variability in response to heterogeneous nutrient loading (Fig. 4.4B). In theory, such environmental gradients should allow species sorting to occur across multiple trophic levels (Whittaker, 1962; Leibold et al., 1997). However, multiple environmental conditions also differed in their mean values between treatment combinations (Fig. 4.4A), even though the total nutrient loading was equivalent in both homogenous and heterogenous meta-ecosystems. Many biological processes that cause imbalances in utilization of resources among sites could lead to changes in mean conditions despite similar loading at the meta-ecosystem scale. Differences in nutrient availability (e.g. N, P) can affect ecosystem productivity (Elser et al., 2007) which in turn can affect the cycling of nutrients in a system and can cause divergence in mean environmental parameters. Nutrient availability can also affect physical characteristics of the system, such as light penetration, which can be caused by variations in DOC concentrations (Pace and Cole, 2002) or algae biomass. For example, species rich algal communities have different resource use efficiencies (Ptacnik et al., 2008) compared to species poor communities. If species responded independently to these mean and spatially structured environmental conditions among meta-ecosystems, this could affect biodiversity patterns both within individual sites, as well as across meta-ecosystem scales.

Community responses

Meta-ecosystem and meta-community theory make different predictions about the mechanisms structuring of biodiversity, as the former concept additionally considers how fluxes of materials are interacting with dispersal of organisms among sites. For example, spatial fluxes of non-living materials (such as nutrients and organic matter) in meta-ecosystems can affect source-sink dynamics and cause sinks to be become sources and vice versa (Gravel et al., 2010a). One possible mechanism is that nutrient fluxes among patches can alter coexistence of species that vary in their competitive and dispersal abilities (Gravel et al., 2010b). In our experiment, the movement of living organisms strongly affected both richness of bacteria and algae (Fig. 4.5), as well as the abundance and temporal dynamics of algae and DOC (Fig. 4.2,4.3,4.4). This implies that the spatial structure of diversity (Fig. 4.5) and community composition (Fig. 4.6+S2, Table 4.6) at lower trophic levels in meta-ecosystems, which experience the same total flux of total organic matter, is nevertheless strongly influenced by the meta-community dynamics (i.e. the movement of living organisms).

Meta-community theory generally assumes that patch heterogeneity is fixed over time, whereas by considering flows of materials between patches, which can also affect temporal variations of environmental heterogeneity, the meta-ecosystem perspective allows for more complex dynamics of niche variation. Heterogenous environments might increase species richness, if the movement of materials opens up new niches or facilitates coexistence. However, if the material fluxes homogenize environments then this might decrease richness over time. Heterogenous environments may also create patches with different productivities, where some patches are net exporters of nutrients and act as keystone ecosystem patches, while other ecosystem patches act as net sinks (Mouquet et al., 2013). We found interactive effects of dispersal and nutrient heterogeneity on phytoplankton (Fig. 4.5B) and micro-zooplankton diversity (Fig. 4.5L), and of nutrient heterogeneity on the composition of bacteria and phytoplankton (Fig. 4.6). Within our heterogeneous treatment, species composition differed strongly among the nature of nutrient loading (i.e. HN, L, DOC; Fig. 4.7), and across all communities, some species were more common in either homogeneous or heterogeneous environments (Fig. 4.8). Interestingly, the distributions of some species were affected by dispersal and its interaction with meta-ecosystem heterogeneity, suggesting evidence of niche differentiation of species within all trophic levels (Fig. 4.8). Although the specific mechanism underlying such effects are still uncertain, one possibility is that the interactive effects of heterogeneity and dispersal on both average (e.g. Chl-a, and DOC absorbance spectra) and spatial variability (Chl-a) of meta-ecosystem conditions influence species distribution patterns.

The relative importance of dispersal and heterogeneity in meta-ecosystems differs among groups of organisms, and can be affected by parameters such as dispersal abilities, body size, niche width, and trophic interactions. The relationship between body size and dispersal capacities has gained a lot of attention (Jenkins et al., 2007), because of its importance in determining biogeographical patterns of species distributions. De Bie et al. (2012) recently performed an observational study across trophic levels in aquatic communities and found that dispersal limitation increases with body size for passive dispersers, while for active dispersers, the dispersal mode and connectivity between patches determines dispersal capacities. Small organisms, such as prokaryotes and small eukaryote species, are considered to be less dispersal limited (Fenchel and Finlay, 2004; Finlay, 2002). Their large population sizes and short generation times presumably allows them to adapt rapidly to environmental changes (Korhonen et al., 2010). However, in our study we found a strong effect of dispersal regime on the richness of small organisms (bacteria and phytoplankton), where live dispersal significantly increased local and regional richness,

while larger organisms (zooplankton) showed no clear signal of dispersal limitation, as richness was equivalent at both dispersal treatments. Our results, thus, suggest, that bacterial and phytoplankton communities were either dispersal limited or limited in some way by what is being dispersed (for example grazers or competitors) within the experimental setup. As for the zooplankton richness, it is possible that either the small dispersal volumes were not sufficient to disperse zooplankton species within meta-ecosystems, or that persistence of zooplankton was not strongly influenced by dispersal treatments, or that richness is not sensitive enough to detect compositional effects of dispersal.

Another important factor for structuring communities in meta-ecosystem is the degree of niche differentiation among different individuals, species, populations and trophic levels (Leibold et al., 1997; Bolnick et al., 2003, 2007). Niche theory originally describes the degree of species specialization in an n-dimensional space (Hutchinson, 1957), and was later formalized to include how organisms both respond to and impact their environment (Leibold, 1995). The fundamental and realized niches may differ among species depending on the surrounding conditions, for example due to competition for space and resources, or predation pressure (Vandermeer, 1972), with generalist species being less restricted in niche requirements possessing larger fundamental niche spaces compared to specialist species (Devictor et al., 2010). Furthermore, environmental heterogeneity can increase niche specialization (Büchi and Vuilleumier, 2014). Organisms at higher trophic levels might also tend to be more generalist and have wider niches than smaller taxa (Farjalla et al., 2012). In our experiment we manipulated environmental heterogeneity, but the effects on richness will depend on how niche variation among species aligns with the environmental gradients we imposed. We found that heterogeneity increased richness of the phytoplankton community, consistent with strong niche differentiation within the phytoplankton community (Fig. 4.6). Nevertheless, there was evidence of niche differentiation at all trophic levels based on community composition (Fig. 4.7), highlighting the importance of niche differentiation across all studied trophic groups. However, the fact that bacterial richness, unlike phytoplankton richness, was not increased in heterogeneous environments might be due to differential niche requirements. One possible explanation for this might be that the heterogenous environments, which we created in our experiment, harbored a larger number of different niches for the phytoplankton community, but less so for the bacterial community. This matches previous results suggesting that niche variation increases with organism body size (Farjalla et al., 2012). For bacteria, even the homogenous meta-ecosystems might have contained heterogenous properties and a wide

distribution of niche spaces, which might have generated variable responses in different phylogenetic and functional groups of bacteria. Bacterial community data furthermore (Fig. 4.6+4.7) underlines strong niche differentiation within the bacterial community within all treatments and nutrient identities, which might have been masked in the richness data.

