Doctoral Thesis

Microbial dynamics during stream ecosystem succession community structure and enzyme activities

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Microbial dynamics during stream ecosystem succession: community structure and enzyme activities

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Summary

In a world increasingly modified by human activities, studying successional trajectories can aid in the interpretation and prediction of ecosystem responses to anthropogenic disturbances, such as changes in biodiversity and fluxes of matter and energy. Emphasis in the past has been placed on the importance of plant community succession, whereas the role of microbial communities as drivers of, and respondent to, ecosystem succession has been largely neglected, in spite of the ubiquity of microbes on earth and their importance for biogeochemical cycles. Owing to their limited complexity, early successional stages of ecosystems may also provide excellent opportunities for testing central ecological questions, such as the linkage between biodiversity and ecosystem functioning.

Concepts of stream ecosystem succession in recently created landscapes emphasize a sequence of three main stages shaped largely by the development of terrestrial vegetation. The initial biofilm stage is characterized by a lack of higher plants, and the subsequent stage by a lack of woody vegetation, which is finally replaced by a forested stage. Changes in energy sources and in a range of environmental factors accompanying the transition among these different stages are likely to have repercussions for stream microbial community structure and activities, such as transformations of organic carbon compounds. However, empirical data about the changes of these processes and communities during succession are not currently available.

The overall aim of this dissertation was to contribute to understanding the effects of changes in biological and physical structures during stream ecosystem succession on microbial community structure and function, and on the relationship between both. In the first project, I took advantage of an artificially created, early successional catchment to investigate the spatial and temporal patterns of bacterial community structure and relationships with potential microbial enzyme activities along the hydrological flow paths of stream corridors formed naturally in the catchment. For the second project, I used experimental stream channels simulating early-successional streams to test the importance of fungal and bacterial communities for stream metabolism during the very first stage of ecosystem succession. The response of microbial community structure and function to changes in the quantity and quality of plant litter delivered to
streams during various successional stages was examined in the third project. Finally, in a companion study to the investigation above, the fourth project assessed the relative importance of effects resulting from changes in litter quality and quantity during succession versus the quality of the substrate (i.e. grass or tree litter) effectively colonized.

Results of the four studies suggest that during stream succession, the input of plant litter strongly influences the structure and function of stream microbial communities, both fungi and bacteria. Moreover, the quality of the substrate on which the microbes grow has a greater influence than the stream environment in general. Conversely, spatial variation in important environmental factors such as water availability appears to have surprisingly little effect on bacterial community structure and microbial enzyme activities during the initial successional stage. However, strong seasonal patterns of potential enzyme activities, but not of microbial communities, were apparent, suggesting a disconnect between community structure and function. Finally, results of this study suggest that fungal communities can affect stream metabolism well beyond their well-known significance for litter decomposition.

Taken together, the results presented here highlight the role of microbial communities in, and the response to, successional changes in a structurally simple ecosystem. They also suggest that microbial communities are likely to affect the trajectories of ecosystem successions, thereby potentially influencing the development of features in mature ecosystems. The approaches taken in the present study, i.e. a survey conducted in early-successional stream corridors combined with experiments in artificial stream channels, proved useful to address a number of specific hypotheses. However, comparisons with other systems undergoing successional changes are needed for broader inferences about the role of microbes during stream succession.
Résumé

Dans un monde de plus en plus modifié par les activités humaines, l’étude de la succession des écosystèmes peut se révéler très utile pour prédire et interpréter les réponses aux perturbations subies par les écosystèmes. Jusqu’à récemment, les recherches faites dans ce domaine ont placés beaucoup d’importance dans l’étude de la succession des communautés végétales. Toutefois les communautés microbiennes, à cause de leur ubiquité sur terre et de leur importance dans les cycles biogéochimiques, sont des facteurs potentiels de changement importants dans la succession des écosystèmes. D’autre part, la complexité limitée des écosystèmes dans les premiers stades de développements fait de ces systèmes d’excellentes opportunités pour tester des questions centrales en écologie, comme la relation entre biodiversité et fonctionnement des écosystèmes.

Des concepts émergent d’études menées sur la succession des écosystèmes lotiques dans des milieux nouvellement créés mettent en évidence trois stades de développements, largement liés au développement de la végétation: un premier stade caractérisé par l’absence de végétation et la dominance de biofilm, un second stade caractérisé par l’absence de végétation boisée, et finalement un troisième stade caractérisé par le développement de forêts. D’un stade de développement à l’autre, des modifications de la source d’énergie et de facteurs environnementaux sont susceptibles d’entrainer des changements dans la structure et les activités des communautés microbiennes telles que la transformation ou l’accumulation du carbone. Toutefois, aucune donnée n’est disponible concernant ces processus.

L’objectif principal de cette thèse est de comprendre les effets des changements de structures biologiques et physiques qui interviennent lors de la succession des écosystèmes lotiques sur la structure et les fonctions des communautés microbiennes et de leurs interactions. Lors du premier projet d’étude, j’ai pris avantage du bassin versant nouvellement créé « Chicken Creek » pour investiguer un écosystème lotique dans une phase initiale de succession. Le projet se concentre sur l’analyse des changements spatio-temporels de la structure de la communauté bactérienne et de son lien avec des activités enzymatiques extracellulaires le long de chenaux d’érosions formés par l’écoulement d’eau de surface dans le bassin versant. Pour les projets suivants, j’ai conduit des expériences dans un système de chenaux simulant des rivières dans
un stade initial de développement. Ainsi, la seconde étude teste l’importance relative des communautés de champignons aquatiques et de bactéries pour le métabolisme des écosystèmes lotiques initiaux. Le troisième projet examine l’effet d’apport de matière organique de qualité et de quantité différente sur la structure et les fonctions des communautés microbiennes dans le lit des rivières. Finalement, en lien avec le projet précédant, le quatrième projet évalue l’importance relative des apports de litières de qualité et de quantité variant lors de la succession des écosystèmes lotiques par rapport à la qualité du substrat directement colonisée (litière) par les communautés microbiennes.

Les résultats que ces projets apportent suggèrent que lors de la succession des écosystèmes lotiques, l’apport nouveau de matière organique influence sérieusement les fonctions et la structure des communautés microbiennes. De plus, la qualité de la litière directement colonisée par les microorganismes a plus d’influence pour les activités microbiennes que les conditions environnementales de la rivière. Au contraire, la structure de la communauté bactérienne et les activités microbiennes sont très peu affectées par la variation d’un important facteur environnemental pour les écosystèmes lotiques dans un stade initial, à savoir la disponibilité de l’eau. Finalement, la composition initiale de la communauté microbienne s’est révélée être importante pour le métabolisme général des écosystèmes lotiques dans les premiers stades de développement.

Ces résultats soulignent l’importance des communautés microbiennes dans la succession des écosystèmes lotiques. La dynamique de la structure et des fonctions microbiennes sont susceptible d’influencer la trajectoire de la succession des écosystèmes et aussi d’avoir des répercussions sur le développement structurel des écosystèmes matures. Les approches choisies, à savoir l’investigation d’un bassin versant dans un stade initial de développement et en parallèle ainsi que des expériences dans des chenaux artificiels se sont avérés être efficaces pour explorer la distribution spatiale des communautés microbiennes. Toutefois, une comparaison avec d’autres systèmes dans des stades primaires de développement est nécessaire pour élaborer de plus larges conclusions sur la succession des écosystèmes lotiques.
Chapter 1

Introduction

1.1 Ecosystem succession

Ecosystem succession, the sequential replacement of species following a disturbance, is a central theme in ecology (Clements, 1916). Although ecosystem succession is a rather old concept (Gleason, 1927; Clements, 1936), its study can aid in the prediction and interpretation of community and ecosystem responses to contemporary environmental stressors. In a world that is increasingly altered by human activities, understanding successional patterns is especially relevant for understanding the consequences of changes in biodiversity and ecosystem function due to invasive species, climate change, or ecosystem restoration (Prach & Walker, 2011).

Primary succession is defined as the ecosystem development on barren surfaces where severe disturbance have removed most biological activity. Primary succession depends fully on the processes occurring very early in the succession and these early processes will have consequence for the trajectory of the succession (Walker & del Moral, 2003). However, predictions of these trajectories are still discussed, notably in the context of deterministic versus stochastic community assembly (Milner & Robertson, 2009).

Despite their great importance to development of community and ecosystem theory, opportunities to study primary succession are rare in natural environments. Most studies on primary succession have been conducted in landscapes that have been completely destroyed, transformed or newly created, such as volcanic areas (del Moral & Wood, 1993; Müller-Dombois & Fostberg, 1998) or glacier retreat areas (Matthews, 1992; Chapin et al., 1994). Anthropogenic large-scale disturbances, such as open-pit mining activities, also provide the possibility to follow the re-establishment and development of a new ecosystem after the mining activity stopped (Hüttl & Weber, 2001; Gerwin et al., 2009; Schaaf et al., 2011). Vegetation development was the central focus of these investigations. Thus, most studies on ecosystem succession have been conducted in terrestrial ecosystems (Walker & del Moral, 2003).
In contrast, very little is known about primary succession in streams or other freshwater ecosystems. Extrapolations of concepts of terrestrial ecosystem succession to aquatic ecosystems were investigated particularly by examining the effect of flash floods (Fischer et al., 1982). Flood disturbance is a well-known agent of disturbance in stream ecosystems. It results in secondary succession by sending mature ecosystems back to earlier stages of development. However, flood disturbance does not generally set the entire ecosystem back to the starting point of primary succession. Primary stream succession can only be studied in catchments that start in newly formed landscape.

1.2 Stages of stream-ecosystem succession

Concepts of ecosystem succession in recently created landscapes such as volcanic lava fields (Vitousek, 2004), forefields of receding glaciers (Milner & Gloyne-Phillips, 2005), or post-mining areas (Mutz et al., 2002) emphasize a sequence of three stages shaped largely by the development of terrestrial vegetation (Fig. 1.1). Below I describe these three stages of stream succession, and discuss the rationale for how microbial community structure and function may influence processes at each stage.

Due to a lack of riparian and in-stream vascular vegetation, the first phase of stream-succession is strongly influenced by hydrology. Very little allochthonous organic matter enters stream beds in early-successional catchments due to the lack of litterfall from riparian trees (Golladay, 1997), and frequent flash floods causing movement and relocation of sediment in the streambed (Maltchik & Pedro, 2001; Riis et al., 2004). Erosion in the catchment leads to the formation of a network of rills including ephemeral and perennial stream channels (Graf, 1988). This successional stage is called the biofilm stage because streams are dominated by autochthonous algal production fuelling heterotrophic organisms present in the biofilm with labile (i.e. easily degradable) organic matter (Jones et al., 1995; Romaní & Sabater, 1999). Consequently, microbial biofilms associated with benthic and hyporheic sediment are key players for carbon transformations in the initial successional stage. Water availability as well as sediment disturbance will influence primary production and other ecosystem processes at this time (Stanley et al., 2010).

The second phase, referred to as macrophyte stage, is characterized by the establishment of macrophytes along and in the stream channels and non-woody
vegetation in the riparian area. The increasing coverage of vegetation provides some stability in the catchment; flashfloods are less severe and less frequent (Ludwig et al., 2005), which in turn facilitates the establishment of macrophytes in the stream channels (Riis & Biggs, 2003). The presence of vegetation introduces a new source of organic matter fuelling stream-bed biofilms and enhancing ecosystem metabolism (Huryn et al., 2001; Wilcock & Croker, 2004; Acuña et al., 2010).

The third and mature stage of succession, referred to as forest stage, is characterized by the presence of wooded riparian vegetation. The development of trees, delivering leaf litter and dead wood to the stream, supplies microbial biofilms with an important allochthonous carbon and nutrient source that is expected to increase stream metabolism (Fisher & Likens, 1973; Vannote et al., 1980; Webster & Meyer, 1997). The presence of riparian woody vegetation also causes shading and thus limits in-stream primary production (Sabater et al., 2000).

![Figure 1.1. Schematic of expected pattern of physical structure and community structure and their effect on carbon transformations during three different stages of stream succession (adapted from Gessner and Mutz, 2007, unpublished).](image-url)
The complexity and stability of stream ecosystems increases with time as succession proceeds. Additionally, interactions between the physical structure of streams and the structure of stream biological communities shift among the different stages, which could affect stream carbon transformations. As this entails changes in energy sources and environmental factors that support shifts in stream microbial community structure, successional stages is likely also to influence carbon transformations, although pertinent data are currently unavailable (Schaaf et al., 2011). Physically and biologically simpler model systems, such as artificially created catchments subject to natural colonization and development from an initially bare stage provide opportunities to assess patterns of microbial community structure and activities and their relation with the environmental conditions and community structure.

1.3. Microbial activities and functions
Although emphasis has been placed on the importance of in-stream and riparian plant communities for stream succession, microbial communities are expected to be important drivers of ecosystem succession, given their ubiquity on earth (Fenchel & Finlay, 2004) and their importance for biogeochemical cycles. Thus, understanding successional patterns of microbial community structure and function can help developing a more comprehensive model of succession and improve our perception of ecological processes (Fierer et al., 2010).

A general principle in microbial and general ecology is that community structure (i.e. composition and diversity of communities) determines ecosystem functioning (i.e. the flux of nutrients and energy through the system) (Cardinale et al., 2009). However, for microbial communities, there are only a few experimental studies that have demonstrated effects of microbial community composition on ecosystem functioning (Bell et al., 2009; Langenheder et al., 2010; Peter et al., 2011). When an environmental factors were modified in these studies (e.g. source or concentration of organic matter), changes in the structure of the microbial community (generally bacterial community structure) were associated with a change in ecosystem functioning. However, other studies found only weak coupling between the structure of microbial communities and the functioning of natural ecosystems (Langenheder et al., 2005; Langenheder et al., 2006; Wertz et al., 2006; Östman et al., 2010), and it is still unclear how the inconsistencies

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among studies examining the relationships between microbial community structure and ecosystem functioning can be resolved.

Investigations of the relationship between microbial community structure and function have been hindered by several technological limitations (Lennon & Jones, 2011). First, the microbial structure-function relationships could be biased because the analysis of microbial communities, often based on molecular fingerprinting techniques, takes into account dormant cells which do not participate actively in ecosystem functioning. Second, the relation between microbial community structure and function might depend on the particular process investigated. Narrow functions such as specific enzyme activities might respond more tightly to environmental and community changes than broader functions (e.g. microbial respiration) which are mediated by widely distributed metabolic capacities within microbial communities (Langenheder et al., 2006). Interactions between community structure and function are thus regulated by a complex combination of factors that can be difficult to disentangle. Therefore, new approaches are needed to elucidate the functional significance of complex microbial communities, where changes in environmental conditions could result in changes to community composition while functioning remains unaltered (Wohl et al., 2004; Kritzberg et al., 2006; Allison & Martiny, 2008; Langenheder et al., 2010).

Investigations into extracellular enzyme activities, mostly excreted by microorganisms, are of great interest for understanding ecosystem functioning, and their analysis gives valuable information about microbial metabolic activities in the environment. Enzyme activities in stream have been observed to vary in space and time (e.g. seasonal variation; Jones & Lock, 1993; Romaní & Sabater, 2001; Wilczek et al., 2005). More specifically, microbial enzyme activities were found to vary according to several environmental conditions (Fig. 1.2), such as the quantity and quality of organic matter sources (e.g. Findlay et al., 2002; Findlay et al., 2003; Romaní et al., 2004; Rulík & Spáčil, 2004; Artigas et al., 2008b; Artigas et al., 2009), the structure of the streambed (e.g. Pusch et al., 1998; Artigas et al., 2004), and changing water flow (e.g. Battin, 2000; Rulik & Spacil, 2004) or water availability (e.g. Romaní & Sabater, 2000a; Romaní et al., 2006; Artigas et al., 2009). Interactions among different biological groups colonizing the substratum, such as bacteria-algae (Francoeur & Wetzel, 2003; Ylla
et al., 2009; Pohlon et al., 2010) or bacteria-fungi (Romaní et al., 2006) are also expected to influence microbial enzyme activities.

![Diagram of interactions](image)

**Figure 1.2.** Schematic of interactions between environmental conditions, biofilms, organic matter and extracellular enzymatic activities. Adapted from Arnosti et al. (2011).

### 1.4. Scope of the thesis

The projects presented in this thesis were conducted as part of the integrative project "Structure and processes of the initial ecosystem development phase in an artificial water catchment" of the German Transregional Collaborative Research Centre (http://www.tu-cottbus.de/sfb_trr/eng/index.htm). The overall aim of this project was to clarify the role of structures and processes during the initial establishment of an ecosystem (Gerwin et al., 2009; Schaaf et al., 2010). To this end, an artificial catchment was constructed in the post open-pit mining area of Lusatia in eastern Germany (Gerwin et al., 2009). The catchment is referred to as "Chicken Creek" ("Hühnerwasser" in German, Fig. 1.3) and was built between 2004 and 2005. It has a surface area of about 6.5 ha and was constructed as a 2-4 m layer of postglacial sandy to loamy substrate overlaying a 1-2 m layer of tertiary clay (Gerwin et al., 2009). No further measures of restoration were taken.
so as to allow natural succession and undisturbed development of the ecosystem (Schaaf et al., 2011).

**Figure 1.3.** Aerial view and main features of the artificial catchment “Chicken Creek” near Cottbus, Germany, in May 2007, 18 months after finishing its construction. From Schaaf et al. (2011).

Next to the study site, experiments were conducted in 16 artificial channels installed at the periphery of the Chicken Creek catchment (Fig. 1.4.). Groundwater collected from the catchment was pumped directly to the channels built to simulate early-successional streams. The number of 16 channels allowed true replication of treatments in multifactorial experiments.

**Figure 1.4.** View of the experimental channels, in the Chicken Creek catchment.
All projects presented in this thesis were accomplished in close collaboration with Linda Gerull, PhD student at the Brandenburg Technical University in Cottbus, Germany. Field work and some of the measurements (respiration, leaf mass loss) were carried out jointly, whereas data analyses were performed independently. Except for the last joint paper, the writing of the dissertation was also carried out independently.

1.5. Projects of the dissertation

This thesis focuses on a central issue in general and microbial ecology, namely the effect of environmental condition on microbial activities and their link to microbial community structure. The aim of the dissertation was to better understand the effects of changes of biological and physical structures on microbial community structure and function during stream succession. Several aspects potentially influencing microbial communities in early-successional streams or during the stream succession were investigated in the Chicken Creek catchment (Chapter 2) or in artificial outdoor stream channels (Chapters 3, 4 and 5).

In the first project (Chapter 2), I took advantage of the recently created Chicken Creek catchment to investigate the spatial and temporal pattern of bacterial community structure and their linkages with potential microbial enzyme activities along the hydrological flow paths of stream corridors formed in the catchment. I hypothesized that water availability and vertical exchange of water between the hyporheic zone and the stream-bed affect both bacterial community structure and microbial activities. Ten potential enzyme activities and fingerprints of the bacterial communities were assessed in three stream corridors of the Chicken Creek catchment at four occasions over a year.

The second project (Chapter 3) tested the relative importance of fungal and bacterial communities for the metabolism of early successional streams. Fungi are important decomposers of leaf litter in forested streams with possible effects on other stream microbes and carbon cycling. Such effects should be limited in early-successional streams in which organic matter within the stream bed is present in only low amounts. Thus, I hypothesized that the presence of fungi in early-successional streams will still have an effect on the entire stream system and on the leaf litter compartment and will affect the structure of the bacterial community. This experiment was conducted in the artificial channels receiving
Chicken Creek water and one of four distinct microbial inocula: 1) bacterial and fungal communities, 2) bacterial communities only, 3) no microorganisms, and 4) dead communities of bacteria and fungi. Community structure, respiration, biomass of fungi and bacteria associated with microbes in the sediment and on exposed leaf litter, as well as respiration and primary production of the whole system were measured several times during 5 weeks following the start of the experiment.

The third project (Chapter 4) investigated the effect of varying quality and quantity of particulate organic matter input on streambed microbial community structure and function. As changes of vegetation type during stream succession can have profound effects on stream ecosystems, it was hypothesized that the structure and activity of microbial communities establishing in the stream bed during different stages of succession will be affected by the quality and quantity of allochthonous litter inputs. To test for such effects, the experimental channels were stocked with leaf litter to mimic five stages of stream succession: 1) an initial biofilm stage (no litter), 2) a subsequent macrophyte stage (grass litter), 3) a transitional stage between the macrophyte and the later forested stage (mix of grass and tree litter), 4) a young forested stage (tree litter), and 5) a mature forested stage (large quantity of tree litter). Bacterial community structure and microbial enzyme activities within the stream-bed were analysed after a period of 10 weeks of litter exposure.

The fourth project (chapter 5) assessed the relative importance of general environmental conditions in streams (i.e. quality and quantity of organic matter input) and the quality of the specific substrate colonized by microbial communities. It was hypothesized that the structure and activity of the microbial communities colonizing two distinct types of leaf litter considerably differ and that the simulated successional stages also affect the microbial communities. The artificial channels were stocked in the same manner as in the previous project (Chapter 4), but microbial community structure and activities were assessed for the microbes colonizing small amounts of either grass or tree leaf litter exposed in the channels in addition to the litter added to simulate different successional stages.
Chapter 2

Disconnect between microbial structure and function: enzyme activities and bacterial communities in nascent stream corridors

Frossard Aline, Linda Gerull, Michael Mutz and Mark O. Gessner

In press in the ISME Journal

Abstract

A fundamental issue in microbial and general ecology is the question to what extent environmental conditions dictate the structure of communities and the linkages with functional properties of ecosystems (i.e. ecosystem function). We approached this question by taking advantage of environmental gradients established in soil and sediments of small stream corridors in a recently created, early successional catchment. Specifically, we determined spatial and temporal patterns of bacterial community structure and their linkages with potential microbial enzyme activities along the hydrological flow paths of the catchment. Soil and sediments were sampled at a total of 15 sites on four occasions spread throughout a year. Denaturing gradient gel electrophoresis (DGGE) was used to characterize bacterial communities, and substrate analogues linked to fluorescent molecules served to track 10 different enzymes as specific measures of ecosystem functions. Potential enzyme activities varied little among sites, despite contrasting environmental conditions especially in terms of water availability. Temporal changes, in contrast, were pronounced and remarkably variable among the enzymes tested. This suggests much greater importance of temporal dynamics than spatial heterogeneity in affecting specific ecosystem functions. Most strikingly, bacterial community structure revealed neither temporal nor spatial patterns. The resulting disconnect between bacterial community structure and potential enzyme activities indicates high functional redundancy within
microbial communities even in the physically and biologically simplified stream corridors of early successional landscapes.

2.1. Introduction

A widespread tenet in microbial and general ecology is that structure determines function. Here structure refers to the composition and diversity of biological communities, and function relates to the processes that the communities drive. Both are directly determined by the environmental setting; in addition, functions can be indirectly affected through altered community structure. Experimental tests with assembled bacterial communities at strictly controlled diversity levels have demonstrated that changes in community attributes such as diversity can affect ecosystem processes in various cases (Bell et al., 2009; Langenheder et al., 2010; Peter et al., 2011), one of them quite spectacular (Bell et al., 2005). These results are in line with evidence from a body of similar experiments conducted with higher plants, invertebrates, algae, and other microorganisms (Gessner et al., 2010; Cardinale et al., 2011). They contrast with outcomes of indirect microbial community manipulations, for instance by means of dilution series (Langenheder et al., 2005, 2006; Wertz et al., 2006; Östman et al., 2010) or fumigation (Griffiths et al., 2000), which have failed to detect similar relationships. The discrepancy can be reconciled, by acknowledging that key differences exist between the two approaches (Bell et al., 2009). Among the most notable ones are the vastly divergent diversity levels considered, and the fact that manipulations such as fumigation or dilution series generate microbial communities that are subsets of the taxa originally present, whereas experiments with assembled communities can independently vary in diversity and taxonomic composition. A drawback of the latter approach, however, is the unknown degree to which the simplified assembled communities represent natural situations.

A third approach that can yield insights into relationships between community structure and ecosystem function consists in simultaneously analysing communities and processes across a gradient of environmental conditions in space or time and examining the data for common patterns (Findlay et al., 2003; Docherty et al., 2006; Findlay & Sinsabaugh, 2006; Comte & del Giorgio, 2009). This strategy is akin to experimentally modifying an environmental factor (e.g. the type, source or concentration of organic carbon or nutrients) before assessing differences in both functional and structural
community parameters (Langenheder et al., 2005, 2006). Four different outcomes are possible in such studies: 1) neither community structure nor function responds to environmental changes, which is suggestive of resistance of both structure and function; 2) ecosystem function but not community structure responds, suggesting greater sensitivity of ecosystem function than community structure; 3) community structure is affected whereas ecosystem function is not, indicating functional redundancy or compensatory effects (Kritzberg et al., 2006; Allison & Martiny, 2008); and 4) both community structure and ecosystem function are altered, in which case it remains unclear whether the latter changes are due to direct environmental influences or to altered communities and their functional capacities. Although the uncertainty associated with the last point is unsatisfactory, the approach can yield valuable insights, especially if one of the first three outcomes is observed.

Stream corridors are heterogeneous environments composed of a mosaic of patches of different metabolic activities (Larned et al., 2010; Winemiller et al., 2010). Microbial activities in these patches also vary over time in response to temporal variation of environmental conditions (Jones & Lock, 1993; Wilczek et al., 2005; Sinsabaugh & Follstad Shah, 2010a, b), changes in water availability (Romaní et al., 2006; Artigas et al., 2009) and varying interactions among diverse microbial groups (e.g. bacteria and algae; Romaní et al., 2006; Ylla et al., 2009; Pohlon et al., 2010). This creates a spatio-temporal template that provides scope for gradients in environmental conditions along hydrological flow paths from upland sites to stream channels and longitudinally in stream corridors (Larned et al., 2010).

One reason evoked to explain the frequent lack of functional consequences to community changes in field studies is that microbial diversity in natural environments is extremely high. As a result, effects of community change on ecosystem functions can be easily masked. However, this masking effect could be alleviated by taking advantage of the physically and biologically simple structure of nascent ecosystems formed by volcanism (Vitousek, 2004), glacier retreat (Milner et al., 2007; Brankatschk et al., 2011) or humans (Gerwin et al., 2009; Gerull et al., 2011), where species numbers are reduced but artifacts caused by greatly simplified laboratory systems are avoided.

Another potential reason for the frequent inability to detect functional consequences arising from community changes is that the broad ecosystem
processes commonly assessed (e.g. microbial respiration or nitrogen mineralization) are too coarse-grained because they integrate across many individual processes. Specific metabolic functions, such as particular biogeochemical reactions catalyzed by microbial enzymes, are likely to be more tightly linked to the particular populations constituting a given microbial community (Langenheder et al., 2006). Therefore, one way to increase sensitivity of tests for effects of altered community structure on ecosystem functions is to assess activities of a suite of enzymes involved in the biogeochemical cycling of important elements such as carbon, nitrogen and phosphorus.

The objectives of the present study were to determine patterns of, and linkages between, bacterial community structure and microbial metabolic activities in soil and sediment along hydrologic flow paths in small nascent stream corridors of a recently created catchment. We hypothesized that long-term water availability and vertical exchange of water between the hyporheic zone and the stream-bed would be major drivers of change of both bacterial community structure and microbial metabolic activity reflected in potential enzyme activities. Accordingly, we expected to find: (1) spatial variation of bacterial community structure and potential enzyme activities in soil and sediments along the environmental gradient defined by the hydrologic flow path in the catchment, (2) similar changes of community structure and potential enzyme activities over time, and (3) systematic relationships between community structure and patterns of the potential enzyme activities.

2.2. Material and Methods

Study site

The study was conducted in an experimental catchment near Cottbus in eastern Germany (51°36’N, 14°16’E), which is referred to as Chicken Creek catchment (Gerwin, et al., 2009). It was deliberately created to study ecosystem succession. Average annual precipitation and air temperature are 559 mm and 9.3°C, respectively (record 1971-2000, Meteorological Station Cottbus, German Weather Service). The catchment, which covers an area of 6 ha (400 m length x 150 m width), was created between 2004 and 2005. A 1-2 m base layer of Tertiary clay was overlain by a 2-4 m top layer of Pleistocene sand. Conditions typically associated with mining (e.g. low pH, or high metal concentrations), were prevented during construction of the experimental catchment. The total
Elevational difference of the catchment was 15 m, resulting in an average slope of 3.5% (Gerwin et al., 2009).

Geomorphological development and biological colonization of the catchment has followed a natural succession from the initially bare quaternary sands. By 2007, the initially plane surface had been shaped by water erosion, resulting in a network of rills and channels. The upper portions of the three major stream corridors in this network were characterized by ephemeral flow, whereas a subterranean clay barrier downstream resulted in groundwater upwelling, a short section with permanent surface flow (10-20 m) and a downwelling zone (Fig. 2.1). The extent of these stream sections was rather stable over time. Sediments in both the stream channels and soil were dominated by fine to medium sand (grain size of 200-300 μm). Further physico-chemical characteristics of the catchment and its streams are summarized in Table 2.1.
Table 2.1. Characteristics of the three main streams corridors in the Chicken Creek catchment. Data on the stream channels refer to the main stem of the drainage networks. Most data from Gerull et al. (2011). Values are means ± 1 SE (n=9 to 12) with ranges in parentheses.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Stream 1</th>
<th>Stream 2</th>
<th>Stream 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catchment area (m²)</td>
<td>950</td>
<td>7473</td>
<td>20496</td>
</tr>
<tr>
<td>Mean discharge (l s⁻¹)</td>
<td>0.02</td>
<td>0.13</td>
<td>0.37</td>
</tr>
<tr>
<td>Length of perennial stream section (m)</td>
<td>20</td>
<td>38</td>
<td>43</td>
</tr>
<tr>
<td>Length of ephemeral stream channel (m)</td>
<td>103</td>
<td>171</td>
<td>249</td>
</tr>
<tr>
<td>Permanently wetted streambed area (m²)</td>
<td>4</td>
<td>99</td>
<td>68</td>
</tr>
<tr>
<td>Ephemeral streambed area (m²)</td>
<td>30</td>
<td>390</td>
<td>432</td>
</tr>
<tr>
<td>Channel slope (%)</td>
<td>6.5</td>
<td>5.1</td>
<td>3.9</td>
</tr>
<tr>
<td>Channel width at ground level (m)</td>
<td>1.2 (0.6-1.9)</td>
<td>2.6 (1.0-5.8)</td>
<td>2.2 (0.2-5.4)</td>
</tr>
<tr>
<td>Organic matter in sediment or soil (mg g⁻¹)</td>
<td>2.5 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>Chlorophyll a in sediment or soil (mg m⁻²)</td>
<td>35.1 ± 0.3</td>
<td>23.8 ± 0.2</td>
<td>11.9 ± 0.1</td>
</tr>
<tr>
<td>DOC surface water (mg L⁻¹)</td>
<td>16.3 ± 0.8</td>
<td>11.2 ± 0.7</td>
<td>15.9 ± 1.1</td>
</tr>
<tr>
<td>DOC pore water (mg L⁻¹)</td>
<td>18.3 ± 0.9</td>
<td>10.8 ± 0.2</td>
<td>14.0 ± 0.5</td>
</tr>
<tr>
<td>DON surface water (µg L⁻¹)</td>
<td>18 ± 6</td>
<td>19 ± 5</td>
<td>42 ± 12</td>
</tr>
<tr>
<td>DON pore water (µg L⁻¹)</td>
<td>17 ± 5</td>
<td>24 ± 7</td>
<td>34 ± 13</td>
</tr>
<tr>
<td>NH₄⁺ surface water (µg N L⁻¹)</td>
<td>116 ± 26</td>
<td>74 ± 10</td>
<td>146 ± 33</td>
</tr>
<tr>
<td>NH₄⁺ pore water (µg N L⁻¹)</td>
<td>98 ± 10</td>
<td>62 ± 3</td>
<td>122 ± 39</td>
</tr>
<tr>
<td>PO₄³⁻ surface water (µg P L⁻¹)</td>
<td>4.3 ± 0.6</td>
<td>4.1 ± 0.3</td>
<td>6.2 ± 0.5</td>
</tr>
<tr>
<td>PO₄³⁻ pore water (µg P L⁻¹)</td>
<td>5.9 ± 1.3</td>
<td>4.9 ± 0.6</td>
<td>7.5 ± 1.6</td>
</tr>
</tbody>
</table>
**Sampling**

Soil and sediment samples were collected once in each season (late November 2007, April 2008, August 2008 and October 2008) in the three major sub-catchments of the Chicken Creek catchment (Gerull et al., 2011). Five sites differing in their hydrological characteristics were selected along each of the three stream corridors (Fig. 2.1): 1) an upland terrestrial site adjacent to the stream channel, 2) an upstream ephemeral channel site, 3) a permanently wet upwelling site, 4) a site with perched surface flow, and 5) a downwelling site. Three replicate samples from the aerobic surface soil or sediment (top 2 cm) were taken at each site (Gerull et al., 2011) and immediately preserved. About 3 g of soil or sediment was preserved in 10ml of 2% formalin containing 0.1% pyrophosphate and stored at 4°C for determination of bacterial abundance. Subsamples for measuring potential enzyme activities were frozen at -20°C as recommended by Sinsabaugh et al. (1991) in case immediate analyses are not possible. Subsamples for molecular analyses were frozen in liquid nitrogen and stored at -80°C until DNA was extracted. However, during shipping on dry ice, the winter samples were delivered with delay, thawed, and were therefore discarded.

**Figure 2.1.** Aerial view of the three main stream networks in the Chicken Creek catchment (A) and position of sampling sites along the hydrological flow path, as illustrated for Stream 2 (B): terrestrial soil (t) and ephemeral (e), upwelling (u), perched flow (p), and downwelling (d) sites in the stream channels.
Enzyme assays

A total of 10 potential enzyme activities were measured with substrate analogues linked to fluorescent molecules, 4-methyllumbiferone (MUB), 7-amino-4-methylcoumarin (AMC), or to 3,4-dihydroxyphenylalanine (L-DOPA) for colorimetric assays of phenol oxidase and peroxidase. Enzymes were chosen based on their metabolic function and use in previous studies (Table 2.2; Sinsabaugh et al., 2008; Sinsabaugh et al., 1991). Enzyme assays were performed as follows: 10 g of soil or sediment sample were placed in 300 ml of acetate buffer (32.1 mM sodium acetate, 0.1% glacial acetic acid, pH 5, autoclaved). The slurry was stirred and volumes of 200 μl were pipetted into 96-well microplates. The 10 different substrate analogues were added to the wells (50 μl of 200 μM stock solutions) and the microplates incubated for 90 min at 12 °C for winter samples, 15 °C for spring and autumn samples, and 18°C for summer samples. There were eight analytical replicates. A volume of 10 μl of 0.5 N NaOH was added to increase pH in the wells before shaking the microplates and measuring fluorescence or absorbance on a microplate reader (Tecan Infinite® 200, Männedorf, Switzerland). The fluorescence emission wavelengths were 445 nm and 450 nm for the cleaved MUB and AMC substrates, respectively. The excitation wavelength was 365 nm for both types of substrate. Absorbance in the phenol oxidase and peroxidase assays was measured at 460 nm using the same microplate reader. Potential enzyme activities are expressed in μmol or mmol substrate per gram sediment or soil dry mass per hour. Potential enzyme activities determined in winter (12 °C) and summer (18 °C) were normalized to the spring and autumn temperature of 15 °C based on a Q_{10} of 2 (Davidson & Janssens, 2006).
Bacterial Abundance
Bacterial abundance of soil and sediment samples was determined by flow cytometry after detaching the cells from sediment or soil grains (Buesing & Gessner, 2002). Cells were detached with a sonifier probe (Branson Digital Sonifier 250, Danbury, USA, flat tip, actual output of 38 W for 3×20 s) as described by Buesing and Gessner (2002). After homogenization, a 1-ml subsample of the bacterial cell suspension was placed on top of 0.5ml of Histodenz® solution (1.3 g ml⁻¹, Sigma-Aldrich, Buchs, Switzerland). Samples were then centrifuged (90 min at 4 °C, 17135 × g) to separate bacterial cells from other particles. The supernatant (layer on top of the Histodenz® layer) was quantitatively removed. The suspended bacterial cells were stained with a solution of 0.1 μl ml⁻¹ SYBR® Green I (Promega, Dübendorf, Switzerland) in anhydrous dimethylsulfoxide (DMSO) and incubated in the dark for 15 min prior to measurement. Samples
were diluted 1:10 or 1:100 with filtered (0.22 μm Millex®-GP, Millipore, Wohlen, Switzerland) mineral water (Evian, France) such that the cell concentration did not exceed $10^6$ ml$^{-1}$. Samples were analyzed with a CyFlow® space Flow Cytometer System (Partec, Görlitz, Germany) equipped with a 200 mW solid state laser emitting light at a fixed wavelength of 488 nm. Green fluorescence was measured at 520 nm (FL1 channel) and red fluorescence at 630 nm (FL3 channel). The flow cytometer was set as follows: gain FL1 = 495, gain FL3 = 650, speed = 4 (implying an event rate never exceeding 1000 events per second). All counts were recorded as logarithmic signals and were triggered on the green fluorescence channel (FL1). Data were processed with Flowmax software (Partec, Germany), using electronic gating to separate the desired events. Presentation of the data as FL1/FL3 dot plots allows for optimal distinction between stained intact microbial cells and instrument noise or sample background (Hammes & Egli, 2005).

**Bacterial Community Fingerprinting**

DNA extraction and purification involved cell breakage, enzymatic digestion of unwanted cell constituent, and DNA purification. Frozen soil and sediment samples (0.5 g fresh mass) were placed in sterile 2-ml reaction tubes containing 0.5 g zirconium beads (0.7 mm diameter, Carl Roth GmbH + Co. KG, Karlsruhe, Germany). Cells were mechanically disrupted by shaking the tubes on a microplate shaker (IKA, VWR, Dietikon Switzerland). A volume of 600 μl phosphate buffer (53 ml L$^{-1}$ of 120 mM NaH$_2$PO$_4$ and 947 ml L$^{-1}$ of 120 mM Na$_2$HPO$_4$, pH=8) and 100 μl of 25% sodium dodecyl sulfate (SDS) was then added. The tubes were fixed horizontally on a shaker, mixed for 10 min, and finally centrifuged for 6 min at 15230 x $g$. The supernatants were transferred to new 2-ml reaction tubes. Enzymatic digestion of the cells was achieved by adding to the reaction tubes 200 μl of lysozyme in TE buffer (10 mg/ml, TE buffer: 10 mM Tris, 1 mM Na$_2$EDTA, pH=8) and placing them on a thermomixer (37°C) for 30 min. Next, 12.5 μl of proteinase K (20 mg/ml) and 150 μl of SDS 25% was added before continuing incubation of the tubes at 55°C overnight. Finally, the DNA was purified and proteins removed from the solution by adding 7.5 M ammonium acetate (0.4x the final volume), placing the tubes on ice for 5 min, and centrifugation for 8 min at 15230 x $g$. The supernatant was transferred to a new reaction tube and a volume of isopropanol corresponding to 70% of the volume...
of the supernatant was added. The tubes were then centrifuged for 60 min at 15230 × g and the supernatant discarded. The pellet was washed twice by shaking the tube with the pellet and with 600 μl of 80% ethanol for 15 s, centrifuging for 5 min at maximum speed (17135 × g), and discarding the supernatant each time. The pellet in the tube was then dried for 1-2 hours in a clean bench and finally re-dissolved in 100 μl of Nanopure water (4°C, overnight). The extracted and purified DNA was stored at -20°C.

PCR amplification of the V3 region of 16S rDNA gene was performed in two steps to obtain enough DNA for the analyses. A first PCR was carried out using forward primer Eub338f (5'-ACT CCT ACG GGA GGC AGC AG-3') and reverse primer Eub518r (5'-ATT ACC GCG GCT GCT GG-3'; Microsynth, Balgach, Switzerland). The same universal bacterial primers were used for the second PCR except that a 40 bp GC-clamp needed for denaturing gradient gel electrophoresis (DGGE) analysis was added to the 3’ end of the forward primer. The PCR reaction mix contained (final concentrations): 1x GoTaq® Flexi reaction buffer (Promega, Switzerland), 3 mM MgCl₂, 0.25 mM dNTPs, 0.25 mM of each primer, and 1 U/μl GoTaq® Flexi DNA Polymerase (Promega, Dübendorf, Switzerland). DNA extract (2 μl) was added to each reaction mix and the final reaction volume adjusted to 20 μl. A first denaturation step was performed at 94°C for 5 min. Each of the following 31 amplification cycles consisted of a heat denaturation step at 94°C for 1 min, primer annealing at 65°C for 30 s and an extension phase at 74°C for 1 min. During primer annealing, a touchdown of 1°C per cycle was programmed for the first 10 cycles. The mix was then maintained at 74°C for 10 min for the final extension. The size and yield of the PCR products were checked on a 2% agarose gel containing a 100 bp ladder (Promega, Dübendorf, Switzerland).

Denaturing gradient gel electrophoresis of 16S rDNA was performed using a D-code electrophoresis system (Bio-Rad Laboratories, Reinach, Switzerland). A 8% (w/v) polyacrylamide gel (acrylamide:bisacrylamide ratio of 37.5:1) was loaded with 600 ng of PCR product. The denaturing gradient increased linearly in the gel from 45% to 60% (top to bottom) of denaturant with 100% of denaturant corresponding to 40% formamide and 7M urea. The first and last lane of each gel contained 2 μl of a commercial ladder for DGGE (DGGE marker II, Wako, Tokyo, Japan), which was used as a reference. The gels were run at 60°C and 38 V for 16h in 1 x TAE buffer (4.84 g L⁻¹ Tris base, 1.14 ml L⁻¹ glacial acetic acid, 2 ml L⁻¹ 0.5 M
Na$_2$EDTA, pH=8). The gels were stained with 0.01% SYBRGreen II solution (Promega, Dübendorf, Switzerland) in 1x TAE buffer in the dark for 25 min. Gel images were taken in a gel documentation system (Gel Doc XR, Bio-Rad) under UV light, and analyzed using the GelCompar II software (Applied Maths NV, Sint-Marteen-Latert, Belgium).

Data analysis
Analysis of variance (ANOVA) was used with the program PASW 18.0 (SPSS Inc., IBM, Armonk, New York, USA) to test for differences in potential enzyme activities and bacterial abundance among seasons, streams and sites. Season and site were treated as fixed factors, and stream as random factor. Samples taken in different seasons were considered independent because the study site is highly dynamic and samples were taken several months apart. QQ-plots and frequency histograms indicated that residuals did not meet assumptions required for parametric tests. Therefore variables (x) were transformed according to ln(x+c), where c = q0.25 x$^2$ / q0.75 x and q = quantile (Statistical Seminar, ETH Zurich, pers. comm.). ANOVAs indicated that the 3-way interactions (season x site x stream) were not significant for most of the enzyme activities, except for aspartate aminopeptidase, phenol oxidase and phenol peroxidase. However, with this design, the assumptions of the model were not met, except for peroxidase. When excluding the 3-way interaction from the design, the resulting model better fit the data and the underlying assumptions were met. Graphical displays showed no indication either that the 3-way interaction was significant, and including or excluding this interaction term produced similar results except for peroxidase and phenol oxidase. Therefore a simplified model was used for the final analyses, in which the 3-way interaction was removed for all enzymes.

Non-metric multidimensional scaling (NMDS) analyses were performed on a matrix of DGGE band information and a matrix including information on all potential enzyme activities. These analyses were performed with the function meta.mds of the package vegan (Oksanen, 2011) of the freeware R (R development Team, 2011) based on Bray-Curtis distances and 1000 permutations. Multivariate analysis of variance was performed with the function adonis in R to test for significant differences among clusters, again based on Bray-Curtis distances.
2.3. Results

**Bacterial abundance**

Bacterial cell abundance varied among sites and seasons without showing any striking pattern (Fig. 2.2). These variations were reflected in a significant interaction between site and season (ANOVA, $F_{12,140}=6.52$, $P<0.001$). Nevertheless, bacterial cell numbers at all sites were highest in summer ($F_{3,6}=47.48$, $P<0.01$) and they were also higher in all seasons at the terrestrial and upwelling sites than at the ephemeral sites of the stream channels ($F_{4,8}=7.05$, $P=0.01$).