Yet another important component of shaping diversity and community compositions is food web structure (Paine, 1966). Trophic interactions and differences between trophic groups have been integrated into meta-community theory, with the aim of understanding how various trophic levels are structured differently by spatial and environmental gradients (Beisner et al., 2006). Longmuir et al. (2007) has shown that the diversity and composition of zooplankton, phytoplankton, and bacteria is regulated independently from each other and that communities respond to different environmental parameters, while a recent study by Verreydt et al. (2012) points out the importance of trophic interactions for dispersal limitations. We did not directly manipulate trophic interactions in this experiment, but we think that some of the observed patterns in community responses might be affected by trophic interactions that were altered by dispersal. For example, responses in phytoplankton densities (Fig. 4.2), diversity (Fig. 4.5), and community composition (Fig. 4.6A) are likely not only affected by dispersal, niche differentiation and body size, but also by predation of the zooplankton community and the availability of resources from lower trophic levels. Similarly, the bacterial community responses supposably interacted other trophic levels, e.g. through predation pressure (Berga et al. 2014; chapter II), resource competition and resource recycling. Further work is needed to disentangle the relative importance of trophic interactions and dispersal, possibly by size fractioning the dispersing community.

Response of individual taxa/ OTUs

Next to analyzing community responses of different trophic levels using richness and community composition data, we also analyzed how individual species across trophic levels responded to our experimental treatments. We did this because species specific responses might be masked by overall community responses. Across all trophic levels, certain taxa, respectively OTUs, were strongly associated with either one or both of out treatments (Fig. 4.8). Even though we cannot explain the mechanisms, we found it interesting that several OTUs of the *Betaproteobacterial* class were strongly associated with heterogeneous environments, while OTUs, that were significantly more abundant in homogenous meta-ecosystems belonged to several different classes (Fig. 4.8A).

Betaproteobacteria are often the dominant class in freshwater systems, constituting up to 70% of total cell counts, and also the most studied freshwater bacterial class (Newton

et al., 2011). Mesocosm experiments have shown that species of this class grow fast when high amounts of nutrients are available, and are prone to size-selective grazing (Newton et al., 2011). Another ubiquitous class in freshwater systems are *Alphaproteobacteria*, which are not as well studied as the *Betaprotoebacteria*, but research suggests that they are more competitive at low nutrient conditions and are more resistant to grazing compared to other bacterial classes (Newton et al., 2011). The high abundance of *Betaproteobacteria* specifically at our low nutrient treatments (L and DOC; Fig. S4) indicates that nutrient availability has not been the main driver of *Betaproteobacteria* abundance in our experiment. Other factors, such as grazing pressure or other ecosystem properties, which were not solely defined by nutrient additions, might have shaped species abundances within this class. *Alphaproteobacteria*, on the other hand, were less abundant at low nutrient conditions (Fig. S4), which also indicates that nutrient availability has not been the driving force for these groups of bacteria. Some OTUs from the *Alphaproteobacteria* class were significantly associated with dispersal regime, which could indicate that they possess traits which allowed them to be good dispersers in our experimental setup. In general, the applied nutrient loading regimes have been the most important shaping force of the bacterial communities (Fig. 4.9+S4), and previous studies have already suggested that habitat filtering can be a main driver of bacterial community assembly (Armitage et al., 2012; Cardinale et al., 2012b). Rank abundance and relative abundance distributions revealed high community similarities in L and DOC tanks (Fig. 4.9+S4), and community composition of these two treatments overlapped (Fig. 4.7+S3). Even though absolute nutrient concentrations might not have been the governing force, other environmental properties, which were affected by the nutrient loading regimes could have created the observed patterns. Analyzing the bacterial community at lower taxonomic resolutions, and comparing differences in environmental parameters between heterogenous tanks might help explain this pattern. As richness and community composition data already revealed, the phytoplankton richness and community composition differed strongly among treatments (Fig. 4.6+S4). Several phytoplankton taxa were also affected by both dispersal and environmental heterogeneity, which might indicate that the dispersal volumes were high enough for phytoplankton taxa to find their preferred niche spaces and that heterogenous environments contained a larger amount of niche variation among species, which could sustain larger regional diversity. Individual zooplankton taxa were also affected by treatment combinations, but patterns were not as clear as for the bacterial and phytoplankton communities. It is possible, that individual zooplankton species responded to both treatment effects and

trophic interactions.

CONCLUSION

Our experiment has been one of the first to test for the importance of material fluxes for meta-community dynamics. We have shown both resource heterogeneity and dispersal regime can affect the diversity and community composition in spatially structured environments, and that responses varied between trophic levels. Using a meta-ecosystem design, in which we could limit suppress the movement of organisms, and high-throughput sequencing, which allows for a high resolution of the bacterial community, we observed that bacterial richness was strongly affected by the movement of live organisms. This indicates that bacterial communities were strongly limited by space or other resident organisms (for example through through priority effects) and less affected by the environmental heterogeneity imposed with our treatments. Overall, this highlights the importance of both biogeography and local environmental conditions (Lindström and Langenheder, 2011). By integrating community responses of three different trophic levels we have furthermore shown that each community exhibited distinct responses to meta-ecosystem dynamics.