![Figure 2.2](image)

**Figure 2.2.** Bacterial abundances (means ± 1SE, $n=9$) in soil and sediments sampled in 4 seasons at 5 sampling sites each along the hydrological flowpath of 3 small stream corridors. Note the logarithmic scale.

**Enzymes activities**

Potential activities of the 10 enzymes analysed varied little among sites and streams in the Chicken Creek catchment (Fig. 2.3). Only the potential activity of glutamate-aminopeptidase differed among sites ($F_{4,8}=6.55$, $P=0.012$), that of celllobiohydrolase among streams ($F_{2,4}=7.45$, $P=0.049$), and that of β-xylosidase among both sites ($F_{4,8}=5.60$, $P=0.02$) and streams ($F_{2,7}=6.68$, $P=0.03$), and these spatial differences did not reveal any clear systematic pattern. Potential peroxidase activity showed significant interactions between site and stream ($F_{8,144}=2.62$, $P=0.01$) and, in the full model, also between site, stream and season ($F_{24,120}=6.02$, $P<0.001$).
When enzyme activities are expressed per amount of organic matter, site differences were slightly more pronounced, with leucine aminopeptidase, glutamate aminopeptidase and phenol peroxidase becoming significant \( (F_{4,9} \geq 5.0, P<0.05) \). This effect was primarily due to higher organic matter contents in terrestrial soil than in stream sediments (Table 2.1; Gerull et al., 2011). However, potential enzyme activities per amount of organic matter were also higher at the perennial stream sites, especially at the upwelling and downwelling sites where sediment organic matter content was low. Cell-specific activities (i.e. activities per bacterial cell abundance) of some enzymes were higher in ephemeral (\( \beta \)-xylosidase and chitinase, \( F_{4,9} = 8.32 \) and \( F_{4,11} = 5.88 \), respectively, \( p<0.01 \)) and terrestrial (\( \beta \)-xylosidase, chitinase and peroxidase, \( F_{4,9} = 8.32, F_{4,11} = 5.88 \) and \( F_{4,9} = 3.89 \), respectively, \( P<0.05 \)) sites compared to the permanently wet sites.

In contrast to rather small spatial differences, temporal differences were large for all enzymes tested except leucine aminopeptidase (Fig. 2.3). However, different enzymes showed markedly different temporal patterns. The potential activity of phosphatase was the highest in summer, with average values up to 250 nmol MUB g\(^{-1}\) h\(^{-1}\), differing greatly from potential activities in other seasons \( (F_{3,6} = 477, P<0.001) \). The polysaccharide-degrading enzymes (\( \beta \)-glucosidase, cellobiohydrolase and \( \beta \)-xylosidase) also showed greater potential activities in summer \( (F_{3,6} = 311, F_{3,6} = 76.2 \) and \( F_{3,6} = 79.4 \), respectively, \( P<0.001 \)), although absolute levels differed among the three enzymes. The potential activity of \( \beta \)-glucosidase in summer \( (43 - 95 \) nmol MUB g\(^{-1}\) h\(^{-1}\)) far exceeded those of cellobiohydrolase and \( \beta \)-xylosidase \( (1.5 - 3.4 \) nmol MUB g\(^{-1}\) h\(^{-1}\)). Chitinase potential activity was also increased in summer \( (F_{3,6} = 24.5, P<0.01) \), ranging from 44 to 55 nmol MUB g\(^{-1}\) h\(^{-1}\), although at some sites, levels of this enzyme were also elevated in winter, when they ranged from 17 to 45 nmol MUB g\(^{-1}\) h\(^{-1}\). Potential peptidase activities (i.e. leucine, glutamate and aspartate aminopeptidase) were rather variable overall and the seasonal differences were less pronounced than for the other types of enzymes (Fig. 2.3). Aspartate aminopeptidase potential activity ranged from undetectable to 13 nmol AMC g\(^{-1}\) h\(^{-1}\) and was highest in summer \( (F_{3,6} = 48.4, P<0.01) \). Glutamate aminopeptidase activity was very low, ranging from undetectable to 0.33 nmol AMC g\(^{-1}\) h\(^{-1}\), with the highest activities occurring in winter and autumn \( (F_{3,6} = 40.76, P<0.01) \). Leucine aminopeptidase, in contrast, showed slightly higher potential activities in summer, although temporal differences were not significant \( (F_{3,6} = 3.6, P=0.085) \).
Figure 2.3. Patterns of enzyme activities (means ± 1SE, n=9) in soil and sediments sampled in 4 seasons at 5 sampling sites each along the hydrological flowpath of 3 small stream corridors.
Phenol oxidase and phenol peroxidase, unlike all other enzymes, showed higher potential activities in spring and autumn compared to winter and summer ($F_{3,6}=41.0$ and $F_{3,6}=36.0$, respectively, $P<0.01$). The potential activities of both enzymes ranged from undetectable to $31 \mu$mol L-DOPA g$^{-1}$ h$^{-1}$. Significant interactions between sampling date and site or stream were observed in 4 of 20 cases but overall these effects were weak.

Temperature-corrected enzyme activities based on a $Q_{10}$ of 2 (see e.g. Davidson & Janssens, 2006) showed similar temporal patterns as uncorrected activities, although seasonal differences tended to be slightly reduced (data not shown). Similarly, the same temporal patterns emerged when enzyme activities were expressed per gram of soil or sediment organic matter, rather than per gram of soil or sediment dry mass, because the organic matter content in soil and sediment fluctuated little over time. Temporal differences in cell-specific enzyme activities were less pronounced than those shown in Fig. 2.3, although still significant for each of the 10 enzymes tested ($F_{4,8-9}=7.3$ to 82, $P<0.01$). Thus, the overall picture that emerges is that temporal differences in potential enzyme activities were pronounced and variable among the 10 enzymes tested, whereas spatial differences were nearly absent.

**Community structure and functional diversity**

Non-metric multidimensional scaling (NMDS) analysis of potential enzyme activities revealed three clusters regrouping most samples taken in winter and spring, in summer, and in autumn, respectively (Fig. 2.4a, $F_{3,141}=135.4$, $P=0.001$), although six autumn samples grouped close to the summer cluster. A similar pattern was not found in the NMDS biplot calculated from DGGE banding patterns, including samples from all 3 streams, 2 sites in each (ephemeral and upwelling sites) and 3 seasons (spring, summer and autumn), which were arranged completely randomly in the NMDS ordination (Fig. 2.4b). As a result, a link between microbial community structure and enzyme activities was not apparent.
Figure 2.4. NMDS ordination of a) potential activities of 10 different enzymes, and b) bacterial community structure inferred from DGGE bands of samples taken at three sampling dates (winter samples were lost during transport) and two types of sites (ephemeral and upwelling sites).
2.4. Discussion

Spatial variability of potential enzyme activities

An important result of our analysis of enzymes in the early successional Chicken Creek catchment is that potential activities varied little across sites. This resemblance for all of the ten enzymes studied is unexpected because sites, ranging from dry soil crusts to permanently submerged stream sediments, differed widely in terms of water availability, a key factor determining microbial metabolism. The unresponsiveness of potential enzyme activities to differences in site characteristics is in accordance with observations on general microbial metabolism measured as respiratory activity at various sites in a barren desert of Antarctica (McMurdo Valley, Zeglin et al., 2009) and in the early successional Chicken Creek catchment (Gerull et al., 2011). This suggests, contrary to our hypotheses, that neither permanence of water availability (perennial versus ephemeral sites) nor direction of vertical water exchange (groundwater upwelling versus channel water downwelling) control potential enzyme activities in early successional catchments with scarce vegetation cover.

A feature of sediments and soils that often correlates with potential enzyme activities is organic matter content (e.g. Romaní & Marxsen, 2002; Sinsabaugh et al., 2008; Artigas et al., 2009). This is corroborated by higher activities of cellulose- and hemicellulose-degrading enzymes in hyporheic sediments of an upwelling zone of a lowland stream with more organic matter than in a downwelling zone (Rulík & Spácil, 2004). However, in line with the lack of spatial variation in potential enzyme activities, organic matter content varied little in the Chicken Creek catchment, and although it was slightly higher at the terrestrial sites (Gerull et al., 2011), this difference was small and apparently insufficient to alter potential enzyme activities.

Temporal variability of potential enzyme activities

More striking than the spatial similarities in potential enzyme activities are the highly congruent changes we observed over time. Seasonal temperature fluctuations accounted for a small fraction of the activity increases of many enzymes in the summer, as revealed by persistent temporal patterns when the data were normalized to a standard temperature. Furthermore, temperature can evidently not account for the often-elevated potential activities in spring, autumn and, in one case (chitinase), in winter. This limited temperature response is
consistent with data from surface water (Sinsabaugh & Follstad Shah, 2010b),
fine sediments (Wilczek et al., 2005), and biofilms (Jones & Lock, 1993; Romaní &
Sabater, 2001) of lowland streams and rivers and suggests important, seasonally
varying determinants of enzyme activities in addition to temperature.

One such factor is light. Although neither particularly strong \( r=0.24-0.33 \)
nor necessarily indicative of cause-and-effect relationships, highly significant
correlations between chlorophyll-\( a \) content in soil and sediments (data from
Gerull et al., 2011) and potential activities of phosphatase and all carbon-
acquiring hydrolytic enzymes suggest that algae were instrumental in increasing
potential enzyme activities in summer. This could be due directly to algal enzyme
production, especially in the case of phosphatase. Two alternative mechanisms
involving heterotrophs are more likely, however, at least for the carbon acquiring
enzymes: heterotrophs may excrete enzymes using carbon compounds of algal
origin as the main substrate, or the organic carbon supplied by algae serves as
priming agent facilitating the enzymatic cleavage of recalcitrant carbon
compounds from other sources (Guenet et al., 2010; Rier et al., 2007). Such
priming might indeed be an important mechanism in the Chicken Creek
catchment, because DOC is abundant (around 14 mg L\(^{-1}\)) but highly recalcitrant
(Gerull et al., 2011). POC also appears to be recalcitrant in the catchment, as
suggested by significant correlations between sediment organic matter content
and potential activities of ligninolytic (phenol oxidase and peroxidase; \( r=0.19 \) and
0.26, \( P<0.02 \)) but not polysaccharide-degrading enzymes (\( r\leq0.3, P>0.7 \), except for
\( \beta\)-xylosidase, \( P = 0.08 \)), suggesting that priming could influence the utilization of
POC as well. Irrespective of the predominant mechanism (i.e. direct use of algal
carbon or priming), the increased bacterial abundances we observed in summer
indicate that heterotrophs contributed to the elevated potential activities of many
of the enzymes we determined.

The tight synchrony of potential enzyme activities observed across sites is
further amplified by stark contrasts in the temporal patterns of the ten individual
enzymes tested. This demonstrates that measures of general metabolic activity of
microbes, such as respiration, merely reflect average responses of microbial
activities to environmental conditions or microbial community structure.
Mechanistic understanding of the link between individual metabolic activity and
ecosystem process rates might require analyses with finer resolution. The
potential enzyme activities measured here are more informative in this regard
than bulk respiration measurements. Nevertheless, they might still be too coarse, given that proteomic analysis has revealed that even individual microbial strains on decomposing leaf litter can simultaneously express nearly two dozen enzymes of a given class (e.g. cellulases; Schneider & Riedel, 2010), let alone the number of enzymes expressed by diverse microbial communities.

Temporal variation of two nitrogen-acquiring enzymes used in our study (aspartate- and glutamate-aminopeptidase) showed strikingly opposite temporal patterns, although the basic function and molecular structure of the two enzymes is very similar. This discrepancy points to the complexity of relationships between microbial activities and environmental conditions and further underlines the difficulty of interpreting spatio-temporal patterns of potential activities of individual enzymes in terms of broad ecosystem processes.

Temporal patterns of oxidases capable of attacking aromatic rings were markedly different from those of all other enzymes. With algal photosynthesis declining in the autumn, the supply of easily degradable organic matter should also decrease, and eventually be completely consumed by heterotrophic microbes. This would leave behind a recalcitrant organic matter pool that includes a large fraction of aromatic compounds. High potential activities of phenol oxidase and peroxidase in this season could reflect the degradation of these compounds. Subsequent declines in winter (along with those of all other enzymes except chitinase) but reestablishment of high activities in spring appear to arise from temperature limitation during the cold season when ice sheets regularly formed in the streams at night.

**Bacterial community structure and ecosystem function**

A particularly intriguing finding of our analysis is that potential activities of the broad suite of carbon, nitrogen and phosphorus acquiring enzymes we measured were unrelated to the structure of bacterial communities in the early successional Chicken Creek catchment. Phrased differently, community structure was unrelated to function (see also Burke et al., 2011). This disconnect arose because of a lack of clear spatial or temporal patterns in bacterial communities, whereas the activities of all ten enzymes measured showed distinct temporal changes (see above). This finding is surprising in view of the general paradigm in ecology and biology that structure determines function.
The complete absence of temporal or spatial community pattern suggests stochastic distributions of populations following the neutral community assembly model (Sloan et al., 2006; Hubbell, 2001). Accordingly, community structure in environmental patches is shaped only by population growth or declines, and by random immigration of individuals of new populations. High dispersal and invasion ability of bacteria in a uniform environment can then lead to ubiquity of communities across space and time (Van Der Gucht et al., 2007; Urban & De Meester, 2009). Our results indicate that high dispersal can override the influence of even substantial environmental differences. This includes strong spatial and temporal variation in water availability, which arguably is one of the most critical constraints of microbial activities.

The striking disconnect between bacterial community structure and potential enzyme activities we observed also appears to be counter to results from controlled experiments with assembled communities, where bacterial diversity (i.e. a component of community structure) has been found to matter for ecosystem functioning (Bell et al., 2005; Langenheder et al., 2010; Peter et al., 2011). However, those experiments primarily focused on assessing effects of varying numbers of species or strains rather than the composition of communities and abundance of dominant strains or species as in the present study. Moreover, in line with our results, field studies that have indirectly manipulated microbial communities have not typically found evidence for strong relationships between community structure and rates of ecosystem processes (Langenheder et al., 2005, 2006; Wertz et al., 2006; Östman et al., 2010).

One reason for these differences might be methodological, specifically the inability of DGGE to sufficiently resolve the structure of diverse communities (Woodcock et al., 2006) that could have rapidly established even in the early successional Chicken Creek catchment. DGGE tends to detect dominant taxa only, resulting in a greatly simplified blueprint of the communities characterized with this kind of fingerprinting methods (Casamayor et al., 2000; Lindström, 2000; Woodcock et al., 2006). This explanation would imply, however, that patterns of ecosystem functioning (i.e. potential enzyme activities) are primarily driven by subordinate as opposed to the dominant species or strains that are best detected by DGGE. Conversely, one cannot rule out the possibility that DGGE detected a significant fraction of dormant bacteria, which have been estimated to account for
an average of about 45% of all bacterial cells in fresh waters (Lennon & Jones, 2011). However, both of these scenarios are rather unlikely.

Alternatively, the lack of congruent patterns between bacterial community structure (DGGE fingerprint pattern) and function (potential enzyme activities) in space and time can arise from functional redundancy of the bacterial communities. If the few taxa dominating in communities are generalists in terms of the functions they perform in a given ecosystem, then shifts in community structure would induce only weak effects on functional characteristics (Östman et al., 2010). This might be particularly relevant in early successional environments, such as the Chicken Creek catchment, where low resource supply could favour bacterial communities composed of a large fraction of generalists that profit from a broad range of scarce resources (Egli, 1995) and, when switching among metabolic pathways, excrete different kinds of enzymes. It is noteworthy in this context that although bacterial diversity was found to affect rates of ecosystem processes (determined as rates of substrate utilization) for example in the experiment by Langenheder et al. (2010), there was pronounced functional redundancy even at the low bacterial richness levels examined in that study. The functional redundancy hypothesis thus appears to be the most likely explanation for the disconnect between bacterial community structure and function (i.e. potential enzyme activities) in environments akin to the Chicken Creek catchment, a conclusion that has recently been drawn also from another microbial system (Burke et al., 2011).

2.5. Acknowledgements
We thank Vattenfall Europe Mining AG for providing the research site; A. Ling, R. Ender, T. Wolburg, M. Weber, C. Kessler, M. Seidel, J. Westphal, K. Westphal, and M. Stange for field or laboratory assistance; G. Lippert, U. Abel, R. Müller and G. Franke for water chemical analyses; F. Hammes and colleagues for support for the use of flow cytometry; L. Rosinus at the Seminar for Statistics of ETH Zurich for statistical advice; and R. Freimann for critically important comment on the manuscript. This study is a contribution to the Transregional Collaborative Research Centre 38 (SFB/TRR 38) funded by the German Research Council (DFG, Bonn) and the Brandenburg Ministry of Science, Research and Culture (MWFK, Potsdam).
Chapter 3

Fungal importance for stream metabolism: an experimental test in early successional stream channels

Frossard Aline, Linda Gerull, Michael Mutz and Mark O. Gessner

Abstract
Fungi are important decomposers of leaf litter in streams with possible knock-on effects on other stream microbes and on carbon cycling. To elucidate such effects, we designed an experiment in outdoor experimental channels simulating sandy-bottom streams in an early successional state. We hypothesized that overall microbial activity would be enhanced in the presence of fungi, accompanied by a shift in the community (fungi and bacteria) on leaf litter. Fifteen experimental channels (4.0 × 0.12 × 0.12 m) filled with sterile sediments and leaf litter were inoculated with one of four different suspensions made with water from forest streams. The suspensions contained either: 1) fungi and bacteria, 2) bacteria only, 3) no microorganisms, or 4) dead microorganisms. Water from an early-successional watershed was then circulated through the channels for 5 weeks. We measured metabolism and sampled sediment and leaf litter at 6 dates during that time. Whole-stream metabolism and microbial respiration associated with leaf litter were higher in experimental streams inoculated with fungi, reflecting higher fungal activity on leaves. There were no significant differences among the two controls and the bacteria treatment, suggesting a minor role of bacteria in microbial metabolism. Bacterial biomass either on leaf litter or in sediments did not vary among treatments at any time, but sediment microbial respiration increased dramatically at the last sampling date in the stream channels that had received a fungal inoculum. Effects of the fungal community on bacterial community structure on leaves were important. Similarly, increases in net primary production and chlorophyll-a content on the sediment surface were
greatest in the stream channels inoculated with fungi. These results point to a major role of fungal communities in stream ecosystems, well beyond the direct involvement in leaf litter decomposition.

3.1. Introduction

A fundamental and well-recognized function of fungi in stream ecosystems is the decomposition of plant litter. Fungal decomposers belonging to a phylogenetically heterogeneous group known as aquatic hyphomycetes generally dominate the microbial communities on decomposing leaf litter in streams (Gessner et al., 2007). When submerged, they produce large numbers of characteristically shaped spores that facilitate microscopic identification and quantitative assessment of fungal reproductive activity (Gessner et al., 2003). They are also thought to play a much more important role in litter decomposition than aquatic bacteria (Gessner et al., 2007; Duarte et al., 2010) This notion is supported by multiple lines of evidence (Gessner et al., 2007), including the observation that fungal biomass in decomposing leaves in streams greatly exceeds bacterial biomass, typically accounting for 90% or more of the total microbial biomass (Baldy et al., 2002; Gulis & Suberkropp, 2003; Gessner et al., 2007; Duarte et al., 2010). Fungi are thus recognized as a major microbial group of decomposers in stream ecosystems.

While dominant on organic substrates such as leaf litter, fungi are not universally important in streams. Their significance relative to that of bacteria rather appears to vary with the type of organic matter colonized. Bacteria increase in relative importance as the particle size of organic matter decreases (Findlay et al., 2002). This has been attributed to increasing surface-to-volume ratios that offer scope for colonization and uptake of dissolved organic matter (Findlay et al., 2002; Docherty et al., 2006). In addition, bacteria and algae effectively colonize mineral surfaces in streams, where they form extensive biofilms, including in sandy sediments (Stevenson, 1996; Artigas et al., 2008a), whereas fungi are not particularly prominent in this habitat (e.g. Gessner et al., 2007; Harrop et al., 2009). Thus, bacteria are assumed to play a major role in the metabolism of stream sediments, although fungal biomass can still be significant in such situations when sediment organic matter content is high (Artigas et al., 2004).
In microcosm experiments, antagonistic interactions between bacteria and fungi have been demonstrated (Wohl & McArthur, 2001; Mille-Lindblom & Tranvik, 2003). Fungal growth was reduced in the presence of bacteria in both experiments. However, bacteria biomass was unaffected by the presence of fungi in the study by Wohl & MacArthur (2001), whereas reciprocal antagonistic effects of bacteria and fungi were detected as early as 2 weeks after inoculation of the microcosms studied by Mille-Lindblom and Tranvik (2003). These antagonistic interactions, however, had no significant repercussions on the decomposition of organic matter (Mille-Lindblom & Tranvik, 2003), and Wohl & MacArthur (2001) even found synergistic effects on organic matter decomposition when microcosms were inoculated with fungi 2 days before bacteria were added.

Microbial diversity on leaf litter tends to peak after 2 to 3 weeks of exposure of the leaves in the stream (Das et al., 2007, Nikolcheva et al., 2003). However, colonization patterns of microbes through time have been shown to be different for bacterial and fungal communities; in a study of leaf litter colonization by microbes, Harrop et al. (2009), observed an increase of the bacterial diversity after initial colonization, suggesting the formation of more complex communities as leaf nutrients became more readily available for bacteria, and as the biofilm development created new microhabitats.

The main objective of the experiment presented here was to assess whether the occurrence of fungi and bacteria in recently created streams has consequence for stream ecosystem metabolism and whether such responses reflect differences in the structure of microbial communities associated with both decomposing leaf litter and stream sediment. We hypothesized that the presence of fungi in such early-successional streams, characterized by low amounts of plant litter present, will still have an effect on stream metabolism as a whole in addition to the leaf litter compartment, but not on microbial activity in stream sediments. Similarly, we hypothesized that the presence of fungi will affect the structure of bacterial communities associated with leaf litter but not with sediments.
3.2. Material and Methods

**Experimental set-up**

We conducted a full factorial experiment in outdoor channels simulating early-successional sand-bed streams. The channels (4.0 x 0.12 x 0.12 m) were set up next to a recently created experimental catchment (Chicken Creek) near Cottbus in Eastern Germany (51°36' N, 14°16' E) situated in a former brown-coal mining area (Gerwin et al., 2009; Gerull, 2011, chapter 3). They were filled with a 4 cm-layer of sterilized sand from the catchment (combustion overnight at 160°C) and fed with water originating from the catchment. Naturally abscised birch leaves (*Betula pendula*) were sterilized by γ-radiation (0.75 Mrad, 52 h), individually weighed, attached to strings, and placed on top of the sediment. Thirty six leaves were introduced in each channel, corresponding to about 2 g dry mass m⁻² of sediment surface, or 100-200 times less than the typical annual input of plant litter to natural forest streams.

Replicate channels were inoculated with stream water from the catchment containing either: 1) bacterial and fungal communities, 2) bacterial communities only, 3) no microorganisms, or 4) dead bacteria and fungi. As we were interested in making general inferences about the effect of microbial community types, we used inocula from four local forest streams rather than from a single stream. This resulted in four true replicates of stream microbial communities for inoculation of 16 experimental channels in total. However, a technical failure of one of the experimental channels reduced the replication level of the control treatment with the killed fungal and bacterial suspension to three. To generate the inocula, birch leaves from the same batch used in the experiment were enclosed in a total of 12 litter bags (100 g air-dried) and three of them were submerged in each of four local streams. After two weeks, the colonized leaves were retrieved, cleaned under water, incubated for 48 h in 5 l of stream water filtered over 5 μm pore size membrane filters (Sartorius, Göttingen, Germany), and vigorously aerated by aquarium pumps to induce fungal sporulation. The resulting suspension was used as Inoculum 1 (fungi plus bacteria). For Inoculum 2 (bacteria only), the suspension was filtered over 5-μm membrane filters to remove fungal spores and larger hyphal fragments, and for Inoculum 3 (no microorganisms), the suspension was filtered over 0.2-μm membrane filters (Sartorius, Göttingen, Germany) and autoclaved to kill any remaining microorganisms. Inoculum 4 consisted of directly autoclaving the suspension containing all particles (i.e.
Inoculum 1) to serve as a second control testing the effect of the presence of organic particle (>0.2 μm), effect not included in the Inoculum 3 where the water suspension was filtrated at 0.2 μm.

After inoculation, the stream channels ran in complete recirculation mode for 24 h with filtered (5-μm pore-size membrane filters) Chicken Creek water to facilitate establishment of the added microorganisms. Subsequently, the channels received well-oxygenated water from a subterranean tank (Gerull, 2011, chapter 3) to replace in the now partially recirculating channels 5 % of the water volume during each cycle. The current velocity was 1.7 ± 0.3 cm s⁻¹ at a water depth of 1 cm. This resulted in an average water renewal time of 6 h. After 7 days, the channels were re-inoculated in the same way to ensure good microbial colonization of sediment and leaf litter.

All stream channels were regularly monitored for temperature, conductivity and pH, which were directly measured in the channels (Table 3.1). Surface water in the channels was collected with a syringe, filtered through prewashed 0.45-μm pore size cellulose acetate filters (Millipore, Zug, Switzerland), frozen (-20 °C) and later analyzed for nutrients (total dissolved phosphorus, NO₂⁻, NO₃⁻, NH₄⁺) and dissolved organic carbon (Table 3.1). Oxygen depth profiles in the sediment were measured at the end of the experiment at three sites in each flume (Table 3.2). An optode microsensor (0.9 mm diameter; Microx TX 3, Presens GmbH, Regensburg, Germany) was used for this purpose. It was protected in a 20-cm steel tube with a 1.7-mm² opening at the tip. The tube was slowly lowered to the desired depths in the sediment before a small volume of pore water was drawn into the tube with a syringe connected to the upper tube end. Volumes of 50-100 μl were sufficient to cover the probe tip and avoid mixing of two successive samples.
**Table 3.1.** Physico-chemical characteristics of water and sediment in experimental stream channels receiving one of four different inocula. Values are means with 1 s.e. in parentheses (n=3-4). n.d.: not determined

<table>
<thead>
<tr>
<th>Elapsed time</th>
<th>Temperature (°C)</th>
<th>pH</th>
<th>Conductivity (μS cm⁻¹)</th>
<th>DOC (mg L⁻¹)</th>
<th>Total P (μg N L⁻¹)</th>
<th>NH₄⁺ (μg N L⁻¹)</th>
<th>NO₂⁻ (mg N L⁻¹)</th>
<th>NO₃⁻ (mg N L⁻¹)</th>
<th>Sediment OM (mg g⁻¹)</th>
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<tbody>
<tr>
<td><strong>4 days</strong></td>
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<td></td>
</tr>
<tr>
<td>Fungi and bacteria</td>
<td>11.7 (0.0)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>28.5 (5.9)</td>
<td>96.8 (24.3)</td>
<td>30.2 (6.7)</td>
<td>2.0 (0.6)</td>
<td>&lt;0.1</td>
<td>0.67 (0.04)</td>
</tr>
<tr>
<td>Bacteria only</td>
<td>n.d.</td>
<td>n.d.</td>
<td>30.0 (4.3)</td>
<td>96.0 (39.4)</td>
<td>18.0 (1.4)</td>
<td>1.7 (0.4)</td>
<td>&lt;0.1</td>
<td>0.74 (0.07)</td>
<td></td>
</tr>
<tr>
<td>No microorganism</td>
<td>11.1 (0.0)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>28.9 (6.5)</td>
<td>138.2 (79.4)</td>
<td>15.5 (7.9)</td>
<td>2.2 (1.5)</td>
<td>&lt;0.1</td>
<td>0.71 (0.08)</td>
</tr>
<tr>
<td>Dead microorganisms</td>
<td>n.d.</td>
<td>n.d.</td>
<td>30.5 (5.3)</td>
<td>188.2 (84.4)</td>
<td>13.1 (4.7)</td>
<td>1.7 (0.5)</td>
<td>&lt;0.1</td>
<td>0.67 (0.08)</td>
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<tr>
<td><strong>11 days</strong></td>
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</tr>
<tr>
<td>Fungi and bacteria</td>
<td>8.6 (0.5)</td>
<td>8.6 (0.05)</td>
<td>972 (104)</td>
<td>12.5 (0.8)</td>
<td>22.5 (6.4)</td>
<td>90.3 (13.5)</td>
<td>8.4 (0.2)</td>
<td>0.16 (0.02)</td>
<td>0.80 (0.16)</td>
</tr>
<tr>
<td>Bacteria only</td>
<td>8.8 (0.0)</td>
<td>8.6 (0.01)</td>
<td>905 (5)</td>
<td>11.8 (0.4)</td>
<td>11.3 (1.8)</td>
<td>66.4 (12.5)</td>
<td>7.4 (0.3)</td>
<td>0.13 (0.01)</td>
<td>0.96 (0.43)</td>
</tr>
<tr>
<td>No microorganism</td>
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<td>8.6 (0.01)</td>
<td>972 (24)</td>
<td>12.3 (1.2)</td>
<td>7.3 (2.0)</td>
<td>55.8 (15.4)</td>
<td>7.6 (1.7)</td>
<td>0.15 (0.02)</td>
<td>0.92 (0.22)</td>
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<tr>
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<td>8.6 (0.00)</td>
<td>924 (28)</td>
<td>12.0 (5.3)</td>
<td>7.5 (2.2)</td>
<td>46.4 (18.8)</td>
<td>6.2 (1.2)</td>
<td>0.16 (0.01)</td>
<td>0.91 (0.10)</td>
</tr>
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<td><strong>18 days</strong></td>
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<td></td>
</tr>
<tr>
<td>Fungi and bacteria</td>
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<td>8.3 (0.04)</td>
<td>989 (14)</td>
<td>12.6 (0.6)</td>
<td>6.1 (0.9)</td>
<td>43.1 (6.2)</td>
<td>8.0 (2.1)</td>
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<td>0.89 (0.10)</td>
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<td>8.4 (0.03)</td>
<td>991 (7)</td>
<td>12.5 (0.5)</td>
<td>8.4 (2.8)</td>
<td>30.4 (8.5)</td>
<td>6.8 (2.8)</td>
<td>0.17 (0.02)</td>
<td>0.74 (0.07)</td>
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<tr>
<td>No microorganism</td>
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<td>8.3 (0.08)</td>
<td>995 (6)</td>
<td>12.2 (0.4)</td>
<td>5.3 (0.9)</td>
<td>33.3 (10.1)</td>
<td>7.7 (1.2)</td>
<td>0.17 (0.02)</td>
<td>0.74 (0.02)</td>
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<td>7.1 (2.4)</td>
<td>26.1 (20.0)</td>
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<tr>
<td>Fungi and bacteria</td>
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<td>8.3 (0.03)</td>
<td>1030 (10)</td>
<td>11.0 (1.5)</td>
<td>9.4 (1.0)</td>
<td>4.3 (0.5)</td>
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<td>0.59 (0.05)</td>
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<td>9.7 (0.6)</td>
<td>7.1 (0.7)</td>
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<td>&lt;0.1</td>
<td>0.66 (0.16)</td>
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<tr>
<td>No microorganism</td>
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<td>8.2 (0.04)</td>
<td>1015 (5)</td>
<td>9.8 (0.1)</td>
<td>5.9 (0.6)</td>
<td>26.1 (13.8)</td>
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<td>0.78 (0.13)</td>
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<td>986 (20)</td>
<td>8.9 (0.0)</td>
<td>7.7 (0.0)</td>
<td>34.9 (0.0)</td>
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<td>&lt;0.1</td>
<td>0.73 (0.20)</td>
</tr>
<tr>
<td><strong>32 days</strong></td>
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</tr>
<tr>
<td>Fungi and bacteria</td>
<td>3.8 (0.5)</td>
<td>8.2 (0.03)</td>
<td>980 (40)</td>
<td>11.6 (0.3)</td>
<td>5.7 (0.5)</td>
<td>22.5 (6.2)</td>
<td>1.7 (0.3)</td>
<td>&lt;0.1</td>
<td>0.86 (0.07)</td>
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<tr>
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<td>8.2 (0.02)</td>
<td>993 (57)</td>
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<td>5.8 (2.3)</td>
<td>24.2 (5.5)</td>
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<td>0.83 (0.05)</td>
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<td>1017 (12)</td>
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<td>3.8 (0.9)</td>
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<td>2.4 (2.0)</td>
<td>&lt;0.1</td>
<td>0.80 (0.11)</td>
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<tr>
<td>Dead microorganisms</td>
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<td>1024 (2)</td>
<td>12.5 (1.8)</td>
<td>8.0 (2.2)</td>
<td>23.3 (10.1)</td>
<td>4.9 (0.0)</td>
<td>&lt;0.1</td>
<td>0.93 (0.10)</td>
</tr>
<tr>
<td><strong>36 days</strong></td>
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</tr>
<tr>
<td>Fungi and bacteria</td>
<td>3.4 (0.2)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>10.8 (1.7)</td>
<td>6.5 (2.3)</td>
<td>18.1 (6.1)</td>
<td>2.0 (0.8)</td>
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<td>n.d.</td>
</tr>
<tr>
<td>Bacteria only</td>
<td>3.3 (0.3)</td>
<td>n.d.</td>
<td>9.3 (0.1)</td>
<td>4.8 (0.8)</td>
<td>19.4 (0.3)</td>
<td>1.4 (0.0)</td>
<td>&lt;0.1</td>
<td>n.d.</td>
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<tr>
<td>No microorganism</td>
<td>3.3 (0.2)</td>
<td>n.d.</td>
<td>9.5 (0.3)</td>
<td>5.3 (1.4)</td>
<td>19.0 (3.7)</td>
<td>1.8 (0.4)</td>
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</tr>
<tr>
<td>Dead microorganisms</td>
<td>3.6 (0.3)</td>
<td>n.d.</td>
<td>9.7 (0.4)</td>
<td>4.6 (0.6)</td>
<td>27.3 (5.2)</td>
<td>1.9 (1.2)</td>
<td>&lt;0.1</td>
<td>n.d.</td>
<td></td>
</tr>
</tbody>
</table>
**Table 3.2.** Oxygen concentrations (mean, 1 s.e. in parentheses, n=3) in the sediment of experimental stream channels receiving one of four different inoculum types and sampled at different sediment depth 36 days after inoculation.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Fungi and bacteria</th>
<th>Bacteria only</th>
<th>No microorganism</th>
<th>Dead microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10.6 (0.2)</td>
<td>10.3 (0.3)</td>
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<td>0.5</td>
<td>11.0 (0.6)</td>
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<td>1</td>
<td>10.9 (0.7)</td>
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<td>2</td>
<td>10.2 (0.7)</td>
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<td>3</td>
<td>8.8 (1.2)</td>
<td>8.9 (1.2)</td>
<td>7.3 (0.2)</td>
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</table>

**Sampling**

Sediment and leaf litter samples were randomly collected in each channel after 4, 11, 18, 25, 32 and 36 days and processed within 12 h after sampling. Sediment samples consisted of three pooled cores that were randomly collected in each channel to the maximum sediment depth of 4 cm. The cores were taken with a syringe whose lower end was cut off (1.57 cm² surface area). Sediment organic matter content (Table 3.1) was determined as the difference between sediment dry mass (12 h at 105°C) and ash-free dry mass (combustion for 4 h at 450 °C). Six leaves were also randomly retrieved from the channels on each sampling occasion. They were gently cleaned from mineral deposits with filtered (5-µm pore size) Chicken Creek water. Sediment and leaf samples were used immediately for respiration measurements, preserved in 2% paraformaldehyde for later bacterial biomass determinations, or shock-frozen in liquid nitrogen and stored at -80 for molecular analyses. Leaf mass loss was calculated as the difference between the initial and final dry mass, following drying of leaves at 105 °C and weighing to the nearest 0.1 mg.

**Whole-ecosystem metabolism**

Whole-stream metabolism was measured by sealing the stream channels with Perspex lids and recording changes in oxygen concentration for 1 h using optodes (10-channel Minisensor Oxygen Meter, Oxy 10 mini, PreSens GmbH, Regensburg, Germany; Gerull, 2011, chapter 3). The channels were covered with opaque tarpaulin to measure respiration for 1 h. Net community production (NCP) was subsequently measured for another hour after the cover had been removed.
These measurements were taken at around 11 a.m. To estimate respiration rates in the stream water, changes in oxygen concentrations were simultaneously determined in an air-tight glass bottle filled with the groundwater delivered to the channels. Temperature in the stream channels was recorded at the beginning and end of the measurements and used to normalize respiration rates for temperature. Daily gross primary production (GPP) was calculated as the NCP integrated over the daylight hours (10.2–8.4 h depending on the sampling day) and the integrated respiration during these periods. Respiration rates were extrapolated to 24 h based on the assumption that daytime respiration equaled respiration at night, and P/R ratios were calculated as the quotient of daily GPP and respiration.

**Respiration associated with sediment and leaf litter**

Microbial respiration associated with sediment and leaf litter was measured at 10°C in the laboratory (Gerull et al., 2011). Respiration associated with sediment was measured by percolating sediment cores (Gerull et al., 2011), whereas respiration associated with leaf litter was measured in 50-ml glass bottles containing two leaves and water from the stream channels. After temperature acclimation (1 h), the decline in oxygen concentrations was recorded overnight (12 h) with an optical oxygen meter (Microx TX3, PreSens GmbH, Regensburg, Germany). The sediment and leaf-litter samples were dried after the measurements (105 °C, 12 h) and combusted in a muffle furnace (430 °C, 3 h) to determine dry mass (DM) and organic matter (OM) content.

**Bacterial, fungal and algal biomass**

Bacterial abundance was determined by flow cytometry (Chapter 2) after detachment of bacterial cells from leaves and sediments with an ultrasonic probe (Buesing & Gessner, 2002). Briefly, the detached bacterial cells were separated from other particles by collecting them in Histodenz® solution (Caracciolo et al., 2005), staining with SYBRGreen I, and counting on a CyFlow® space Flow Cytometer System (Partec, Görlitz, Germany) equipped with a 200-mW solid-state laser (light emission at 488 nm) and volumetric counting hardware (Hammes & Egli, 2005). A factor to convert the number of counted bacteria to biomass was determined by epifluorescence microscopy. Biovolumes of a total of 13,000 bacterial cells were measured from 12 different stream sediment samples.
(Frossard et al., unpublished data) and converted to biomass using the biovolume-biomass relationship established by (Loferer-Krössbacher et al., 1998).

Fungal biomass was determined by extracting and quantifying ergosterol (Gessner & Newell, 2002). Leaves were freeze-dried and weighed before extracting lipids in alkaline methanol (80 °C, 30 min) with stirring. The extract was cleaned and concentrated by solid-phase extraction (SPE; Waters Sep-Pak®, Vac RC, tC18, 500 mg; (Gessner & Schmitt, 1996). The extraction efficiency was routinely monitored with external ergosterol standards. Ergosterol in the extracts eluting from the SPE cartridges were separated and quantified on a high-performance liquid chromatograph (HPLC) consisting of two Jasco PU-980 (Tokyo, Japan) pumps, a Jasco AS-950 autosampler, a LichroSpher 100 RP-18 column (0.46 × 25 cm; Merck Inc., Darmstadt, Germany), and a Jasco MD 2010 Plus multiwavelength detector set at 282 nm. The column temperature was 33 °C. A factor of 5.5 mg ergosterol g⁻¹ fungal dry mass was used to convert ergosterol values to dry fungal biomass (Gessner & Chauvet, 1993).

Chlorophyll a was used as a proxy of algal biomass in sediments. It was extracted with ethanol (90%) for 4 min at 70 °C and subsequent sonification for 2 min in an ultrasonic bath set at 40% of its maximum power (Elma Transsonic Digital T790/H, Singen, Germany). The extracts were filtered through two paper filters (MN 619, Macherey-Nagel, Düren, Germany) before measuring absorbance at 665 nm.

**Fungal sporulation**

Sporulation of aquatic hyphomycetes was induced by submerging two leaves from each channel in 47 ml filtered (5 µm pore size) Chicken Creek water kept at 10 °C with constant shaking (Gessner et al., 2003). Three ml of 37% formalin was added after 48 h to preserve the samples. Three to 5 ml of the spore suspension was filtered on a membrane filter (5 µm pore size), the filter placed on a slide and the spores of aquatic hyphomycetes stained with Trypan blue. About 200 spores in 10-30 microscopic fields on each filter were identified and counted at a magnification of 200×. Total spore biomass was calculated as the sum of individual spore masses of the dominant species (Hieber & Gessner, 2002).
Fungal and bacterial community profiles

Fungal and bacterial communities were assessed by automated ribosomal intergenic spacer analysis (ARISA). DNA was extracted from frozen sediment and leaf litter as previously described (Chapter 2). Briefly, cells were mechanically disrupted and enzymatically digested before DNA was separated by centrifugation, purified in multiple steps, and stored at -20 °C.

The complete intergenic region of fungal rDNA (ITS1, 5.8S and ITS2) was amplified using the labeled forward primer ITS1F-FAM (5’FAM-CTT GGT CAT TTA GAG GAA GTA A-3’) and reverse primer ITS4A (5’CGC CGT TAC TGG GGC AAT CCC TG-3’; Microsynth, Balgach, Switzerland; Torzilli et al., 2006). The PCR reaction mix (25 μl) contained (final concentrations): 1x GoTaq® Flexi reaction buffer (Promega, Dübendorf, Switzerland), 2.5 mM MgCl₂, 0.25 mM dNTPs, 0.5 mM of each primer, 0.1 mg ml⁻¹ of bovine serum albumin (BSA), 0.05 U μl⁻¹ GoTaq® Flexi DNA polymerase (Promega), and 2 μl of DNA extract. The initial DNA denaturation occurred at 94 °C for 11 min. Each of the following 35 amplification cycles involved a denaturation step at 94 °C for 1 min, primer annealing at 48 °C for 1 min and an extension phase for 2 min at 72 °C. The final extension occurred at 72 °C for 45 min.

PCR amplification of the intergenic region of bacterial rDNA was carried out using the labeled forward primer 1406F-FAM (5’FAM-TGY ACA CAC CGC CCG T-3’, T = T, C) and reverse primer 23Sr (5’-GGG TTB CCC CAT TCR G-3’, B = G, T, C and R = G, A; Microsynth; Yannarell et al., 2003). The PCR reaction mix (25 μl) and PCR conditions slightly differed from those used for fungal DNA. The PCR mix contained 1x GoTaq® Flexi reaction buffer, 3 mM MgCl₂, 0.25 mM dNTPs, 0.4 mM of each primer, 0.25 mg ml⁻¹ BSA, 0.05 U μl⁻¹ GoTaq® Flexi DNA Polymerase, and 1 μl of DNA extract. The initial denaturation step (94 °C for 2 min) was followed by 30 amplification cycles consisting of a denaturation step at 94 °C for 35 s, primer annealing at 55 °C for 45 s and an extension phase at 72 °C for 2 min. The final extension was completed at 72 °C for 2 min.

Size and yield of the PCR products was checked on a 2% agarose gel containing a 100 bp ladder (Promega). A 1-μl volume of PCR product was mixed with 9 μl of HiDi formamide and 0.5 μl of standard LIZ1200 (Applied Biosystems, Rotkreuz, Switzerland) before exposing it to 95 °C for 3 min and subsequent cooling on ice. The intergenic spacer fragments were on a 3130XL Capillary Genetic Analyzer (Applied Biosystems) using a 50-cm capillary and a standard
genemapper protocol (Applied Biosystems). The peak pattern was analysed with the software Peak Scanner V1.0 (Applied Biosystems). Relative peak area of the fragments with lengths between 200 and 1200 bp were extracted and the profiles of the different samples were compared using the script interactive_binner.r (Ramette, 2009) implemented in the statistical software R (R Development Core Team, 2011).