ACKNOWLEDGMENTS

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APPENDIX

Supplementary Tables

Table S1: ***Summary of ANOVA results from the last sampling (week 19) of the meta-ecosystem experiment.*** *F and p results for the effect of dispersal (Disp), nutrient identity (NutrID), and interactive effects. Significant values highlighted wild bold letters.*

	F and p values of ANOVA analysis		
Metrics	**Disp**	**NutrID**	**Interaction**
Bacteria	1.73 (0.20)	0.42 (0.74)	0.65 (0.59)
Phytoplankton	**7.10 (0.01)**	**8.68 (<0.01)**	1.26 (0.31)
Macro-Zooplankton	0.00 (1.00)	1.89 (0.16)	0.53 (0.67)
Micro-Zooplankton	2.91 (0.10)	0.27 (0.85)	0.54 (0.66)

Supplementary Figures

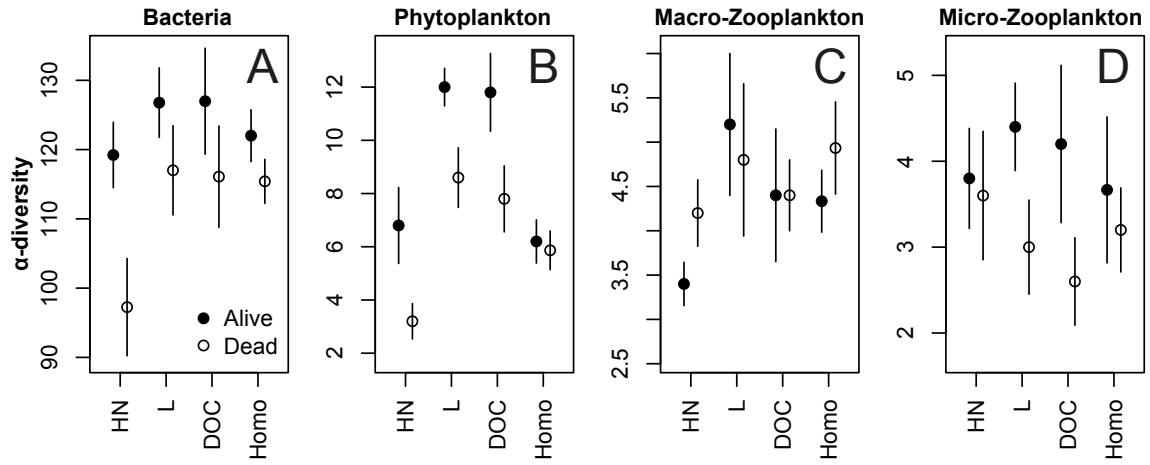

Figure S1: ***Average α-diversity for the different nutrient identities (NutrID).***

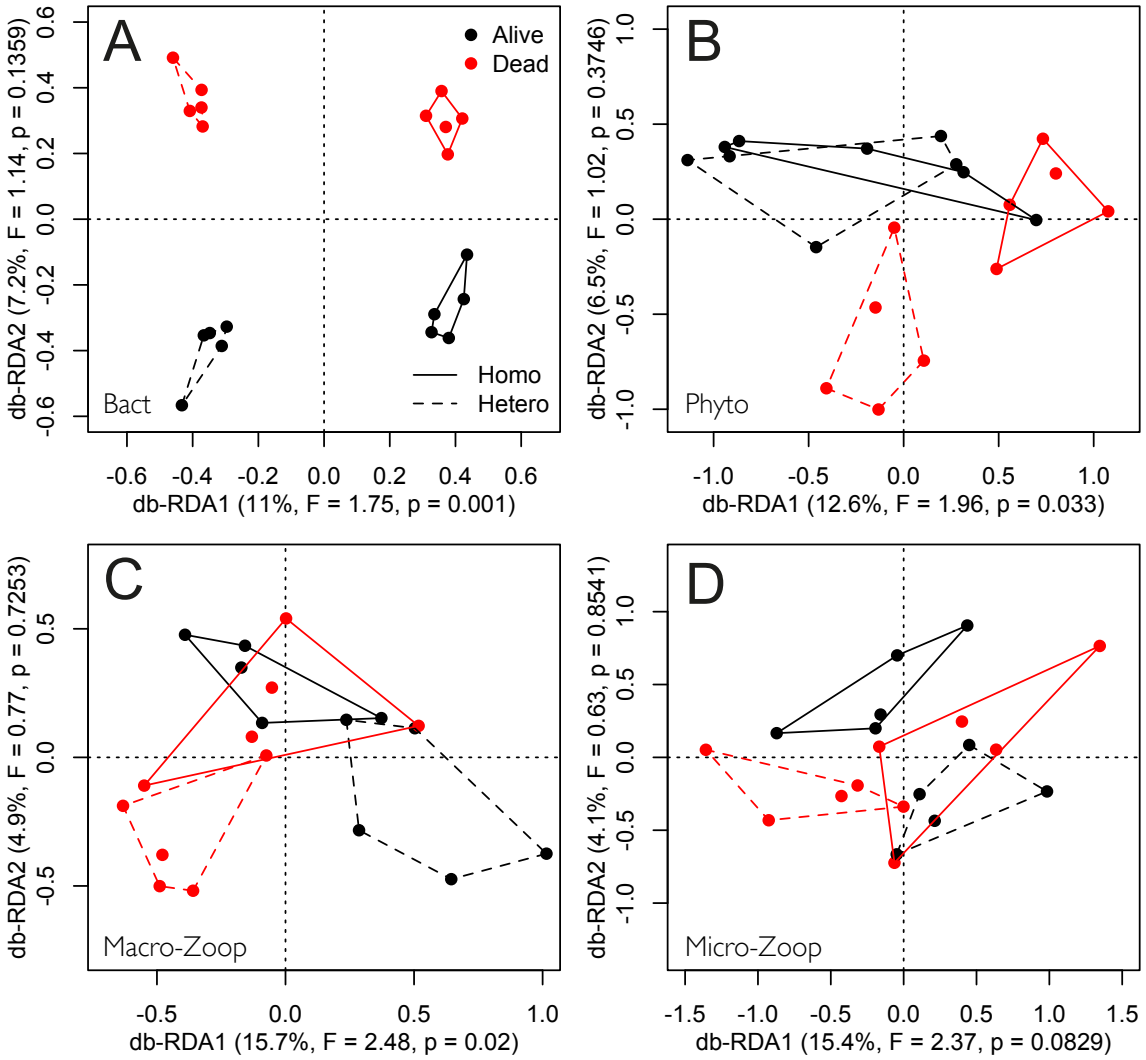

Figure S2: ***db-RDA of Dispersal x Nutrient Loading using abundance data (showing the first two axes).*** *A: Bacterial community, B: Phytoplankton, C: Macro-Zooplankton, D: Micro-Zooplankton (Rotifers).*

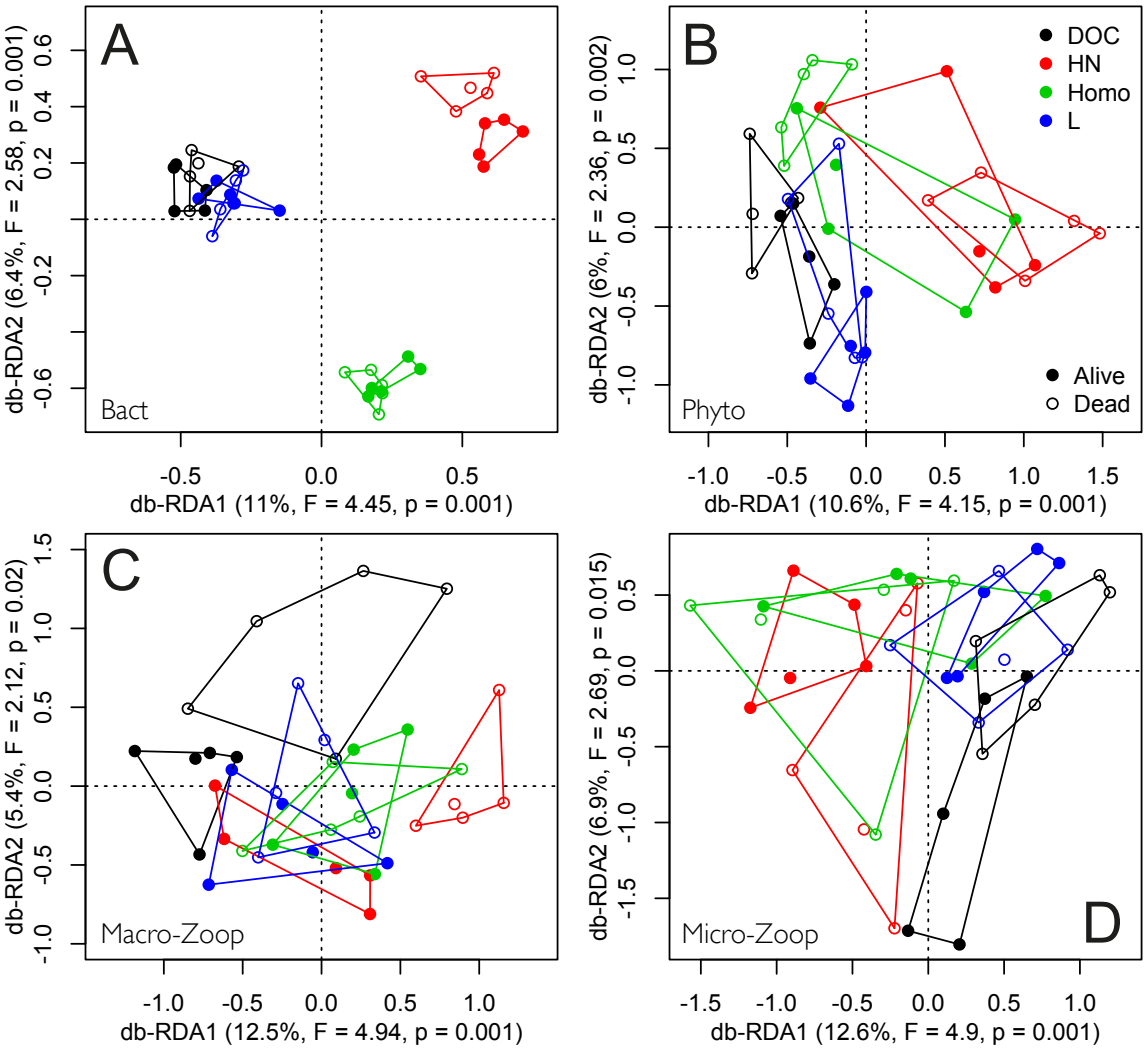

Figure S3: ***db-RDA of Dispersal x Nutrient identity using abundance data (showing the first two axes).*** *A: Bacterial community, B: Phytoplankton, C: Macro-Zooplankton, D: Micro-Zooplankton (Rotifers).*