**Statistical analysis**
Linear mixed-effect models were fitted with the function `lme` from the package `nlme` (Pinheiro et al., 2001) in R (R Development Core team, 2011) to test for differences among types of microbial communities used to inoculate the stream channels. Inoculation type and time were treated as fixed effects. A random intercept effect was included for replicate channels (inoculum type from different streams). QQ-plots and frequency histograms indicated that residuals did not meet assumptions required for parametric tests, but this limitation was overcome by applying a logarithmic transformation, \( \ln(x+1) \), to the data.

Non-metric multidimensional scaling (NMDS) was used to detect patterns of fungal and bacterial communities based on the relative abundances of the operational taxonomic unit (OTU) detected by ARISA. NMDS analyses were performed with the function `meta.mds` of the R package `vegan` (Oksannen et al., 2011) based on Bray-Curtis distances and 1000 permutations. Permutational multivariate analysis of variance (PERMANOVA) was performed with the function `adonis` in the same package. Significance of Bray-Curtis distances among group centroids were assessed with specific contrasts of treatments: 1 vs 2+3+4 to test for the effect of fungal spore addition and 2 vs 3+4 to test for the effect of bacteria.

3.3. Results

**Whole-ecosystem metabolism**
Temperature-normalized respiration rates measured at the whole-system level ranged from 2.7 to 30.9 mg O\(_2\) m\(^{-2}\) h\(^{-1}\) (Fig. 3.1A) and were significantly higher when the channels were inoculated with fungi in addition to bacteria (\( F_{3,31}=8.13, P<0.001 \)). However, on two of four measurement dates, channels inoculated with bacteria only showed similar respiration rates as the channels receiving the full
inocula with fungi (Fig. 3.1A). This patterns is reflected in a significant interaction between inoculum type and time ($F_{9,31}=2.34, P=0.038$).

Net community production shifted from initially negative to positive values in all channels ($F_{3,44}=24.1, P<0.001$). NCP measured at day 25 was significantly higher in channels inoculated with fungi compared to dead microorganisms treatment (Tukey HSD post-hoc test, $P=0.003$; Fig. 3.1B). All channels were clearly dominated by heterotrophic metabolism ($P/R < 1$) for up to 25 days after inoculation, but GPP and respiration became more balanced by day 32, when mean $P/R$ ratios for channels receiving different inoculum types ranged from 0.7 to 1.1 (Fig. 3.1C).

**Figure 3.1.** Whole-stream ecosystem respiration (A) and gross primary production (B), and the ratio between respiration and production or $P/R$ ratio (C) in experimental stream channels receiving one of four inoculum types. Histograms show means ± 1 SE of either 4 replicate channels, or 3 channels for the controls inoculated with a suspension of dead microorganisms.
**Leaf litter mass loss**

Mass loss of birch litter ranged from 20 to 38% after 36 days (Fig. 3.2). It did not differ among channels receiving different inoculum types ($F_{3,78}=0.25, P=0.86$).

![Figure 3.2](image)

**Figure 3.2.** Leaf mass remaining in experimental stream channels receiving one of four inoculum types. Histograms show means ± 1 SE of either 4 replicate stream channels, or 3 channels for the controls inoculated with a suspension of dead microorganisms.

**Respiration associated with leaf litter and sediment**

Rates of microbial respiration associated with birch leaves increased from initially undetectable to 1.3 mg O$_2$ mg$^{-1}$ litter DM h$^{-1}$ after 18 days ($F_{5,66}=23.1, P<0.001$; Fig. 3.3A). Except for the last sampling date, respiration was higher in channels inoculated with fungi (repeated measure on days 11, 18 and 25 $F_{3,33}=7.56, P<0.001$). Respiration rates in sediment ranged from 0.018 to 0.50 μg O$_2$ g$^{-1}$ sediment DM h$^{-1}$ until 25 days and varied little among channels receiving different inoculum types (Fig. 3.3B). On day 32, however, sediment respiration rate more than doubled in the channels inoculated with fungi, reaching 1.09 μg O$_2$ g$^{-1}$ sediment DM h$^{-1}$ ($F_{3,8}=14.1, P=0.0015$).
Figure 3.3. Fungal (A) and bacterial (B) biomass in the sediment of experimental stream channels receiving one of four inoculum types. Histograms show means ± 1 SE of 4 replicate channels, or 3 channels for the controls inoculated with a suspension of dead microorganisms.

Fungal, bacterial and algal biomass and fungal sporulation

Fungal biomass in leaf litter increased over time from 1.05 mg g\(^{-1}\) litter DM on day 4 to 98.8 mg g\(^{-1}\) after 36 days (F\(_{5,78}=78.2\), P<0.001), indicative of pervasive fungal colonisation (Fig. 3.4A). The increase in fungal biomass was greatest in channels inoculated with fungi and varied little among the other three treatments. This difference was significant in later stages of decomposition (F\(_{3,36}=4.05\), P=0.014, repeated-measures ANOVA restricted to days 25, 32 and 36). Bacterial biomass in leaf litter at the end of the experiment (day 36) ranged from 0.74 to 2.7 mg g\(^{-1}\) litter DM, and was more than 20 times lower than the maximum fungal biomass (Fig. 3.4B). These estimates of bacterial biomass are based on a conversion factor of 58 fg per bacterial cell, as determined from various stream-bed sediments biofilms (Frossard, unpublished data) to derive bacterial biomass from numbers. There were no significant differences in bacterial biomass on leaves among channels receiving different inoculum types (F\(_{3,8}=0.77\), P=0.54).

Bacterial biomass in sediments initially declined before increasing again in later stages of the experiment (F\(_{4,64}=7.85\), P<0.001; Fig. 3.4C). There were no clear patterns among channels receiving different inoculum types. Algal biomass estimated at the end of the experiment at the sediment surface was about three
times higher in channels inoculated with fungi than in the other channels ($F_{3,8}=15.5$ $P=0.0011$; Fig. 3.4D).

![Figure 3.4](image.png)

**Figure 3.4.** Biomass of fungi (A) and bacteria (B) associated with birch leaf litter and of bacteria (C) and algae (D) in sediments of experimental stream channels receiving one of four inoculum types. Histograms show means ± 1 SE of either 4 replicate channels, or 3 channels for the controls inoculated with a suspension of dead microorganisms.
Sporulation rates of aquatic hyphomycetes at the end of the experiment was nearly 10 times higher in the channels inoculated with fungi (Tukey HSD post-hoc test, $t=8.15$, $P<0.001$; Fig. 3.5A). There were also more species of aquatic hyphomycetes in those channels ($t=6.17$, $P<0.001$), whereas a tendency towards higher species richness in channels inoculated with bacteria only was not significant ($t=1.44$, $P=0.19$; Fig. 3.5B).

**Figure 3.5.** Sporulation rates (A) and spore richness (B) of fungi colonizing birch leaf litter after 36 days in experimental stream channels receiving one of four inoculum types. Histograms show means ± 1 SE of 4 replicate channels, or 3 channels for the controls inoculated with a suspension of dead microorganisms.

**Fungal and bacterial community profiles**

Fungal diversity of leaf-litter samples assessed by molecular fingerprinting (ARISA) increased with time. The number of detected OTUs averaged 16 ± 5 at day 4 to 68 ± 17 at day 36 of the experiment. No significant difference in fungal richness was observed among channels receiving different inoculum types. However, fungal community structure in channels inoculated with fungi and bacteria differed from the control treatments (PERMANOVA for each sampling date, $F_{1,41}=2.92$, $P=0.007$, Fig 3.6A). Richness of fungal OTUs in surface sediment was low; only 5.9 ± 4.2 taxa were detected per sample (data not shown). Bacterial community structure on leaf litter also differed among treatments (Fig. 6B). The communities in channels inoculated with fungi and bacteria were different from those in other channels ($F_{1,55}=6.1$, $P=0.001$), and this difference was clearly
visible by day 25 (Fig. 3.7). Communities in channels inoculated with bacteria only differed from those developing in the control channels ($F_{1,55}=2.85$, $P=0.003$). A constant average of $91 \pm 6$ bacterial OTUs was detected on leaf litter throughout the experiment. Numbers did not differ among channels receiving different inoculum types or inocula of different origin. The structure of bacterial communities in sediment differed in the channels inoculated with both fungi and bacteria ($F_{1,55}=4.1$, $P=0.001$, Fig 3.6C).

**Figure 3.6.** NMDS ordination of A) fungal communities associated with leaf litter, B) bacterial communities associated with leaf litter, and C) bacterial communities in sediment of experimental stream channels receiving one of four inoculum types (colour coded) and sampled at four different times (days) after inoculation (coded by different symbols).
Figure 3.7. NMDS ordination of fungal communities associated with leaf litter (left column), bacterial communities associated with leaf litter (middle column), and bacterial communities in sediment of experimental stream channels receiving one of four inoculum types (colour coded) at four different sampling dates. Stress value: 2.4 - 11.3 for fungi litter, 12.3 - 15 for bacteria on litter, and 14.8 - 18 for bacteria in sediment.
3.4. Discussion

**Effectiveness of experimental manipulations**

Although conducted in experimental channels, the experiment presented here was conducted under conditions close to the natural situation in the early successional Chicken Creek catchment. The experimental outdoor stream channels were filled with sediment and received water and solutes from the adjacent catchment, and they were subject to all natural environmental influences, including temperature, light, rain, atmospheric deposition, and immigration by microbes and other organisms. Moreover, flow conditions in the experimental channels were similar to those experienced in the three natural streams of the catchment during baseflow conditions. Fungal biomass and sporulation rate on leaves also indicate that the experimental inoculation of channels with fungal propagules was effective. Addition of inocula containing fungi clearly enhanced fungal litter colonization, as revealed by significantly higher fungal biomass and substantially higher sporulation rates in the channels receiving fungal inocula.

**Fungal and bacterial litter colonization**

Notwithstanding the enhanced colonization of leaf litter in channels inoculated with fungal propagules, aquatic hyphomycetes also developed in the four channels receiving bacteria only and even in the seven control channels to which no living microbes had been added. In fact, fungal biomass of 45 mg g⁻¹ litter dry mass after 36 days in the control channels (where no living microorganism were present at the start of the experiment) is in the range of values commonly found in forested streams (Gessner, 1997). For example, 90 mg of fungal dry mass per gram of alder and oak leaves was measured after 25 days of decomposition in a natural forest stream (Ferreira et al., 2006) and significantly lower values have been found elsewhere (Gessner, 1997). Given the relative remoteness of the study area from forested streams, the early successional stage of the Chicken Creek Catchment (Gerwin et al., 2009; Gerull et al., 2011), and the delicate nature of aquatic hyphomycete spores (Gessner & Van Ryckegem, 2003; Bärlocher, 2009), the effective colonization of litter in uninoculated channels might seem surprising. However, the success of fungi observed here is consistent with the development of fungal biomass and sporulation on leaf litter exposed in alpine catchments (Gessner & Robinson, 2003), including in streams immediately
downstream of glaciers (Gessner et al., 1998) and on relatively recalcitrant larch needles (Robinson et al., 2000). This illustrates that the colonization potential of leaf litter by fungi is important even in early-successional catchments, despite the absence of plant litter and low amounts of organic matter in general to support fungal growth. It appears that as soon as suitable substrates enter streams, aquatic hyphomycetes are capable of rapidly capitalizing on the resource. This suggests that dispersal limitation is not a major issue for these fungi even in fairly extreme conditions such as in glacial streams (Gessner et al., 1998) or bare, recently created catchments devoid of vegetation cover (this study).

Fungi dominated the microbial communities on leaf litter independent of inoculum type, their biomass exceeding that of bacteria in all channels by 22-53 times at the end of the experiment, similar to the situation found in forest streams (Gessner et al., 2007; Duarte et al., 2009). This suggests that the role of bacteria in the decomposition of leaf litter was limited, and corroborates results by Gulis & Suberkropp (2003), who found minor participation of bacteria in the carbon degradation of organic matter originating from decaying leaves, independently of the presence of fungi or nutrient availability. The marginal importance of bacteria reinforces the conclusion above that similar to forested streams that receive large amounts of litter inputs from riparian vegetation, fungi are capable of assuming dominance in the colonization and decomposition of leaf litter even in rather unfavourable conditions such as the experimental channels in the present study that simulate early-successional streams.

**Role of microorganisms in overall stream metabolism**

The similar pattern of litter-associated respiration and fungal biomass across experimental treatments, with faster increases in the channels receiving a fungal inoculum, suggests an important contribution of fungi to total microbial respiration associated with the leaf litter. Rates in the channels inoculated with fungi averaged 0.6 mg O$_2$ g$^{-1}$ AFDM, which is similar to the rate observed for birch litter in a forested stream (0.75 mg O$_2$ g$^{-1}$ AFDM; Royer & Minshall, 2001). However, fungal inoculation of the stream channels had no effect on leaf-litter mass loss, possibly because the experimental period was too short to detect differences, since mass loss never exceeded 38% by the end of the experiment. Although not deliberately excluded, macro-invertebrate shredders were absent from the experimental channels (personal observation), which most likely
explain, by their absence, the low leaf decomposition rates (e.g. Hieber & Gessner, 2002).

Although litter-associated microbial respiration rates were similar to rates measured in forest streams (see above), the contribution of litter-associated respiration to whole-system respiration in the stream channels was very small, contributing only 0.01 % even in the channels inoculated with fungi. This notwithstanding, whole-stream metabolism was increased in the channels receiving a fungal inoculum, which is suggestive of a stimulating metabolic effect of fungi beyond the leaf-litter compartment. To our knowledge, such an indirect effect of fungi on the overall metabolism of streams, independent of fungal activity in leaf litter, has not previously been demonstrated. This outcome was facilitated, however, by the fact that overall respiration rates in the experimental stream channels (0.32 - 0.74 g O$_2$ m$^{-2}$ day$^{-1}$) were at a low level. This is true even in comparison to natural streams with reduced organic matter contents in sediments (range of mean respiration rates of various desert and temperate streams from 0.56 to 16 g O$_2$ m$^{-2}$ day$^{-1}$; see compilation in Gerull et al., 2011).

The observed decline in respiration rates towards the end of the experiment (day 32) in the channels inoculated with fungi is unlikely to have been caused by a limitation of resources for microbial activity. One more likely reason is that low night-time temperatures, dropping down to -0.9 °C between days 25 and 32 after inoculation, caused the surface water in the stream channels to freeze (personal observation). This could have had negative consequences on fungal viability (Sridhar & Bärlocher, 1994; Bärlocher, 2009), which appear to be more susceptible to freeze-thaw cycles than bacteria (Feng et al., 2007; Schmitt et al., 2008). That decrease in whole-stream and litter-associated respiration in the channels inoculated with fungi corresponds to greatly increased respiration rates and algal biomass in sediments, which might have been due to fungal death caused by freezing and the resulting release of labile carbon and nutrients to the benefit of algae. Higher algal biomass and labile carbon release could then have boosted bacterial metabolism in sediments, as has been concluded based on observations in natural streams (e.g. Rier et al., 2007; Artigas et al., 2009).

**Dynamics of fungal and bacterial communities**

Following submersion of leaf litter in streams, fungal diversity assessed as the number of phylotypes detected in fingerprints profiles has been found initially
to decrease with time, whereas bacterial diversity remained constant (Nikolcheva et al., 2005; Harrop et al., 2009). This contrasts with observations in the present study, where the number of phylotypes increased over time. The discrepancy is readily explained, however, because the leaves used in our investigation were sterilized by γ-radiation before exposure in the stream channels to eliminate any influence of phylloplane or other terrestrial fungi (e.g. Bärlocher, 2010). Because the sterilization was very effective, as tested by plating out leaf pieces on agar (unpublished data), the birch leaves introduced in our experimental stream channels initially had zero fungi associated with them.

There was no evidence from our experiment that subsequent increases in fungal diversity limited or stimulated the diversity of bacteria on the decomposing birch leaves. Bacterial richness neither decreased nor increased with time as fungal communities developed and became more species rich. Furthermore, richness of the bacterial communities was not lower than that of fungal communities, in contrast to some other recent findings (Das et al., 2007; Kominoski et al., 2011). Nevertheless, NMDS showed that the structure of bacterial communities was affected by the inoculation of stream channels with fungi. By day 25 after inoculation, bacterial communities on leaf litter were distinct in stream channels that received fungal inocula, corresponding to a doubling of fungal taxa numbers between days 18 and 25, a large increase in fungal biomass, and an increase in whole-stream and litter-associated respiration. Thus, the development of fungal communities (i.e. biomass and diversity) appears to have significantly influenced microbial metabolism on leaf litter, sediment and the stream channels as a whole.

**Fungi as key organisms in early-successional streams**

In conclusion, we found that fungi can play a prominent role in the metabolism of streams even in early successional stages when leaf litter derived from riparian vegetation is scarce. Fungi dominated the microbial communities establishing on leaf litter regardless of whether inoculation of stream channels initially included fungal propagules. More important, however, the role of fungal communities in early-successional stream ecosystems appears to go well beyond the well-established importance in leaf litter decomposition. The present results suggest that it involves indirect effects on the structure of bacterial communities associated with both leaves and sediments, microbial respiratory activity in
sediments, algal biomass, and whole-stream metabolism, although litter-associated respiration only accounted for a minute fraction of total-system respiration. This indicates that the significance of fungi for stream metabolism in general might be greatly underrated at present.

3.5. Acknowledgments
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Chapter 4

Dynamics of microbial community structure and enzyme activities in stream-bed sediments during simulated stream-ecosystem succession

Aline Frossard, Linda Gerull, Michael Mutz and Mark O. Gessner

Abstract

Successional changes of terrestrial vegetation can have profound influences on stream ecosystem structure and function. We hypothesized that this involves microbial responses, specifically that microbial enzyme production and the structure of bacterial communities establishing during different stages of succession depend on the quality and quantity of terrestrial litter inputs. To test for such effects, we used outdoor stream channels supplied with leaf litter of varying quantities and qualities to mimic five distinct successional stages. Experimental treatments included 1) an initial biofilm stage without any litter added, 2) a subsequent open-land stage with grass litter (*Calamagrostis epigejos*) added, 3) a transitional open-land and forested stage with a mix of grass and birch (*Betula pendula*) litter, 4) an early forest stage with birch litter only, and 5) an advanced forest stage with a larger amount of birch litter than in the three earlier stages. Potential activities of nitrogen- and phosphorus-acquiring enzymes (leucine-aminopeptidase and phosphatase) in the bed sediments were much greater than those of a range of carbon-acquiring enzymes, both hydrolytic and oxidative, and were lower when tree litter was added to the experimental channels. This suggests persistent, though gradually diminishing, nutrient limitation of microbial activity during succession. Shifts in potential enzyme activities over successional stages were reflected in the structure of bacterial communities, which showed a smooth gradient from early to late stages of experimental ecosystem succession. In contrast, activities of carbon-acquiring enzymes (β-glucosidase, β-xylosidase, and chitinase) did not vary among stream...
Chapter 4  Stream bed microbial communities during stream ecosystem succession

channels receiving different types or amounts of leaf litter. These results clearly show that both the type and density of terrestrial vegetation control bacterial community structure and function during stream ecosystem succession, particularly enzyme activities related to nitrogen and phosphorus cycling.

4.1. Introduction

Concepts of ecosystem succession in recently created landscapes such as volcanic lava fields (Vitousek, 2004), forefields of receding glaciers (Milner et al., 2011), or post-mining areas (Hüttl & Weber, 2001) emphasize a sequence of successional stages shaped largely by the development of terrestrial vegetation (Walker & del Moral, 2003). Streams forming in these landscapes are intimately linked to their terrestrial surroundings (Webster, 2007), implying that their metabolism and communities vary profoundly along the successional path from bare land to mature forests. In open-land streams where riparian vegetation is absent or restricted to herbs, metabolism is initially dominated by biofilm processes. This includes instream primary production by benthic algae (Golladay, 1997; Bernot et al., 2011) fuelling heterotrophic biofilm communities (Jones et al., 1995; Romaní and Sabater, 1999), in addition to mobilized ancient organic matter stores (Bardgett et al., 2007) and aerial inputs (Hodkinson et al., 2002). Later, as herbal vegetation encroaches, plants near and within streams provide increasing amounts of terrestrial plant litter, while sunlight still reaches the stream bottom and enables continued algal growth (Huryn et al., 2001; Acuña et al., 2010). Eventually, however, shrubs and trees will form a closed canopy above stream channels up to a few metres wide, limiting light penetration and algal development (Sabater et al., 2000) while delivering large amounts of litter (Benfield, 1997). In addition to these quantitative changes, shifts occur in the quality of plant litter along the successional trajectory, especially when grasses are replaced by woody vegetation.

How will microbial communities and activities respond as landscapes and their streams undergo such successions? Will changes be idiosyncratic or smooth, reflecting the gradual changes in environmental conditions and resource supply occurring during the successional trajectory? How important is the quantity and quality of litter in determining microbial community composition and activities? These and similar questions can be addressed by analysing microbial communities and activities along chronosequences, for example in glacier
forefields (Milner et al., 2011, Brankatsch et al., 2010) or on oceanic volcanic archipelagos (Vitousek, 2004). However, a weakness of the chronosequence approach is that many factors vary simultaneously, making it difficult to pinpoint the significance of a particular factor such as litter supply. An alternative approach is to conduct manipulative experiments in model systems, where all factors but the one of interest are kept constant. Although such model systems are necessarily simplified, they stand out by the advantage of precluding influences of any known or unknown confounding factors.

A commonly used measure of microbial activity in ecosystems is respiration (e.g. Crenshaw et al., 2002; Williams & Del Giorgio, 2005; Gerull et al., 2011). Integrating across all aerobic metabolic processes, respiration is often an effective measure of overall activity in ecosystems. However, respiration essentially reflects only carbon transformations and, due to the integrative nature of this measure, specific responses to changing environmental conditions can go undetected. Assessing enzymes has potential to yield more detailed information, including insights into biogeochemical processes other than those involved in the carbon cycle (Sinsabaugh et al., 2008). Moreover, ratios of potential enzyme activities have proved useful to make inferences about the relative importance of different biogeochemical processes (Sinsabaugh et al., 2009; Sinsabaugh & Follstad Shah, 2010a).