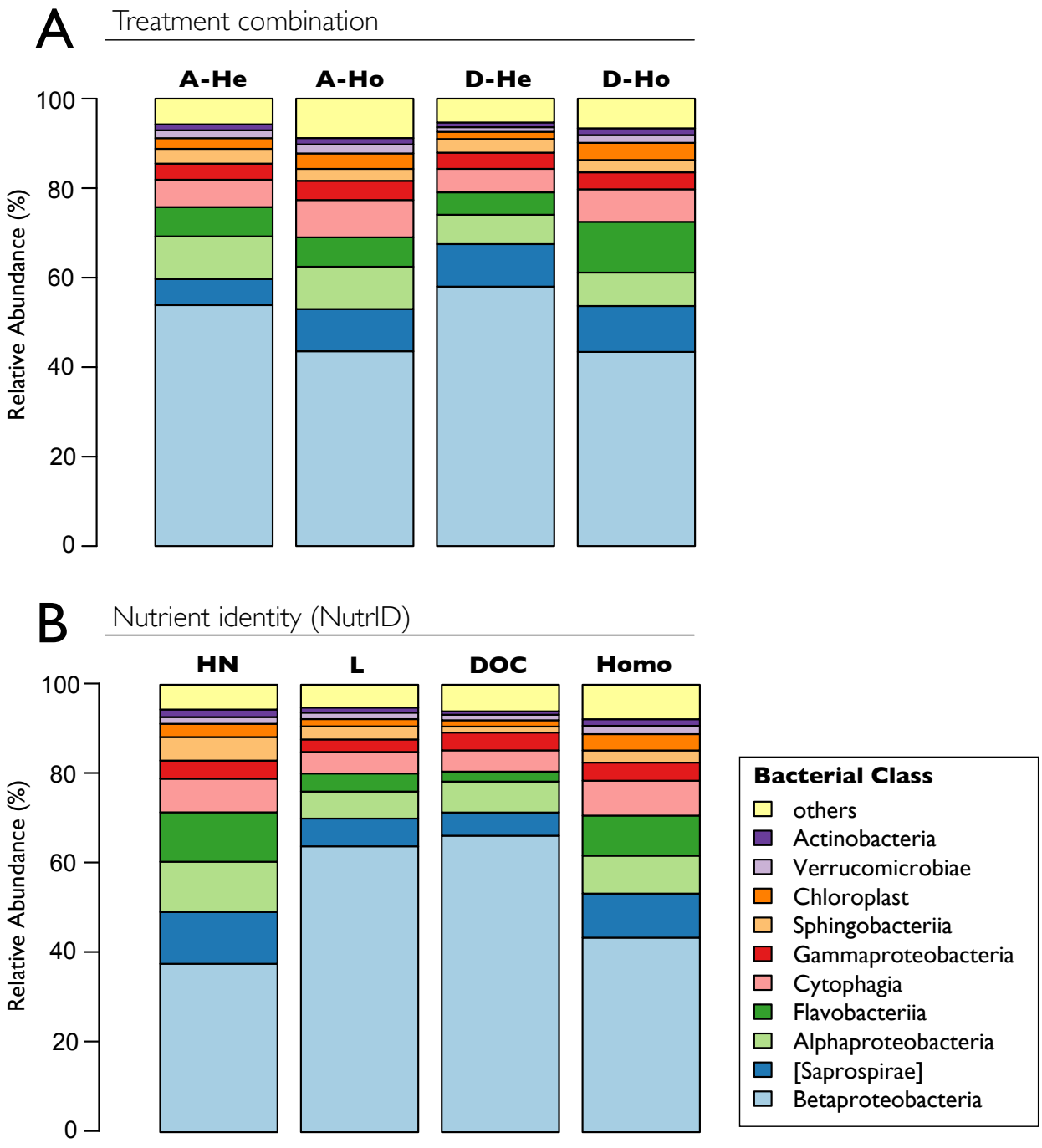

Figure S4: ***Class affiliations of the 100 most abundant OTUs divided by treatment combination (A) and nutrient identity (B).***

5

CONCLUSION AND OUTLOOK

The aim of this thesis was to investigate how biogeography and environmental variation affect the diversity and functioning of aquatic ecosystems, focussing specifically on bacterial communities. The microbial community (which includes microscopic organisms such as bacteria and archaea, but also small eukaryotes and fungi) comprise a bulk of the phylogenetic diversity and biomass on earth (Fierer and Lennon, 2011) and is essential for biogeochemical cycling (Falkowski et al., 2008). However, ecological theory is less commonly tested using microbial compared to other communities (Prosser et al., 2007), partly due to methodological constraints in analyzing microbial diversity. Using large scale mesocosm experiments, which allow for a balance between realism and experimental control, I have investigated community responses to food-web and ecosystem manipulations across multiple trophic levels (bacteria, phytoplankton and zooplankton).

Measuring microbial diversity

Methods for measuring microbial diversity and community composition have evolved rapidly over the last few decades, allowing for ever greater resolution and precision (Zinger et al., 2012). Early methods for characterizing microbial communities included cultivation and microscopy, and while still valuable for certain ecological questions, such approaches only capture a fraction of the microbial community and miss a vast diversity of microbial community present in natural environments. A substantial breakthrough for analyzing microbial diversity has been the use of DNA based methods (Amann et al., 1995). Community fingerprinting techniques and rRNA-targetting microscopy already allowed for better analyses of microbial communities, but recent advances in the sequencing of the small-subunit ribosomal RNA gene (prokaryotes: 16S rRNA, eukaryotes: 18S rRNA) have revolutionized the abilities to analyze microbial communities. These new methods sequence the hypervariable regions of the 16S rRNA gene (Ward et al., 1990), and sequences can then be compared to databases for phylogenetic matching. Unfortunately,

standard methods do not yet allow for sequencing of the complete 16S rRNA gene (circa 1550 bp long) of entire communities at an affordable cost, and researchers therefore have to restrict themselves to sequencing only parts of the 16S rRNA. Currently, there is no consensus among research groups about which regions of the 16S rRNA is best suited for community analysis. In part, this is due to the fact that different regions are better suited for characterizing certain bacterial groups, but this can become a problem when comparing whole communities from natural environments. Currently, a growing number of large scale microbiome projects are sequencing microbial communities from a large variety of habitats to explore the the composition and dynamics of microbial communities across the globe (Nelson, 2013). Examples are the Earth Microbiome Project (EMP) (Gilbert et al., 2014), the Human Microbiome Project (HMP) (Peterson et al., 2009), and the Brazilian Microbiome Project (Pylro et al., 2014). While each of these projects has chosen a standard region of the 16S rRNA for measuring microbial diversity, the projects do not use the same variable regions, making broad scale comparisons difficult. The EMP for example uses the V4 region, while the HMP is using the the V3-V5 regions of the 16S rRNA gene. In order to be able to compare data across these studies, it is important to know if this difference will affect comparisons of biodiversity among studies.

Lack of ecological theory in microbial studies

The field of microbial ecology has focussed intensively on method development and generating large amounts of sequence information, but has made less progress in developing and testing ecological theory (Prosser et al., 2007). My project tries to bridge ecological theory and microbial community ecology, which have developed more or less independently from one other. In order to apply ecological measures to microbial communities, which is what I did in the experiment described in **chapters II and III**, it was first important to know if sequencing different regions of the 16S rRNA affects ecological conclusions about microbial biodiversity. I therefore explored how the choice of variable region influences common ecological measures of diversity in **chapter I**.

Results of chapter I: Influence of variable region on ecological measures

In **chapter I** I shown that ecological measures of bacterial diversity can be strongly influenced by the choice of variable region of the 16S rRNA gene. The three compared regions (V3, V4, and V5) differed strongly in terms of absolute estimates of diversity (species richness, phylogenetic diversity, β-diversity), rank-abundance distributions, and phylogenetic classification of operational taxonomic units (OTUs). When comparing individual lake

samples, species richness estimates were correlated among the three variable regions, but the same was not true for phylogenetic diversity. Overall, results from the V3 and V5 region were more similar to each other than to the V4 region. Additionally, I also compared my Illumina sequences to fingerprinting data (ARISA) and did not find a correlation in species richness between methods. This part of my thesis has shown that ecological conclusions can differ depending on which variable region of the 16S rRNA gene is chosen for characterizing bacterial diversity. I was not able to make recommendations on which region might be best suited for ecological studies due to a several reasons, including the fact that my study has only analyzed three of the nine variable regions. However, I could show that caution is warranted when selecting a region and when comparing results across studies which have used different variable regions. For example, comparisons of diversity from the EMP and the HMP should be treated with caution. If read length remains a limitation due to sequencing techniques, future research should address this issue across the complete 16S rRNA and researchers should discuss if defining a standard 16S region for ecological studies might be useful to increase the robustness of meta-analyses.
For pragmatic reasons I have chosen to use the V3 region to quantify bacterial diversity in **chapters II and III**. Species richness of the V3 region is better correlated with phylogenetic diversity then for the V4 region (Fig. 2.2C), and the V3 region has a longer read length compared to the V5 region (chapter I, Table S2).