The experiment presented here aimed at assessing the responses of microbial community structure and activities to a key component of landscape and stream succession: the development of riparian vegetation leading to increasing amounts and quality of plant litter and concomitant increases in shading. We hypothesized that microbial communities and patterns of enzymatic capacities in stream sediments (i) are strongly influenced by the presence of leaf litter, (ii) depend on litter quality, and (iii) shift when amounts of litter increase during ecosystem succession.

### 4.2. Material and methods

**Experimental design**

We conducted an experiment in 15 outdoor channels simulating sand-bed streams receiving allochthonous litter inputs at five stages of ecosystem succession: 1) an initial biofilm stage, 2) an open-land stage characterized by grass and forb vegetation, 3) a transitional open-land and forested stage, 4) an
early forest stage, and 5) an advanced forest stage. The experimental channels (4.0 x 0.12 x 0.12 m) were set up in the field to ensure natural light, temperature and other environmental conditions, next to a recently created experimental catchment (Chicken Creek; 51°36'N, 14°16'E) in eastern Germany (Gerwin et al., 2009; Gerull et al., 2011). The channels were filled with a 4cm-layer of sand collected from a dry stream reach in the catchment and supplied with well-oxygenated groundwater from the catchment, which was collected and stored in a subterranean tank (Gerull et al., 2012). Dissolved organic carbon (DOC) was abundant in the water but of old age and hardly bioavailable (Gerull et al., 2011). Other physico-chemical characteristics of the water in the channels are summarized in Table 4.1.

To simulate plant litter input into streams during the five stages of stream succession, the experimental channels were stocked with grass or tree litter. Treatments included 1) no litter addition, 2) 100 g m\(^{-2}\) of grass litter 3) 50 g m\(^{-2}\) of grass litter and 50 g m\(^{-2}\) of tree litter, 4) 100 g m\(^{-2}\) of tree litter, and 5) 250 g m\(^{-2}\) of tree litter. The five treatments were replicated 3 times, resulting in a total of 15 experimental channels. One fifth of the litter was buried to mimic natural mixing of litter into sandy sediments. The rest was distributed on the sediment surface. Wood small-reed (Calamagrostis epigejos (L.) Roth) was used as grass litter and silver birch (Betula pendula (L.) Roth) as tree litter. Wood small-reed was one of the early successional plant species at the site. Birch is a common tree species in the area. Both types of litter were collected from single stands in autumn 2007. The tree litter had higher concentrations of both nitrogen and phosphorus (6.9 mg N g\(^{-1}\) DM, 1.2 mg P g\(^{-1}\) DM) than the grass litter (3.4 mg N g\(^{-1}\) DM, 0.16mg P g\(^{-1}\) DM).

Stream water suspensions containing natural microbial assemblages were poured in all channels as natural inoculum to boost microbial colonization of the channels. To generate the inoculum, 24 g DM of mixed grass and tree litter (various species) were collected in three local open-land and forest streams. To detach bacterial cells from the leaf surfaces, half of the litter was sonified in an ultrasonic bath (3 min, 35W output) containing 8 L of Chicken Creek water. The other half was aerated for 48 h in 8 L of Chicken Creek water to induce fungal sporulation. Each of the 15 channels received 500 ml of either suspension. After inoculation, water was completely recirculated in the channels for 24 h to facilitate establishment of the added microorganisms. Subsequently, the channels
were supplied with water from the catchment such that 5% of the water volume in the channels was replaced during each cycle. The current velocity was $1.7 \pm 0.3 \text{ cm s}^{-1}$ at a water depth of 1 cm. This resulted in an average water renewal time of 6 h.

**Sampling and basic analyses**

Water and sediment samples were randomly collected after 6, 8 and 10 weeks. Three sediment cores were taken with a syringe (2.8 cm inner diameter, lower end cut off) to a depth of 4 cm, pooled and frozen for later analyses. Separate sediment cores for chlorophyll-$a$ analyses were collected to a depth of 1 cm. Surface water was collected with a syringe, immediately filtered through pre-washed 0.45-µm pore size cellulose acetate filters, frozen at -20°C and later analyzed for DOC and nutrients ($\text{TDP} = \text{total dissolved phosphorus}$, $\text{TDN} = \text{total dissolved nitrogen} = \text{NO}_2^- + \text{NO}_3^- + \text{NH}_4^+$). Conductivity, pH and temperature were measured directly in the channels with WTW probes (Weilheim, Germany). Sediment organic matter content was determined as the difference in mass of dried ($105^\circ\text{C}$) and combusted ($4 \text{ h at } 450^\circ\text{C}$) samples.

**Extracellular enzyme activities**

The potential activities of seven enzymes involved in the degradation of organic matter (phosphatase, leucine-aminopeptidase, $\beta$-glucosidase, $\beta$-xylosidase, chitinase, phenol oxidase and phenol peroxidase) were assessed with substrate analogues linked to fluorescent molecules (4-methylumbelliferone, MUB; or 7-amino-4-methylcoumarin, AMC), or to 3,4-dihydroxyphenylalanine (L-DOPA) for the spectrophotometric assay of phenol oxidase and peroxidase as described in Chapter 2. Assays involved placing 2 g of sediment in 60 ml of 0.1 mM tri(hydroxymethyl)aminomethane buffer (pH 7.5, autoclaved), stirring the slurry, and adding 200 µl to 96-well microplates. The substrate analogues were added (50 µl of 200 µM stock solutions) and fluorescence or absorbance measured after incubation at $10^\circ\text{C}$ for 1.5 (chitinase and phosphatase) or 4 h ($\beta$-glucosidase, $\beta$-xylosidase, leucine-AP, peroxidase). NaOH (0.5 N, 10 µl) was added before shaking the microplates and measuring fluorescence on a microplate reader (Tecan Infinite® 200, Männedorf, Switzerland) at an emission wavelength of 445 nm and 450 nm, respectively, and an excitation wavelength of 365 nm for both types of substrate. Absorbance in the phenol oxidase and peroxidase assays was
measured at 460 nm. Background fluorescence or absorbance from the sediment and substrate analogue was subtracted by measuring sample and substrate controls. Declines of the fluorescence signal due to sediment sample background were taken into account by correcting it with a quench coefficient calculated for each sample.

**Bacterial and algal biomass**

Bacterial abundance was determined by flow cytometry as described in Chapter 2. Bacterial cells were detached from the sediment with an ultrasonic probe (Buesing and Gessner, 2002), separated from other particles by collecting them in Histodenz® solution (Caracciolo et al., 2005), staining with SYBR Green I, and counting on a CyFlow® space Flow Cytometer System (Partec, Görlitz, Germany) equipped with a 200-mW solid-state laser (light emission at 488 nm) and volumetric counting hardware (Hammes and Egli, 2005). A conversion factor of 58 fg per cell was used to calculate bacterial biomass from abundance data (Frossard et al., unpublished data) and assuming that 50% of dry mass was carbon.

Chlorophyll $a$ was used as a proxy of algal biomass and was determined spectrophotometrically after extraction with 90% ethanol for 4 min in the dark at 70°C (Gerull et al., 2011). A conversion factor of 60 mg of C per g chlorophyll $a$ was used (Romaní and Sabater, 2000).

**Bacterial community structure**

The structure of bacterial and the fungal communities was assessed by automated ribosomal intergenic spacer analysis (ARISA). DNA was extracted from frozen (-80°C) sediment following steps described in Chapter 2. The extraction involved mechanical cell breakage, enzymatic digestion of unwanted cell constituents, and DNA purification. The purified DNA was stored at -20°C. PCR amplification of the intergenic region of bacterial rDNA was carried out using labelled forward primer 1406F-FAM (5’FAM-TGY ACA CAC CGC CCG T-3’, T=T, C) and reverse primer 23Sr (5’-GGG TTB CCC CAT TCR G-3’, B=G, T, C and R=G, A; Microsynth, Balgach, Switzerland; Yannarell et al., 2003). The mix contained 1x GoTaq® Flexi reaction buffer, 3 mM MgCl$_2$, 0.25 mM dNTPs, 0.4 mM of each primer, 0.25 mg ml$^{-1}$ BSA, 0.05U μl$^{-1}$ GoTaq® Flexi DNA Polymerase, and 1 μl of DNA extract. The initial denaturation step (94°C for 2min) was followed by 30
amplification cycles consisting of a denaturation step at 94°C for 35 s, primer annealing at 55°C for 45 s and an extension phase at 72°C for 2 min. The final extension was completed at 72°C for 2 min.

Size and yield of the PCR products was checked on a 2% agarose gel containing a 100bp ladder (Promega). A 1-μl volume of PCR product was mixed with 9 μl of HiDi formamide and 0.5 μl of standard LIZ1200 (Applied Biosystems, Rotkreuz, Switzerland) before exposing it to 95°C for 3 min and subsequent cooling on ice. The intergenic spacer fragments were separated on a 3130XL Capillary Genetic Analyzer (Applied Biosystems) using a 50-cm capillary and a standard Genemapper protocol (Applied Biosystems). The peak pattern was analysed with Peak Scanner V1.0 (Applied Biosystems). Relative peak area of the fragments with lengths between 200 and 1200bp were extracted and the profiles of the different samples were compared using the script interactive_binner.r (Ramette, 2009) implemented in the statistical software R (R Development Core Team, 2011).

Data analysis

Linear mixed effects models were fitted with the function lme from the package nlme (Pinheiro et al., 2011) for the statistical software R (R Development Core Team, 2011) to test for differences among litter treatments at different sampling dates. Litter treatment and time were treated as fixed effects, with the time variable centred on the 10-week sampling date so that the estimated intercepts represent the situation at the end of the experiment when the largest effects were expected. A random intercept effect was included for replicates to account for repeated measures on the same channels. QQ-plots and frequency histograms indicated that residuals did not meet assumptions required for parametric tests. Therefore, variables (x) were transformed according to ln(x+1). Planned contrasts were calculated to test our hypotheses by comparing selected combinations of the five treatments. Specifically, we compared treatment 1 with treatments 2+3+4+5 (Contrast A), 1+2 vs. 3+4+5 (Contrast B), 2 vs. 4 (Contrast C), 4 vs. 5 (Contrast D), and 3 vs. 2+4 (Contrast E).

Non-metric multidimensional scaling (NMDS) analyses were performed on the bacterial community matrix (relative abundance of each OTU detected by ARISA) and the matrix regrouping the seven potential enzyme activities measured with the function meta.mds of the package vegan implemented in R (R
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Development Core Team, 2011). Calculations were based on Bray-Curtis distances and 1000 permutations. Permutational multivariate analysis of variance was performed with the function *adonis* in R. Significance of Bray-Curtis distances among centroids of treatment clusters within each community sampled at the same date was assessed among all treatment and also depending of the specific contrasts described above. Environmental factors were fitted in the ordination plot as vectors.

4.3. Results

*Physicochemical parameters*

DOC, NO$_3^-$, NH$_4^+$, and PO$_4^{3-}$ concentrations were significantly higher directly after the start of the experiment (Table 4.1). However, none of the physicochemical parameters varied among channels receiving different litter treatments.

**Table 4.1**: Means and standard deviations of sediment organic matter (OM) and physico-chemical parameters of water in 15 experimental stream channels that were sampled on four occasions. DOC = dissolved organic carbon, TDN = dissolved nitrogen, TDP = dissolved phosphorus.

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>pH</th>
<th>Temperature (°C)</th>
<th>DOC (mg L$^{-1}$)</th>
<th>TDN (mg L$^{-1}$)</th>
<th>TDP (μg L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>7.66 ± 0.09</td>
<td>24.4 ± 2.8</td>
<td>22.88 ± 9.19</td>
<td>0.53 ± 0.05</td>
<td>14.6 ± 8.7</td>
</tr>
<tr>
<td>6 weeks</td>
<td>7.42 ± 0.04</td>
<td>7.6 ± 2.4</td>
<td>12.46 ± 0.33</td>
<td>0.34 ± 0.04</td>
<td>5.1 ± 1.4</td>
</tr>
<tr>
<td>8 weeks</td>
<td>7.43 ± 0.05</td>
<td>4.9 ± 0.3</td>
<td>8.02 ± 0.39</td>
<td>0.25 ± 0.05</td>
<td>4.2 ± 0.9</td>
</tr>
<tr>
<td>10 weeks</td>
<td>7.37 ± 0.06</td>
<td>10.2 ± 0.4</td>
<td>8.05 ± 0.13</td>
<td>0.15 ± 0.01</td>
<td>2.4 ± 0.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>NO$_2^-$ (μg L$^{-1}$)</th>
<th>NO$_3^-$ (μg L$^{-1}$)</th>
<th>NH$_4^+$ (μg L$^{-1}$)</th>
<th>PO$_4^{3-}$ (μg L$^{-1}$)</th>
<th>OM (mg g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>1.4 ± 0.6</td>
<td>84.0 ± 45.8</td>
<td>18.4 ± 12.5</td>
<td>8.0 ± 6.9</td>
<td>0.89 ± 0.03</td>
</tr>
<tr>
<td>6 weeks</td>
<td>&lt;1.0 ± &lt;1.0</td>
<td>37.8 ± 4.6</td>
<td>17.5 ± 13.2</td>
<td>2.0 ± 0.4</td>
<td>0.80 ± 0.09</td>
</tr>
<tr>
<td>8 weeks</td>
<td>9.2 ± 2.7</td>
<td>54.2 ± 7.3</td>
<td>16.4 ± 5.1</td>
<td>2.1 ± 0.2</td>
<td>1.01 ± 0.09</td>
</tr>
<tr>
<td>10 weeks</td>
<td>2.1 ± 0.6</td>
<td>39.8 ± 3.5</td>
<td>9.8 ± 1.2</td>
<td>2.0 ± 0.2</td>
<td>0.83 ± 0.08</td>
</tr>
</tbody>
</table>
**Extracellular Enzyme activities**

Phosphatase showed the highest potential activity among the enzymes tested. Average rates exceeded 150 μmol MUB g⁻¹ sediment AFDM h⁻¹ in channels without leaf litter, whereas rates in the channels stocked with litter were three times lower (Fig. 4.1A). Leucine-aminopeptidase (AP), which was used as an indicator of N acquisition, showed the second highest potential activity, ranging from 5.5 to nearly 50 μmol AMC g⁻¹ AFDM h⁻¹ (Fig. 4.1B). Chitinase potential activities were lower, with values not exceeding 2.9 μmol MUB g⁻¹ AFDM h⁻¹ (Fig. 4.1C). Potential activities of the carbon-acquiring enzymes such as β-glucosidase and β-xylosidase ranged from 0.4 to 1.8 μmol MUB g⁻¹ AFDM h⁻¹ (Fig. 4.1D, E). Finally, the potential activity of lignin-degrading enzymes ranged from 1.1 to 1.7 μmol L-DOPA g⁻¹ AFDM h⁻¹ for phenol oxidase (Fig. 4.1F) and from 1.5 to 2.4 μmol L-DOPA g⁻¹ AFDM h⁻¹ for phenol peroxidase (Fig. 4.1G).

Potential activities of phosphatase and leucine-AP significantly differed among the five litter treatments ($F_{4,10}=6.21$ and 11.6, $P<0.001$); while phosphatase abruptly dropped when any type of litter was present (Fig. 4.1A), the potential activities of leucine-AP gradually declined along the successional gradient from channels receiving no litter to those receiving 2.5 times the standard amount of tree litter (Fig. 4.1B). Leucine-AP was higher in the bare channels without litter (Contrast A: $P=0.006$). Both enzymes had higher potential activities in the simulated open-land stage (no litter or grass litter added) compared to the channels receiving tree litter (Contrast B: $P=0.032$ and $P<0.001$, respectively). Leucine-AP was the only enzyme reflecting difference in the quality (Contrast C: $P=0.005$) and quantity (Contrast D: $P=0.009$) of added litter. Mixing of grass and tree litter had no significant effect for these both potential enzyme activities. Potential activities of C-acquiring enzymes (β-glucosidase, β-xylosidase, as well as chitinase) and lignin-degrading enzymes (phenol oxidase and phenol peroxidase) did not significantly differ among treatments, nor were significant interactions observed between litter treatment and time. Ratios of C-acquiring and lignin-degrading enzymes to P- and N-acquiring enzymes showed a general increasing trend along the gradient from no litter to larger amounts of tree litter in the channels, although the exact patterns slightly differed among the different enzyme ratios (Fig. 4.2).
Figure 4.1. Potential activities of seven selected enzymes in the sediment of experimental stream channels supplied with different types and amounts of leaf litter: 1) no litter, 2) grass litter, 3) mixed grass and tree litter, 4) tree litter, 5) larger amount of tree litter. Bars are means of three sampling dates (6, 8 and 10 weeks after starting the experiment), error bars denote standard errors (n=3).
Figure 4.2. Ratios of potential enzyme activities in experimental stream channels supplied with different types and amounts of leaf litter: 1) no litter, 2) grass litter, 3) mixed grass and tree litter, 4) tree litter, 5) larger amounts of tree litter. A) β-glucosidase to leucine-AP plus chitinase, B) β-glucosidase to phosphatase, C) phenol oxidase plus phenol peroxidase to leucine-AP plus chitinase, and D) phenol oxidase plus phenol peroxidase to phosphatase.

Fingerprints of potential enzyme activities in sediment from stream channels stocked with different types and amounts of leaf litter did not form clearly separated clusters (Fig. 4.3), but samples were mostly arranged along a gradient from early (no litter) to late (larger amount of tree litter) stages of ecosystem succession (F_{4,27}=2.9, P=0.001). Phosphatase and leucine-AP had the greatest influence on the ordination. When included in the same ordination, algal biomass was significantly correlated to the potential enzyme activity fingerprints (r^2=0.44, P=0.002).
Figure 4.3. NMDS ordination of potential enzyme activity patterns of sediments sampled at three occasions in experimental stream channels supplied with different types and amounts of leaf litter. Three outliers (treatment 5, 10 weeks (2 ×) and treatment 4, 8 weeks (1×)) have been removed for the ordination. Potential enzyme activities (black arrows) and environmental variables (grey arrows) are fitted on the ordination plot as vectors. Asterisks (*) indicate that Alg (algal biomass) is significantly related to the arrangement of the potential enzyme activity patterns in the ordination. Phos = phosphatase, Leu = leucine-AP, Chit = chitinase, Gluc = β-glucosidase, Xylo = β-xylosidase, PO = phenol oxidase, PP = phenol peroxidase, Bact = bacterial biomass, Alg = algal biomass, DOC = dissolved organic carbon, TDN = total dissolved nitrogen, TDP = total dissolved phosphorus, OM = organic matter in sediment.
**Bacterial and algal biomass**

Bacterial biomass in sediment ranged from 0.18 to 0.84 mg C g\(^{-1}\) AFDM and was twice as high in the open-land stages with grass litter only than at later successional stages with tree litter only (Fig. 4.4A), although this difference was not significant due to considerable variation among replicate stream channels. Algal biomass in sediment ranged from 9.6 to 31.2 mg C g\(^{-1}\) AFDM and showed a similar decline along the successional gradient. However, the shift to lower biomass was delayed to stage 4 when only tree litter was supplied to the stream channels (Fig. 4.4B). Open-land stages (no litter added or grass litter only) showed higher algal biomass than later successional stages with tree litter present (Contrast B: P=0.005).

![Graph showing bacterial biomass and algal biomass](image_url)

**Figure 4.4.** Bacterial biomass (A) and algal biomass as chlorophyll-\(\alpha\) content (B) in the sediment of experimental stream channels supplied with different types and amounts of leaf litter: 1) no litter, 2) grass litter, 3) mixed grass and tree litter, 4) tree litter, 5) double amounts of tree litter. Bars are means of three sampling dates (6, 8 and 10 weeks after starting the experiment), error bars denote standard errors (n=3).

**Bacterial community structure**

NMDS analysis revealed similarities of bacterial communities in sediment sampled after 6 and 8 weeks (Fig. 4.5). They were distinctly separated along the first axis (with two exceptions) from the communities established 10 weeks after the start of the experiment (\(F_{2,30}=7.97, P=0.001\)). In addition, the bacterial communities within both clusters were broadly arranged along a gradient defined by axis 2, ranging from the initial stage (no litter) to the late stage (large...
amounts of tree litter) of stream succession ($F_{4,30}=3.32$, $P=0.001$). As a result, bacterial communities differed between channels with and without litter (Contrast A, $F_{1,43}=2.54$, $P=0.008$), with and without tree litter (Contrast B, $F_{1,43}=4.15$, $P=0.002$), with either grass or tree litter (Contrast C, $F_{1,43}=2.43$, $P=0.008$), and with different amounts of tree litter (Contrast D, $F_{1,43}=2.17$, $P=0.025$).