SPATIALLY STRUCTURED TROPHIC INTERACTIONS

Chapters II and III focus on the effects of food-web structure in a spatially structured landscape on various trophic levels, and particularly on the bacterial community. As bacteria are key drivers of ecosystem functions and services (Falkowski et al., 2008), it is important to investigate if spatially structured variations in food-web structure can affect the phylogenetic and functional diversity of bacterial communities. In **chapter II**, I investigated the effect of a specific consumer (*Daphnia*) on bacteria and phytoplankton, while in **chapter III**, I included a more diverse zooplankton community in my experimental ecosystems (Fig. 1.5), and tested for the importance environmental heterogeneity as well as fluxes of either materials alone, or materials and organisms, among ecosystem patches.

Importance of environment and space

Community ecologists commonly estimate the relative importance of both environmental variation and space for shaping species distributions. Environmental variability across the landscape can interact with niche differentiation among species, leading to species sorting.

A recent study by Soininen (2014) has analyzed the relative importance of species sorting across multiple trophic levels and ecosystems and found that the importance of species sorting varied between trophic groups, but was relatively unpredictable based on body size and dispersal mode. The author found that species sorting was most pronounced for autotrophs and omnivores, and substantially lower for decomposers and herbivores. In a different study, Soininen (2015) investigated the importance of of spatial scale and found that body size, thermoregulation, and interactions between body size and dispersal mode were important factors for predicting the importance of spatial scale across meta-ecosystems.

While analyzing the importance of niche differentiation has a long tradition for larger organisms, it is more difficult to determine if spatial patterns of bacterial communities are caused by niche differentiation among species. This is partly due to the enormous diversity of bacterial taxa, technical limitations in measuring bacterial diversity, and the ability of bacterial species to adapt rapidly to environmental conditions. Bacteria are known to exhibit high levels of phenotypic plasticity (Justice et al., 2008), particularly associated with changes in cell size, cell wall structure, morphology, mobility patterns, toxin release, or gene expression (Pernthaler, 2005). Unlike other organisms, bacteria also have genotypic plasticity, because they are able to exchange DNA with surrounding cells through horizontal gene transfer (Thomas and Nielsen, 2005) and take up and incorporate extracellular DNA from the surrounding environment (Lorenz and Wackernagel, 1994). Overall, these characteristics of bacteria allow for rapid adaptation and the persistence in response to environmental change.

In my second mesocosm experiment (**chapter III**), resource identity has lead to clear separations of community composition across all trophic groups, and was particularly pronounced for the bacterial community (Fig. 4.7). Even though the initial bacterial source pool was the same in all mesocosms, the nutrient loading had clear and repeatable effects on bacterial composition, which indicates that the treatments have imposed strong selection pressure on the bacterial communities.

Trophic interactions

When studying the responses of groups of organisms to environmental and spatial gradients, we should not forget that trophic interactions are important shaping forces for community composition in natural environments. Under laboratory conditions, it is common to study how individual species or groups of organisms respond to selected abiotic or biotic conditions, but natural communities are always affected by complex interactions

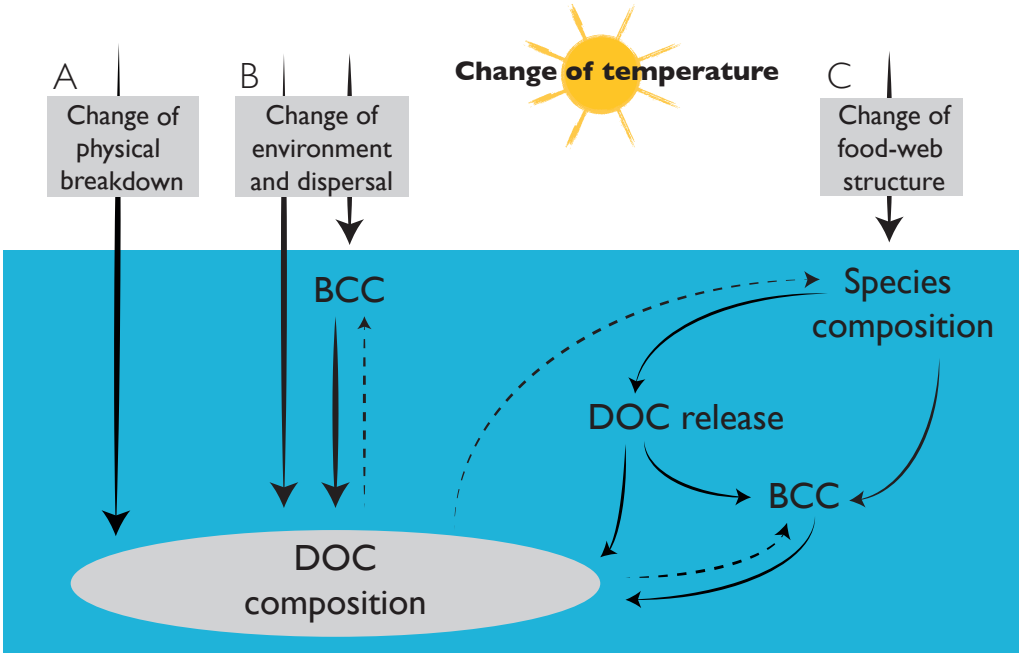

Figure 5.1: ***Possible effects of environmental change on the composition of dissolved organic carbon (DOC).*** *Environmental change, such as increased temperature, might alter DOC composition through multiple pathways. Temperature changes can directly affect the physical breakdown of DOC (A), or lead to changes in environmental properties and dispersal, which can either directly affect the DOC composition, or change the bacterial community (BCC), which can lead to indirect changes of the DOC pool (B). Furthermore, food-web structure can affect the DOC composition (C). Here, the bacterial community (BCC), which is the main biological driver of DOC decomposition, can be altered in indirectly by the food-web structure due to differential DOC release, or directly, if organisms can use bacteria as a food-source (grazing).*

with the environment, spatial structure, and the community structure of other groups of organisms at the same and other trophic levels. For understanding natural ecosystems, it is therefore important to integrate food-web complexity. While food-web interactions have intensively been studied for phytoplankton, zooplankton, and fish (Polis and Strong, 1996), the importance of bacterial diversity for food-web interactions is less well understood. Bacteria are considered an important link for re-mobilizing organic carbon for higher trophic levels through the 'microbial loop' (Azam et al., 1983), but it is not yet known how the aquatic food-web structure can influence such bacterial-mediated functions. There are different pathways in which food-web structure might affect the bacterial community structure and nutrient cycling. The classical theory describes that microbes can return carbon to the food-web by incorporating excess carbon released by other organisms. The source pool of dissolved organic carbon (DOC) might then shape the bacterial community as it adapts to the nutrient conditions. Alternatively, if grazers can directly feed on bacterial taxa, this can affect the bacterial community composition and DOC cycling (Fig. 5.1).