Total dissolved nitrogen, $\text{NH}_4^+$, $\text{NO}_2^-$ and $\text{NO}_3^-$ in stream water were correlated to the ordination of bacterial communities ($r^2=0.67$, 0.25, 0.55, and 0.48; $P<0.001$, 0.034, >0.001, and <0.001), and vectors of these environmental factor (Fig. 4.5) are all arranged roughly to the direction of early successional stages (no litter and grass litter) and of early sampling dates (6 and 8 weeks). In contrast, vector of total dissolved phosphorus pointed to the direction of late successional stages (Fig. 4.5; $r^2=0.27$, $P=0.022$). Furthermore, potential activities of phosphatase, phenol peroxidase and leucine-AP were significantly correlated with bacterial community structure ($r^2=0.28$, 0.23 and 0.34 ; $P=0.004$, 0.004 and <0.001, respectively), these enzyme activities being higher in the early successional stages. Arrows of fitted vectors of these potential enzyme activities on Fig. 4.5 were all roughly parallel to Axis 2, suggesting that differences in potential enzyme activities were largely driven by changes in bacterial communities along the successional gradient. Fitted vector of bacterial biomass was also significantly correlated to bacterial community structure but points to no particular cluster ($r^2=0.38$, $P=0.004$) in contrast to the vector of algal biomass, pointing towards early successional stages ($r^2=0.53$, $P<0.001$).
Figure 4.5. NMDS ordination of bacterial communities sampled at three dates in sediment of experimental stream channels supplied with different types and amounts of leaf litter. Potential enzyme activities (black arrows) and environmental variables (grey arrows) are superimposed on the ordination plot as arrows. Asterisks (*) indicate variables that are significantly related to the arrangement of the bacterial communities in the ordination. Phos = phosphatase, Leu = leucine-AP, Chit = chitinase, Gluc = β-glucosidase, Xylo = β-xylosidase, PO = phenol oxidase, PP = phenol peroxidase, Bact = bacterial biomass, Alg = algal biomass, DOC = dissolved organic carbon, TDN = total dissolved nitrogen, TDP = total dissolved phosphorus, OM = organic matter in sediment.
4.4. Discussion
The results of our experiment mimicking a key aspect of ecosystem succession in simple experimental stream channels clearly show that riparian plant development exerts strong effects on microbial community structure and patterns of enzymatic capacities in stream sediments. Specifically, we observed a smooth directional change in bacterial communities along the simulated successional trajectory from open-land streams without riparian vegetation to forested streams receiving massive litter input. The same gradual changes of the bacterial communities were apparent 6 and 8 as well as 10 weeks after initiation of the experiment, although the communities also clustered according to sampling dates. This indicates that short-term dynamics (i.e. differences between sampling dates) did not mask the influence on the communities caused by our experimental litter treatments.

Enzymatic capacities can be viewed as indicators of functional microbial responses to the mimicked successional gradient. The patterns shown by several of the measured enzyme potentials, especially those of leucine-AP and phenol peroxidase, are consistent with the bacterial community changes in that both were gradual across the five simulated successional changes. This finding contrasts with field observations in real early-successional streams in the study area, where variation of bacterial community structure across sites and over time appeared to be completely random (Chapter 2). Enzymatic capacities, in contrast, showed pronounced temporal changes, suggesting a decoupling between community structure and ecosystem function, as has been observed in field surveys in the catchment (Chapter 2). Given that the recently formed streams sampled in the field study where completely devoid of plant litter, the discrepancy between those and the present results reinforces our conclusion that riparian plant development is a strong structuring force of bacterial communities in streams.

Part of the microbial response to the mimicked successional gradient was likely a result of varying resource supplies for microbial metabolism in the form of plant litter. It must be borne in mind, however, that the five litter addition treatments also defined a gradient of light availability, because grass litter covered much smaller areas of stream bed than birch leaves even when the added amounts were equal. Therefore, in line with the gradual decrease in the chlorophyll-a content observed in the top sediment layer of the experimental
channels, the litter treatments also reflected changes in the degree of shading occurring during succession of natural streams.

Two observations indicate that the successional pattern observed for various structural and functional variables was primarily driven by nutrient acquisition rather than carbon demand. The first observation is that trends of the nutrient-acquiring enzymes, but not of the hydrolytic C-acquiring enzymes, were consistent with the overall successional changes. Leucine-AP, in particular, showed a gradual decrease as succession proceeded, and chitinase also declined after the two early open-land stages. Similarly, phosphatase potential activity dropped sharply as soon as litter was available, suggesting that the supply of litter mitigated a P deficiency for microbial growth (although it did not necessarily remove it; see below). This mitigation could be a result of massive leaching of P from dried leaves, which does not generally occur to a similar extent for N (Gessner, 1991). The second observation is that the potential activities of P- and N-acquiring enzymes, phosphatase and leucine-AP, were substantially higher than those of the major carbon-acquiring enzymes measured. This resulted in mean ratios of 1:2.8 and 1:2.0 between the natural log values of potential activities of C-acquiring to P- and N-acquiring enzymes, respectively. Analysis of a large set of empirical data yielded an average ratio of 1:1:1 for the natural log values of potential activities of enzymes involved in C, N, and P acquisition (Sinsabaugh et al., 2009). If this ratio reflects balanced relationships between elemental demand and supply (Sinsabaugh et al., 2009), then the much lower ratios observed in the present study suggest a large disequilibrium between the elemental stoichiometry of microbial biomass and the resources available for growth and respiration. Taken together, our data thus indicate that the sediment-associated microbial communities in our stream channels were strongly nutrient-limited throughout the succession mimicked in the experiment.

As enzyme activities and most other variables were determined in integrated samples over the entire sediment depth of 4 cm and dissolved oxygen was depleted below 0.5 to 1.5cm depth (Gerull et al., 2011; A. Frossard & L. Gerull, unpublished data), aerobic microbial metabolism was limited to the top sediment layer. However, since oxygen depletion in sediments did not affect potential activities of hydrolytic enzymes in other studies (e.g. Hakulinen et al., 2005; Taylor et al., 2009), it is unlikely that lack of oxygen had a strong influence on the excretion of microbial extracellular enzymes in the present investigation.
Gradual declines in the biomass of algae – from the open-land stream channels receiving no litter or grass litter only to stream channels stocked with tree litter – corresponded to decreases in the potential activities of leucine-AP and, more abruptly, also of phosphatase (see above). This indicates either a stimulating effect of algal carbon supply on bacterial enzyme excretion, or extracellular enzyme production for nutrient acquisition by algae themselves. Potential enzyme activities in stream biofilms are generally attributed to bacteria. Moreover, under dark condition, microbial potential enzyme activities in heterotrophic stream biofilm were shown to react to augmentation of carbon, nitrogen and phosphorus addition (Van Horn et al., 2011). However, under daylight conditions, higher potential activities of hydrolytic enzymes have been measured in autotrophic as opposed to heterotrophic biofilms (e.g. Romaní and Sabater, 2000; Wilczek et al., 2005; Rier et al., 2007; Ylla et al., 2009), and algal excretion of proteases and phosphatases has also been reported (Jones, 1972; Cotner and Wetzel, 1991; Jacobsen and Rai, 1991). Therefore, and given that the biomass of algae in surface sediment was significantly higher than bacterial biomass, both algae and bacteria were likely to contribute to the observed enzyme patterns, although their relative importance may have shifted towards bacteria as litter inputs provided additional carbon sources and increased shading. Algae can also influence potential activities of bacterial enzymes for C-acquisition (Jones and Lock, 1993; Romaní and Sabater, 1999; Espeland et al., 2001) through the release of organic compound (e.g. Romaní and Sabater, 1999) or the increase of pH by photosynthetic activity (e.g. Rier et al., 2007), however the mechanism of the stimulation of enzyme activities by algae remains unclear (Espeland et al., 2001). However, in contrast to the P- and N-acquiring enzymes, potential activities of the hydrolytic C-acquiring enzymes did not decrease with algal biomass along the successional gradient. This indicates either a limited influence of algal presence, or that the algal influence was compensated by other factors (e.g. large supply of carbon by the added litter) during late stages of succession.

NMDS revealed that changes in bacterial communities during ecosystem succession were mainly related to the potential activity of phosphatase, leucine-AP, and phenol peroxidase, suggesting a strong link between bacterial community structure and function. Changes in environmental conditions (OM, DOC, TDN, TDP), in contrast, did not influence the successional pattern but, according to the
results of NMDS, accounted for the observed differences in bacterial communities between sampling dates. Thus, the drivers of long-term successional changes and short-term changes during the 10 weeks of the experiment were distinctly different.

Finally, our results show that the quality of riparian vegetation influences bacterial communities in streams as well. This observation partly supports the home-field advantage hypothesis proposed for microbial litter decomposers in terrestrial environments (Ayres et al., 2009). It states that microbial communities are adapted to the quality of locally available plant litter, which therefore is most effectively utilized and decomposed. This effect can arise even among genetically distinct plants within species (Madritch & Lindroth, 2011). The different responses of various parameters observed in response to grass and tree litter additions in our 10-week experiment suggest that such “adaptations” might occur extremely fast. The evidence for a home-field advantage is not conclusive, however, because the grass and tree litter treatments involved differences also in light availability and algal growth (see above). Furthermore, the dynamics of N-, P- and hydrolytic C-acquiring enzymes during the simulated succession from grass to tree vegetation indicates effects on nutrient cycling, but not on the decomposition of sediment organic matter.

In conclusion, the results presented here from experimental channels mimicking five stages of stream ecosystem succession suggests that successional changes in riparian vegetation induce gradual shifts in bacterial community structure, microbial activities related to nutrient cycling, and several other variables. Evidently, caution is in order in attempts to project results from a greatly simplified model system to ecosystem succession in real streams, which occurs over decades or hundreds of years. This notwithstanding, the presented evidence provides a proof of principle and attests to the possibility that both structural and functional shifts of microbial communities arise over very short time scales in response to changes in riparian vegetation that reflect ecosystem succession.

4.5. Acknowledgement
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Chapter 5

Leaf quality as driver of microbial metabolism and community structure during simulated stream ecosystem succession

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Abstract
Changes in in-stream and riparian vegetation during the succession of newly created landscapes are accompanied by differences in the quantity and quality of plant litter supplied to stream channels. Inputs into open-land streams are dominated by grass litter, which tend to have low quality as a resource for aquatic microbial communities. Forested streams are dominated by inputs of tree litter with are often of higher quality. Here we set out to elucidate whether the substrate quality of grass or tree litter is more important in determining microbial activity and community structure than differences in the quality and quantity of the main litter type (background litter) deposited in streams at different stages of experimentally simulated ecosystem succession in 15 outdoor stream channels supplied with leaf litter in varying qualities and quantities that reflected different stages of stream succession: 1) a biofilm stage without any litter, 2) a macrophyte stage with grass litter (Calamagrostis epigejos), 3) a transitional stage between open-land and forested stage with a mix of grass and tree litter (Betula pendula), 4) an early forested stage with tree litter, and 5) an advanced forested stage with double amounts of tree litter. Microbial activity on tree and grass litter was unaffected by the quantity and type of background litter, whereas major differences were apparent between grass and tree litter within the same stream channel. In contrast, bacterial and fungal community structure differed strongly not only between the litter types colonized, although this was the dominant effect, but also among channels stocked with different amounts and
types of background litter. Such patterns indicate that microbial diversity and functions are strongly influenced by the increasing quality of the litter source directly associated during succession from open-land streams with low quality grass litter to forested streams with higher quality tree litter.

5.1. Introduction
Various investigations in early successional ecosystems in newly formed landscapes such as volcanic lava fields (Vitousek, 2004), glacier forefields (Milner & Gloyne-Phillips, 2005), or post-mining areas (Mutz et al., 2002), suggest that ecosystem succession follows a sequence of three stages that are largely controlled by changes in vegetation. Particularly relevant for streams, are changes in the quality and quantity of plant litter. Open-land streams are initially deprived of litter supplies until they begin to receive inputs from in-stream macrophytes and grasses establishing in the riparian zone (Mackay et al., 1992; Huryn et al., 2001; Wilcock & Croker, 2004; Menninger & Palmer, 2007; Acuña et al., 2010). Eventually, with woody riparian vegetation encroaching, large amounts of leaf litter and woody debris are delivered to stream channels (Fisher & Likens, 1973; Vannote et al., 1980; Webster & Meyer, 1997). The dominance of either grass or tree litter is likely to change the environmental conditions within the stream, and affect the activity and structure of leaf-associated microbial communities.

There are two assumptions regarding microbial activity and diversity associated with leaves during decomposition in aquatic environments. Some studies have emphasized the importance of leaf quality for determining the activity and structure of bacterial (McArthur et al., 1985; Mille-Lindblom et al., 2006) and fungal (Gulis, 2001) communities associated with decomposing leaves. Others have focussed on environmental conditions such as water chemistry and stream morphology (Harrop et al., 2009; Marks et al., 2009). However, these hypotheses have been assessed by comparing various field sites, at which several environmental factors vary simultaneously, thus potentially obscuring the cause of any relationships that have been found among variables.

Leaf species differ in their concentrations of nutrients (nitrogen, phosphorus), lignin, and phenolics such as tannins, and these quality differences can lead to differences in decomposition rates (Gessner & Chauvet, 1994; Ostrofsky, 1997; Ardón et al., 2009; Tank et al., 2010). It is unclear however, how
qualitative differences between grass and tree leaves affect decomposition. Decomposition of tree litter in streams has been extensively studied (Webster & Benfield, 1986; Ostrofsky, 1997), whereas information on the decomposition of grass leaves is rather scarce (Scarsbrook & Townsend, 1994; Menninger & Palmer, 2007; Shaftel et al., 2011). An analysis across a broad range of plants showed that deciduous woody plants had high N and P contents (i.e. low C:N and C:P ratios) and high decomposition rates, whereas graminoids showed the opposite characteristics (Vendrameni et al., 2000). However, monocotyledons are not always of low quality, as shown in a comparative study by Griffith et al. (2009), who found faster decomposition of, and higher microbial activity on, maize compared to maple leaves.

Differences in leaf quality can also influence leaf-associated fungal and bacterial communities. There is some evidence suggesting that distinct quality differences between tree and grass litter lead to specific abilities to colonize the different leaf types (Gulis, 2001) or use grass or tree litter leachates (McArthur et al., 1985). Enzymatic activities of microbial communities colonizing leaf litter also vary with leaf chemical composition (Griffin, 1994). Similarly, shifts in the chemical composition during decomposition have been found in one leaf species to induce changes in microbial communities and enzyme activities (Snajdr et al., 2011).

Streams surrounded by sparse woody vegetation receive less litter input than mature forested streams (Schade & Fisher, 1997). Few studies have tested the effect of litter quantity on decomposition (Tiegs et al., 2008). However, greater litter inputs and storage result in a greater inoculum potential of aquatic hyphomycetes (Laitung et al., 2002), which are effective microbial decomposers of leaf litter in streams (Gessner et al., 2007) and are characterized by rapid sporulation following initial establishment and growth on leaves (Gessner & Chauvet, 1994). The abundance of hyphomycete spores in stream water peaks after litter fall (Bärlocher, 2000) and is related to the quantity of litter deposited in streams (Laitung et al., 2002). Given the tight correlation between spore concentration and fungal colonization speed on leaves (Treton et al., 2004), faster microbial colonization and decomposition of leaves is expected when background litter is abundant. However, in a field study designed to test this effect, microbial leaf decomposition in litter-augmented or depleted stream reaches showed no differences (Tiegs et al., 2008).
During the transition from open-land to forests, streams will receive a mix of litter types, including grass and tree litter. Studies assessing decomposition rates of mixed and single-species litter have observed variable effects (Gessner et al., 2010). Decomposition rates have been found to be additive, i.e. that rates achieved in mixtures are predictable from the rates of the component species decomposing alone (e.g. Taylor et al., 2007), or non-additive, which refers to situations were rates are either with higher (e.g. Kominoski et al., 2007) or lower (e.g. Swan & Palmer, 2004) in mixtures than expected based on the rates of the component species decomposing alone. Effects of litter mixing on microbial diversity are also unclear. An increase in microbial diversity was found in mixed litter compared to single-species litter on a forest floor (Chapman & Newman, 2010), whereas in streams fungal diversity was unaffected by litter mixing (Taylor et al., 2007).

In this experiment, we aimed at elucidating the relative importance of quality differences between leaves colonized by microbes and the differences in the quality and quantity of the main litter types (background litter) deposited in streams at different stages of succession. Inputs of different amounts and types of litter at various successional stages was simulated in 15 outdoor experimental channels: (1) an initial biofilm stage without litter input, (2) an open-land stage with input of grass litter, (3) a transitional stage between open-land and forested stage with mixed grass and tree litter input, (4) a young forested stage with tree litter input, and (5) a mature forested stage with a larger amount of litter than in treatment (2) to (4). We expected higher activities and diversity of microbial communities associated with the leaves when (i) background litter was present (treatment 1 vs. 2+3+4+5), (ii) tree litter accounted for at least part of the background litter (treatment 1+2 vs. 3+4+5). Similarly, we expected differences in the activities and diversity of microbial communities associated with the leaves when (iii) litter of different quality but the same quantity constitutes the background litter (treatment 2 vs 4), (iv) the quantities of background litter differ (treatment 4 vs 5), and (v) when a mix of litter types constitutes the background litter (treatment 3 vs 2+4).
5.2. Material and Methods

**Experimental design**

We conducted an experiment in 15 outdoor channels simulating early-successional sand-bed streams. The channels (4.0 x 0.12 x 0.12 m) were set up next to a recently created experimental catchment (Chicken Creek) near Cottbus in Eastern Germany (51°36’ N, 14°16’ E) situated in a former lignite mine (Gerwin et al., 2009). The channels were filled with 4 cm of sand collected from a dry ephemeral stream reach of the Chicken Creek catchment. They were set up in the catchment with open tops to ensure natural light and temperature conditions. Concentrations of nutrients (especially phosphorus) in the ground water used to supply the channels were low (Table 4.1, chapter 4). Dissolved organic carbon had high concentrations (8-12 mg L\(^{-1}\)) but was of old age and hardly bioavailable (Gerull et al., 2011).

We simulated successional input of plant litter by the following treatments (each replicated 3 times): 1) no litter addition, 2) 100 g m\(^{-2}\) of grass litter, 3) mix of 50 g m\(^{-2}\) of grass litter and 50 g m\(^{-2}\) of tree litter, 4) 100 g m\(^{-2}\) of tree litter and 5) 250 g m\(^{-2}\) of tree litter. One fifth of the litter was buried in the sediment to simulate the natural distribution of POM in sand-bed streams (Fuss & Smock, 1996). Standing-dead Wood small-reed (*Calamagrostis epigejos* (L.) Roth) was used as grass litter and autumn-shed leaves of Silver birch (*Betula pendula* Roth) as tree litter. The litter used for the experiment was collected from one stand for each species in autumn 2007. Birch litter had higher concentrations of nitrogen and phosphorus than the grass litter (Table 5.1).

**Table 5.1.** Carbon, nitrogen and phosphorus concentrations of leaf litter used to stock experimental stream channels mimicking five stages of ecosystem succession. Values are means ± 1 SD, n=4.

<table>
<thead>
<tr>
<th>Species</th>
<th>C (mg g(^{-1}) DM)</th>
<th>N (µg g(^{-1}) DM)</th>
<th>P (µg g(^{-1}) DM)</th>
<th>C/N</th>
<th>C/P</th>
<th>N/P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Calamagrostis epigejos</em> (grass litter)</td>
<td>368 ± 12</td>
<td>3418 ± 159</td>
<td>158 ± 28</td>
<td>126</td>
<td>6020</td>
<td>48</td>
</tr>
<tr>
<td><em>Betula pendula</em> (tree litter)</td>
<td>469 ± 5</td>
<td>6900 ± 2365</td>
<td>1176 ± 64</td>
<td>79</td>
<td>1029</td>
<td>13</td>
</tr>
</tbody>
</table>
Stream channels were inoculated with stream-water suspensions containing a mix of microorganisms from open-land and forest streams to facilitate microbial colonization. To generate the suspensions, 24 g (dry mass) of mixed leaf litter (grass and tree leaf litter) was collected in three streams (open-land and forest streams) in northeastern Germany. Half of the leaves was placed in an ultrasonic bath containing 8 L of water from Chicken Creek and was sonified for 3 min with an energy output of 35 W to detach bacterial cells from the litter. The other half of the leaves was exposed for 48 h in 8 L of water from the Chicken Creek with vigorous aeration to induce fungal spore production. A volume of 500 mL of each suspension was poured in each channel. After inoculation, the water in the channels ran in closed-circulation mode for 24 h to facilitate establishment of microorganisms (Dang et al., 2005). Subsequently, the channels received oxygen saturated water to replace 5 % of the water volume during each cycle. The current velocity was 1.7 ± 0.3 cm s\(^{-1}\) and the water depth of 1 cm. This resulted in an average water renewal time of 6 h.

**Sampling and basic analyses**

Pieces of small-reed and discs of birch (both 1 cm\(^2\)) were exposed in six separate litter bags in each channel. Litter bags were collected after 6, 8 and 10 weeks and processed within 12 h. The leaf discs were gently cleaned from mineral deposits with filtered (5 µm, membrane filters, SMWP, Millipore, Zug, Switzerland) ground water before drying (105 °C) and weighing them to the nearest 0.1 mg. Leaf mass loss was calculated as the difference between the initial and final dry mass. Surface water was collected in each channel with a syringe, filtered through prewashed 0.45µm pore size cellulose acetate filters (membrane filters, HA, Millipore, Zug, Switzerland), frozen and later analyzed for dissolved organic carbon (DOC) and nutrients (TDN = total dissolved nitrogen, NO\(_2^–\), NO\(_3^–\), NH\(_4^+\), TDP = total dissolved phosphorus), pH and conductivity (Table 4.1 in Chapter 4).

**Respiration associated with leaf litter**

Respiration associated with leaf litter was measured in 50 mL glass flasks containing water from the channels and 6 leaf discs. After temperature adaptation (1 h), the oxygen decline was recorded overnight (12 h) with an optical oxygen meter (Microx TX3, PreSens GmbH, Regensburg, Germany). The leaf litter samples were dried to constant weight after the respiration
measurements (105 °C). Organic matter content was acquired by a conversion factor derived from measuring the ash free dry mass (AFDM) content of the leaf type after combustion of 10 leaves (550 °C, 4 h).