In **chapter II** I have investigated the effects of consumers (*Daphnia*) and the initial abun-

dance of bacterial cells (i.e. dilution) on bacterial and phytoplankton communities, and found that the richness both communities decreased in the presence of *Daphnia*. The bacterial community composition was furthermore strongly affected by food-web structure and both the presence and the absence of *Daphnia* selected for specific bacterial groups, suggesting niche differentiation among bacteria species. This shows that the presence of grazers, or more generally speaking food-web structure, can profoundly affect community structure through multiple pathways with possible effects on ecosystem functioning. It also indicates that that the species identities of higher trophic levels can affect ecosystem functioning and should motivate further work investigating how higher trophic levels affect bacteria mediated ecosystem functions.

Phylogenetic structure

One novel aspect of the first mesocosm experiment (**chapter II**) was that I investigated the phylogenetic structure of changes in bacterial abundances in response to a grazer and the dilution treatment (Fig. 3.7, Table S2). The concept of phylogenetic signal (Blomberg et al., 2003) investigates the dependence of species traits on phylogenetic relatedness (Revell et al., 2008) without knowing the exactly which specific species traits are affected. Phylogenetic signal is a statistical association between species traits and phylogenetic relatedness. The presence of phylogenetic signal in the response of species to an environmental manipulation can suggest niche differentiation among species, though says little about the evolutionary process underlying such differentiation (Revell et al., 2008). Numerous studies of larger organism have investigated how phylogenetic relatedness can help detect how species respond to environmental variation or disturbance (Helmus et al., 2010). However, it is a relatively new concept for microbial ecology, partly because of the prevalence of phenotypic plasticity and rapid adaptations (Krause et al., 2014), which might preclude its usefulness. My results, however, suggest that phylogenetic patterns might be useful response variables for microbial communities, and might help to understand community dynamics without knowing very much about trait variation among species. In other words, phylogenies might serve as a proxy for trait variation among microbial species. The presence or absence of *Daphnia* led to very distinct bacterial communities (Fig. 3.5), and closely related species responded similarly to the *Daphnia* manipulation (Fig. 3.7, Table S2). This indicates niche differentiation within the bacterial community, and suggests that phylogenetic signal in bacterial communities can be detected even when using the 16S rRNA gene. Such approaches might help ultimately help us to predict

bacterial community responses to environmental change.

Environmental change and ecosystem functioning

Understanding the relationship between biodiversity and ecosystem functioning (BEF) is a pressing issue in ecological research, particularly in the era of environmental change (Cardinale et al., 2012a). It is generally acknowledged that biodiversity loss will decrease the functioning, stability, and productivity of ecosystems (Hooper et al., 2005), but the evidence is controversial and varies between ecosystems and organisms (Cardinale et al., 2012a). Because bacteria are main drivers of biogeochemical cycles (Falkowski et al., 2008), it is especially important to understand the BEF for bacterial communities. Bacterial communities are considered metabolically plastic and functionally redundant, but the link between diversity and functioning is suggested to vary along a continuum, from being strong to weak, or even absent (Comte et al., 2012). Interestingly though, rates of change in community composition and functional capacities appear to be correlated (Comte and del Giorgio, 2010). In a comparative study, they found that large changes in composition were associated with large changes in a standardized set of ecosystem functions (measured by Biolog plates).

Moreover, studies of BEF relationship have mostly focussed on single trophic levels, and have disregarded the importance of trophic interactions for ecosystem functioning and services (Hooper et al., 2005; Barnes et al., 2014). Meta-community and meta-ecosystem theory can help investigating the BEF relationship, as they specifically analyze the importance of spatial structure and, as shown in this thesis, allow for the integration of multiple trophic levels.

In **chapter II**, I have measured two ecosystem functions, namely respirations and phosphatase activity, and quantified if treatments affected these functional metrics. Phosphatase activity was not affected by treatment combinations, but respiration responded interactively to *Daphnia* and dilution treatments. Understanding the relationship between bacterial community composition and ecosystem functioning could be improved by analyzing a larger set of of ecosystem functions, for example by using assays that simultaneously analyze several enzymes (Sala et al., 2006), by the sequencing of functional genes (Burke et al., 2011), or transcriptomic analyses (Stewart et al., 2011). The reason why I did not find an effect of treatment on phosphates activity may be due to the fact that phosphatase is a very important enzyme, especially at low nutrient concentrations (Chróst and Overbeck, 1987), and therefore commonly distributed across bacterial groups.

Using mesocosms for studying bacterial communities

Natural ecosystems are highly complex and finding general patterns across ecosystems is challenging. The development of sequencing techniques allows for much better resolution of species at lower trophic levels, and phylogenetic analyses have vastly increased our understanding of such communities. However, as described above, there are numerous factors controlling community patterns (such as environmental variation, distance between habitats, and trophic interactions), and we will probably never be able to determine all parameters governing community composition and functioning within natural environments. The bacterial community is particularly diverse, and understanding natural patterns of bacterial community composition (Fig. 5.2) is not trivial.

My results have shown that mesocosm can be a good model system for investigating community responses to environmental change across multiple trophic levels. Mesocosms allow for a high degree of replication and the possibility of manipulating specific ecosystem parameters, in near-natural ecosystems. The fact that I found clear responses of the bacterial community to the imposed treatments is promising and encourages the use of outdoor mesocosm to study bacterial communities.

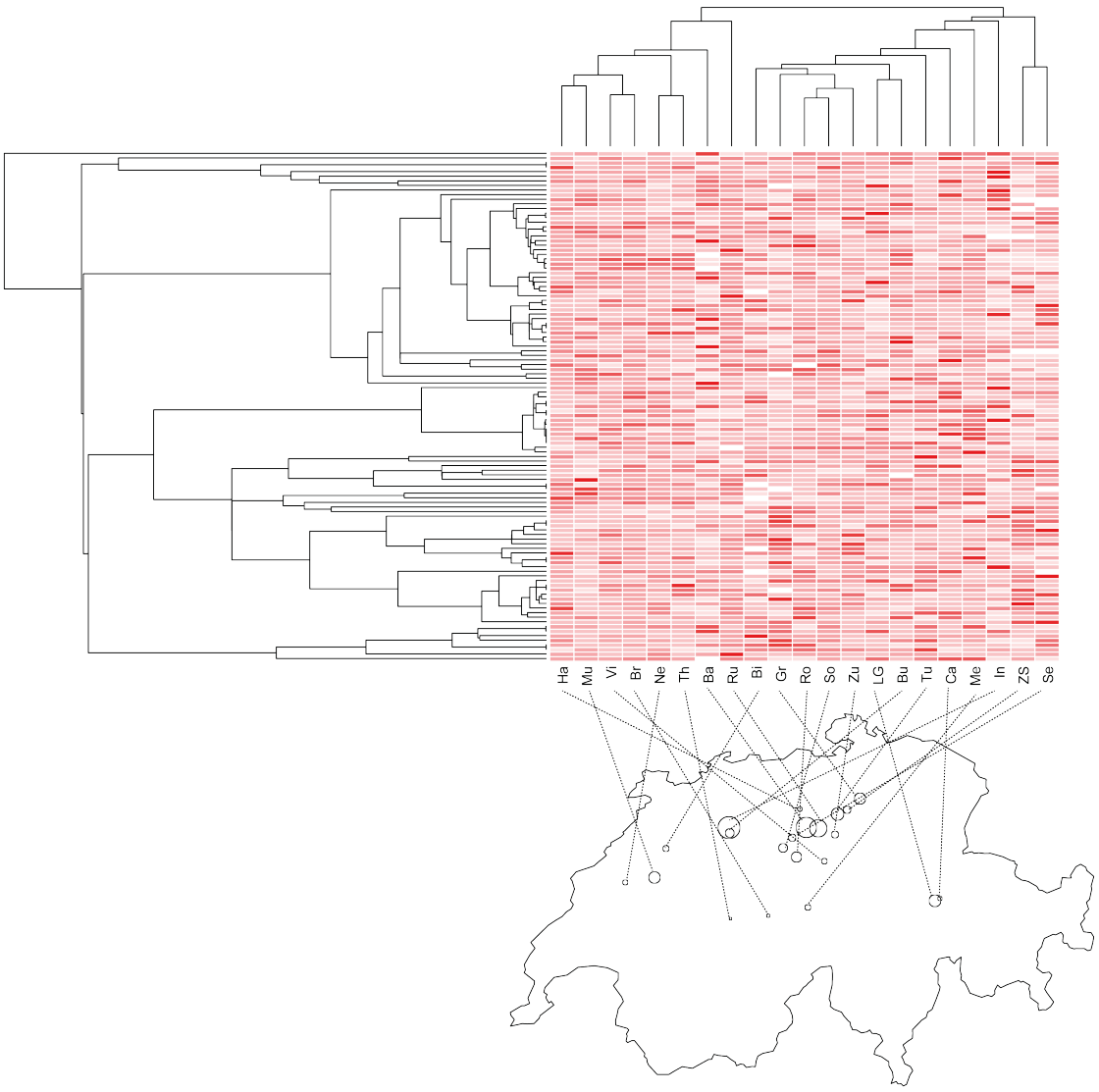

Figure 5.2: ***Comparison of community composition of the lakes included in the Swiss lake survey.*** *The dendogram at the top is constructed from a hierarchical cluster analysis based on the matrix of community dissimilarities among lakes, using the relative abundances of different sequences. The tree on the left is constructed using Qiime, and colors denote the relative abundance of each sequence in the lake sample, from low (white) to, medium abundance (light red), to high abundance (dark red). The circle size is proportional to the number of bacteria measured with flowcytometry.*

IMPLICATIONS AND OUTLOOK

My thesis has investigated meta-ecosystem responses to environmental change across multiple trophic levels. I have highlighted the importance of method selection for analyzing bacterial communities (**chapter I**), investigated the importance of spatially structured food-web interactions and resource heterogeneity for multiple trophic levels (**chapters II and II**), and have demonstrated that mesocosms are a good model system for studying how environmental change affects community responses. To progress our understanding of how trophic interactions affect bacterial community composition and functioning, I propose to use even more complex food webs, including for example fish as top predators, and increase the use of methods for determining functional capacities. My work has been part of a larger survey of lakes across Switzerland, Austria and Germany (Fig. 5.3), which is determining community responses across multiple trophic levels. Applying concepts described in this thesis might help to understand how spatial and environmental parameters affect natural communities.

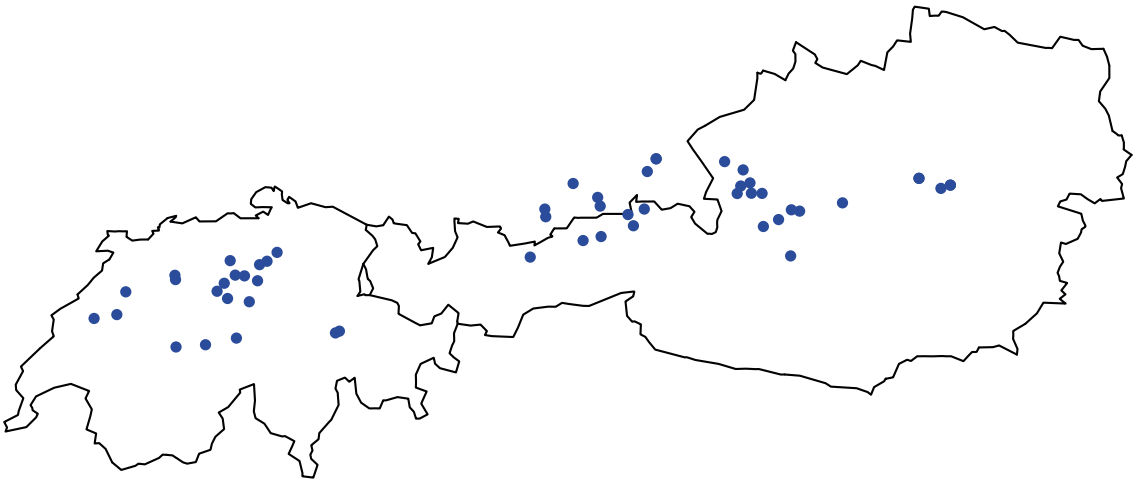

Figure 5.3: ***Lakes sampled across Switzerland, Austria, and Germany.***

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