**Extracellular Enzyme Activities**

The potential activities of seven enzymes involved in the degradation of organic matter were assessed with substrate analogues linked to fluorescent molecules. Information about the substrate analogues used for phosphatase, leucine-aminopeptidase (AP), β-glucosidase, β-xylosidase, chitinase, phenol oxidase and phenol peroxidase, and the function of each of these enzymes are summarized in Table 2.2 of Chapter 2. Fluorometric assays were performed as follows: 3 pieces of grass or 3 leaf discs of birch were placed in 60 mL of tri(hydroxymethyl)aminomethane (Tris) buffer (Tris 0.1 mM, adjusted to pH 7.5 with HCl, autoclaved). The suspension was homogenized with a blender (Ultra-Turrax®, 1700 W, IKA Werk, Staufen im Breisgau, Germany) for 30 s. The slurry was stirred and 200 μl was pipetted into 96-well microplates. The substrate analogues were added (50 μl of 200 μM stock solutions) and fluorescence or absorbance measured after incubation at 10 °C for 1.5 (chitinase and phosphatase) or 4 h (β-glucosidase, β-xylosidase, leucine-AP, peroxidase). NaOH (0.5 N, 10 μl) was added before shaking the microplates and measuring fluorescence on a microplate reader (Tecan Infinite® 200, Männedorf, Switzerland) at an emission wavelength of 445 nm and 450 nm, respectively. The excitation wavelength was 365 nm for both types of substrate. Absorbance in the phenol oxidase and peroxidase assays was measured at 460 nm using the same microplate reader. Background fluorescence or absorbance from the litter and substrate analogue was subtracted by measuring sample and substrate controls. Declines of the fluorescence signal due to sediment sample background were taken into account by correcting it with a quench coefficient calculated for each sample. Potential enzyme activities were expressed in μmol substrate per g AFDM of sediment per h.

**Bacterial and Fungal biomass**

Bacterial abundance was determined by flow cytometry (Chapter 2) after detachment of bacterial cells from leaves and sediments with an ultrasonic probe (Buesing & Gessner, 2002). Briefly, the detached bacterial cells were separated
from other particles by collecting them on top of Histodenz® solution (Caracciolo et al., 2005), staining with SYBRGreen I, and counting on a CyFlow® space Flow Cytometer System (Partec, Görlitz, Germany) equipped with a 200 mW solid-state laser (light emission at 488 nm) and volumetric counting hardware (Hammes & Egli, 2005). A conversion factor of 58 fg per cell was used to calculate bacterial biomass from abundance data (Frossard et al., unpublished data).

Fungal Biomass was determined by extracting and quantifying ergosterol, a lipid specific to fungi (Gessner & Newell, 2002). Briefly, leaves were freeze-dried and weighed before extracting lipids in alkaline methanol (80 °C, 30 min) with stirring. The extract was cleaned and concentrated by solid-phase extraction (SPE; Waters Sep-Pak®, Vac RC, tC18, 500 mg; (Gessner & Schmitt, 1996). The extraction efficiency was routinely monitored with external ergosterol standards (Fluka, Neu-Ulm, Germany). The extract eluted from the SPE cartridges was purified and ergosterol was quantified on a high-performance liquid chromatograph (HPLC) consisting of two Jasco PU-980 (Tokyo, Japan) pumps, a Jasco AS-950 autosampler, a LichroSpher 100 RP-18 column (0.46 × 25 cm; Merck Inc., Darmstadt, Germany), and a Jasco MD 2010 Plus multiwavelength detector set at 282 nm. The column temperature was 33 °C. A factor of 5.5 mg ergosterol per g fungal dry mass was used to convert ergosterol values to fungal biomass (Gessner & Chauvet, 1993).

**Fungal and bacterial community fingerprints**

Fungal and bacterial communities were also assessed by automated ribosomal intergenic spacer analysis (ARISA). DNA from 3 frozen leaf discs (-80 °C) was extracted as previously described for sediment samples (Chapter 2). Briefly, DNA was extracted in 3 steps, first by mechanically disrupting cells, then by an enzymatic digestion, and finally with a DNA purification. The purified DNA was stored at -20 °C. PCR amplification of the intergenic region of bacterial rDNA and analysis of the resulting fragments by ARISA was carried out as described in Chapter 3. Relative peak area of the fragments with lengths between 200 and 1200 bp were determined and the profiles of the different samples compared by using the interactive binning script *interactive_binner.r* (Ramette, 2009) implemented in the software R.
Sporulation

Sporulation of aquatic hyphomycetes was induced by submerging six pieces of reed litter or leaf discs of birch from each channel in 47 ml filtered (5 µm membrane filters, Sartorius, Goettingen, Germany) Chicken Creek water kept at 10°C with constant shaking (Gessner et al., 2003). Three ml of 37% formalin was added after 48 h to preserve the samples. One to three ml of the spore suspension was filtered on a membrane filter (5 µm, Millipore, Zug, Switzerland), the filter placed on a microscope slide and the spores of aquatic hyphomycetes stained with 0.1 % Trypan blue in 60 % lactic acid. About 200 spores were identified and counted in 10-30 microscopic fields on each filter at a magnification of 200×. Sporulation rates were converted to conidial production based on conidial dry mass determined for individual species (Hieber & Gessner, 2002).

Data Analysis

In the text, data is given in ranges with the mean in brackets. Linear mixed effects models were fitted with the function lme from the package nlme (Pinheiro et al., 2011) for the statistical software R (R Development Core Team, 2011) to test for differences among the treatments and litter types during the different sampling dates. Treatment, litter type and time were treated as fixed effects with the time variable centred on sampling date 3 after 10 weeks so that estimated intercepts represented the situation at the end of the experiment when the largest effects were expected. Response variables (x) were transformed (ln(x+1)) if QQ-plots and frequency histograms indicated that residuals did not meet assumptions required for parametric tests. Contrast tests were then performed to distinguish differences between the 5 treatments. We applied contrasts according to our hypotheses, comparing treatment 1 vs. 2+3+4+5 (contrast A), 1+2 vs. 3+4+5 (contrast B), 2 vs. 4 (contrast C), 3 vs. 2+4 (contrast D) and 4 vs. 5 (contrast E).

Non-metric multidimensional scaling (NMDS) were performed on a matrix regrouping relative abundance of each operational taxonomic unit (OTU) detected by ARISA with the function meta.mds of the package vegan (Oksanen et al., 2011) implemented in R (R Development Core Team, 2011). Calculations were based on Bray-Curtis distances and 1000 permutations. Permutational multivariate analysis of variance (PERMANOVA) was performed with the function adonis in R. Significance of Bray-Curtis distances among centroids of treatment clusters within each community sampled at the same date was assessed among
all treatment and also depending of the specific contrasts described above. Environmental factors were fitted in the ordination plot as vectors.

5.3. Results

**Physicochemical parameters**

All physicochemical parameters were similar among the treatments of different background litter input. Dissolved organic carbon, NO$_3^-$, NH$_4^+$, and PO$_4^{3-}$ were temporarily increased after the start of the experiment due to the addition of the inoculum and leaf leaching (Table 4.1, chapter 4).

**Extracellular enzyme activities**

Different background litter inputs had no effect on enzyme activities except for leucine-AP ($F_{4,10}=7.44$, $P=0.005$). This N-acquiring enzyme had a lower activity in the open-land stage treatments (without or with only grass litter input) compared to the treatments with tree litter present (contrast B: $P=0.43$).

All potential enzyme activities clearly differed between grass and tree litter with P- and C-acquiring enzymes showing higher activity associated with grass compared to tree litter, and the opposite pattern for the N- acquiring enzyme (Fig. 5.1). Phosphatase generally showed the highest potential activity among the enzymes with higher values for grass litter ($F_{1,60}=14.7$, $P<0.001$; Fig. 5.1A), ranging from 55.7 to 247.4 (110.0) nmol MUB h$^{-1}$ g$^{-1}$ AFDM than for tree litter, with values ranging from 31.8 to 217.5 (84.7) nmol MUB h$^{-1}$ g$^{-1}$ AFDM. Potential activity rates of β-glucosidase were highest among the C-acquiring enzymes and ranged between 0.0 and 7.4 (2.7) nmol MUB h$^{-1}$ g$^{-1}$ AFDM for tree litter and between 6.4 and 63.3 (20.7) nmol MUB h$^{-1}$ g$^{-1}$ AFDM for grass litter ($F_{1,60}=231.3$, $P<0.001$; Fig. 5.1B). Potential activity of β-Xylosidase ranged from 0.0 to 4.1 (1.4) nmol MUB h$^{-1}$ g$^{-1}$ AFDM for tree litter and from 2.9 to 23.0 (8.2) nmol MUB h$^{-1}$ g$^{-1}$ AFDM for grass litter (Fig. 5.1C). Potential activity of chitinase ranged between 0.0 and 15.6 (5.3) nmol MUB h$^{-1}$ g$^{-1}$ AFDM for tree and between 1.9 and 30.7 (30.6) nmol MUB h$^{-1}$ g$^{-1}$ AFDM for grass litter ($F_{1,60}=54.3$, $P<0.001$) with the smallest difference between litter types among all C-acquiring enzymes (Fig. 5.1D). Contrastingly, potential activity of leucin-AP was higher on tree litter ($F_{1,60}=89.0$, $P<0.001$), with values ranging between 7.9 and 21.5 (13.0) nmol MUB h$^{-1}$ g$^{-1}$ AFDM of tree litter and between 4.2 and 15.4 (6.5) nmol MUB h$^{-1}$ g$^{-1}$ AFDM of grass litter (Fig. 5.1E).
Higher C:N (β-glucosidase + β-xylosidase to leucine-AP) and C:P (β-glucosidase + β-xylosidase to phosphatase) ratios of enzyme activities were found for the microbial communities on grass litter compared to the communities on tree litter (Fig. 5.2). These ratios corresponded to the nutrient contents of the litter substrate, with a lower C:N and C:P ratio for tree litter than for grass litter. Even though N:P (leucine-AP to phosphatase) enzymatic ratios for both litter types were below 1, the ratio was higher for microbial communities on tree litter compared to communities on grass litter. This suggests that communities on tree litter invested more in the synthesis of N-acquiring enzymes compared to P-acquiring enzymes despite the fact that communities on grass litter were probably even more limited by the substrate (N:P ratio on grass bigger than on tree litter, Table 5.1).

**Microbial respiration**

Respiration of the microbial communities colonizing tree and grass litter showed no differences among treatments ($F_{4,10}=0.23$, $P=0.91$) but did between both litter types ($F_{1,59}=80.8$, $P<0.001$). Microbial respiration was higher for tree litter, ranging between 14.2 and 132.4 (68.4) µg C g$^{-1}$AFDM h$^{-1}$ than for grass litter ranging between 18.6 and 74.0 (34.7) µg C g$^{-1}$AFDM h$^{-1}$ (Fig. 5.1F).
Figure 5.1. Potential enzyme activities and respiration of microbial communities associated with tree and grass litter in experimental channels mimicking five stages of stream ecosystem succession. Bar plots show means ± 1 SE pooled across 3 sampling dates in each of three channels, n=3.
Figure 5.2. Ratios of potential activities of enzymes involved in the acquisition of carbon, nitrogen and phosphorus by microbial communities associated with grass and tree litter in experimental channels mimicking five stages of stream ecosystem succession. Symbols show means ± 1 SE pooled across 3 sampling dates in each of three channels, n=3. Note the logarithmic scale in panel A. gluco = β-glucosidase, xylo = β-xylosidase, leu = leucine-AP

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**Leaf mass loss**
Mass loss of tree litter (*Betula pendula*) was more important than of grass litter (*Calamagrostis epigejos*) ($F_{1,58}=4.63$, $P=0.035$) and ranged from 23 to 35% (Fig. 5.3A). Mass loss of grass and tree litter did not vary among the different treatments ($F_{4,10}=0.28$, $P=0.88$) or the different sampling dates ($F_{1,58}=0.56$, $P=0.46$).

![Figure 5.3](image)

**Figure 5.3.** Leaf mass loss (A) and fungal sporulation rate (B) associated with grass and tree litter in experimental channels mimicking five stages of stream ecosystem succession. Histograms show means + 1 SE pooled across 3 (leaf mass loss) or 2 (sporulation rate, 8 and 10 weeks) sampling dates in each of three channels, $n=3$.

**Fungal sporulation rate**
No differences between treatments of sporulation rates measured from tree litter could be observed ($F_{4,10}=1.2$, $P=0.4$) (Fig. 5.3B). Sporulation rates of fungi were constantly low on grass litter, ranging between 0.0 and 8.9 (0.9) pg h$^{-1}$ mg$^{-1}$ DM. They were higher on tree litter ($F_{1,30}=7.6$, $P=0.009$), ranging after 8 weeks between 2.5 and 1059.8 (179.3) pg h$^{-1}$ mg$^{-1}$ DM and then strongly decreased to values ranging between 0.8 and 58.6 (20.5) pg h$^{-1}$ mg$^{-1}$ DM after 10 weeks. This decline of sporulation rates for tree litter was reflected by the interaction of time and type of litter ($F_{1,30}=4.9$, $p=0.03$)
**Fungal and bacterial biomass**

Biomass of the bacterial communities colonizing tree and grass litter showed no differences among treatments ($F_{4,10}=1.73$, $P=0.22$), (Fig. 5.4A). However, bacterial biomass was higher on tree litter than on grass litter ($F_{1,60}=112.0$, $P<0.001$) with values ranging between 291 and 1929 (846) µg cells g$^{-1}$ AFDM on tree litter and between 245 and 834 (466) µg cells g$^{-1}$ AFDM on grass litter. Analysis of variance also showed an interaction between time and litter type, as the increase of biomass in time was higher on grass than on tree litter ($F_{1,60}=10.8$, $p=0.002$).

Similar to bacterial biomass, fungal biomass showed no differences among treatments ($F_{4,10}=1.2$, $P=0.39$) but between litter types with values ranging between 6.4 and 38.2 (19.4) mg cells g$^{-1}$ AFDM on tree litter and between 2.3 and 19.3 (6.5) mg cells g$^{-1}$ AFDM on grass litter ($F_{1,60}=174.1$, $P<0.001$). On average, fungal biomass was 11 to 42 times more important than bacterial biomass on tree litter and 7 to 29 times higher on grass litter (Fig. 5.4B).

**Figure 5.4.** Biomass of bacteria (A) and fungi (B) associated with grass and tree litter in experimental channels mimicking five stages of stream ecosystem succession. Histograms show means + 1 SE pooled across 3 sampling dates in each of three channels, n=3.
Bacterial and fungal community structure

Bacterial communities on grass and tree litter varied among sampling dates and among treatments (treatment x sampling, $F_{8,30} = 1.74$ and 1.5, $P = 0.008$ and 0.041, Fig. 5.5A). Bacterial community structure also differed between the two types of litter ($F_{1,80} = 6.77$, $P = 0.001$) at each sampling date but the communities on both litter types sampled after 6 and 8 weeks (Fig. 5.6A,C) were more similar among litter type than after 10 weeks (Fig. 5.6B,D). The cluster regrouping communities sampled after 6 and 8 week was clearly separated along the first NMDS axis from communities sampled after 10 weeks. The bacterial communities in these two clusters were further ordered along the second NMDS axis reflecting the treatments of different background litter. Specifically, the bacterial community structure on grass and tree litter was different depending on the presence of background litter (contrast A, $F_{1,44} = 1.41$ and 1.47, $P = 0.002$ and 0.001) and on the presence of tree litter (contrast B, $F_{1,44} = 1.95$ and 1.60, $P = 0.001$). Furthermore, the bacterial structure on grass and tree leaves differed depending on the quality of the litter in the channel (contrast C, $F_{1,44} = 1.42$ and 1.27, $P = 0.001$), and depending on the quantity of tree litter in the channel (contrast D, $F_{1,44} = 1.2$ and 0.94, $P = 0.005$ and 0.032). The mix of grass and tree litter had no effect on the structure of the bacterial community compared to single species litter treatments (contrast E).

Fungal community structure differed between the two litter types ($F_{1,70} = 2.06$, $P = 0.002$), and changed with time (Fig. 5.5B). The communities sampled after 6 and 8 weeks clustered more closely than the one collected after 10 weeks ($F_{2,24}$ and 26 = 4.5 and 7.74, $P = 0.001$ and 0.019). Fungal community composition varied among treatments ($F_{4,24}$ and 26 = 2.28 and 1.56, $P = 0.001$ and 0.019), although these differences were less pronounced than for the bacterial community (Fig. 5.7). Specifically, fungal community on exposed grass and tree litter was differently structured depending on the presence of background tree litter in the channels (contrast B, $F_{1,37}$ and 39 = 1.82 and 1.4, $p = 0.02$ and 0.024). Only fungal community composition on grass litter was differently structured depending on the presence of background litter in the channel (contrast A, $F_{1,37} = 1.62$, $P = 0.034$) and on the litter type of background litter (contrast C, $F_{1,37} = 1.77$, $P = 0.022$). The quantity of tree litter in the channels and the mix of grass and tree litter had no effect on the structure of the fungal community.
Both bacterial and fungal communities were not significantly related to enzymatic activities. However, when fitted on the fungal or bacterial ordination plot (Fig. 5.5), the vector of leucine-AP pointed in the opposite direction of β-glucosidase, β-xylosidase and chitinase, indicating opposite relations of these two groups of enzymes with the structure of microbial communities. Vectors of environmental variables as DOC, TDN and TDP all pointed to the direction of microbial communities of both litter types sampled after 6 and 8 weeks and were related to fungal ($r^2=0.17, 0.37, 0.21$, $P=0.002, <0.001, <0.001$) and bacterial ($r^2=0.21, 0.46, 0.22$, $P=0.002, <0.001, <0.001$) communities.
**Figure 5.5A.** NMDS ordination of bacterial community structure inferred from ARISA profiles in relation to a suite of potential enzyme activities associated with tree and grass litter in experimental channels mimicking five stages of stream ecosystem succession. Asterisks (*) indicate variables that are significantly related to the arrangement of the bacterial communities in the ordination. Phos = phosphatase, Leu = leucine-AP, Gluco = β-glucosidase, Xylo = β-xylosidase, PO = phenol oxidase, PP = phenol peroxidase, DOC = dissolved organic carbon, TDN = Total dissolved nitrogen, TDP = Total dissolved phosphorus, OM = organic matter in sediment.
Figure 5.5B. NMDS ordination of fungal community structure inferred from ARISA profiles in relation to a suite of potential enzyme activities associated with tree and grass litter in experimental channels mimicking five stages of stream ecosystem succession. Asterisks (*) indicate variables that are significantly related to the arrangement of the bacterial communities in the ordination. phos = phosphatase, leu = leucine-AP, gluco = β-glucosidase, xylo = β-xylosidase, PO = phenol oxidase, PP = phenol peroxidase, DOC = dissolved organic carbon, TDN = Total dissolved nitrogen, TDP = Total dissolved phosphorus, OM = organic matter in sediment.
**Figure 5.6.** NMDS ordination of bacterial communities inferred from ARISA profiles in A) grass litter, sampled after 6 + 8 weeks, B) grass litter sampled after 10 weeks, C) tree litter sampled after 6 + 8 weeks, and D) tree litter sampled after 10 weeks from experimental channels mimicking five stages of stream ecosystem succession.
Figure 5.7. NMDS ordination of fungal communities inferred from ARISA profiles in A) grass litter, sampled after 6 + 8 weeks, B) grass litter sampled after 10 weeks, C) tree litter sampled after 6 + 8 weeks, and D) tree litter sampled after 10 weeks from experimental channels mimicking five stages of stream ecosystem succession.
5.4. Discussion

Effect of the quality and quantity of litter input on microbial decomposition activities

The main finding of this study is that the quality and quantity of the background litter standing stock in the stream as occurring during stream succession had no direct effect on the substrate associated microbial metabolism, which is consistent with the findings of Mille-Lindblom et al. (2006). In contrast to treatment effects on microbial activities in sediment found in the similar study (Chapter 4; Gerull, 2011, chapter 4), no difference in biomass and respiration of leaf associated microbial communities among treatments were observed here. This is surprising regarding the fact that higher amount of decomposing litter should facilitate a larger microbial inoculum, increase colonization, and consequently accelerate microbial decomposition (Treton et al., 2004). Only a trend of higher fungal sporulation rates in week 8 could be observed in the tree litter treatments before rates declined. It is possible that, by measuring after 8 weeks, we have missed the facilitating effect of the litter input, as fungi are known to be rapid colonizers and reach high productivity within 2 to 8 weeks after colonization (Gessner & Chauvet, 1994). However, the general null-effect of the different litter inputs indicates that decomposition processes on a leaf are independent of the quantity and the quality of surrounding leaves. A missing effect of quantity of background litter on microbial decomposition has been reported before (Tiegs et al., 2008). However, studies revealing faster decomposition in litter augmented streams attributed this effect to higher stream water nutrient content (Young et al., 1994; Niyogi et al., 2003). Effect of quality of background litter was assessed by exposing leaves in forested and pasture streams, but beside background litter, many other variables differed between the studied streams (Hladyz et al., 2010). Thus, our results show for the first time the general lack of an effect of background litter standing stock on microbial activity under otherwise constant conditions.

The only microbial metabolic response to the treatments detected in this study was the potential activity of leucine-AP in the microbial community associated to tree litter, which was lower in the simulated open-land streams without or just with grass litter compared to forested streams containing tree litter (contrast B). In this particular case, the quality of the background litter had an effect on the need of microbial communities associated with tree litter for N-
acquisition. Although we did not detect differences in nutrient concentrations among the treatments in the channel outlet, nitrogen concentrations within the voids and on the leaf surfaces of tree litter layer stocked in later successional stage treatments and colonized by active microbial communities might have been reduced (Suberkropp & Chauvet, 1995). This could have resulted in the increased excretion of N-acquiring enzymes in these treatments with input of tree litter.

The expected effect of mixed litter on decomposition in the transitional treatment between open-land and forested streams was not found. This output was consistent with the findings in leaf pack decomposition experiments comparing mixed to single species litter (Swan & Palmer, 2004; Taylor et al., 2007). However, other studies reported an effect of mixing litter species on decomposition rates (Kominoski et al., 2007; Lecerf et al., 2007; Abelho, 2009) and hence, the general effect of mixing litter on decomposition is still unclear. We can state from this experiment that input of mixed type of litter do not trigger larger decomposition rates and other factors besides the quality of the leaf litter input in stream seem to play an important role (Gessner et al., 2010).

**Effect of the quality of leaf litter directly associated to microbial communities on microbial activities**

The distinct differences in microbial activity parameters and biomass of fungi and bacteria associated either with grass or tree litter reflected the quality dissimilarity of these both types of litter (i.e. differences in P, N, C, C:N and C:P ratios; table 5.1). Decomposition rates have been shown to be correlated to condensed tannins, lignin, N and C:N content of litter material (Gessner & Chauvet, 1994; Ostrofsky, 1997; Ardón et al., 2009; Hladyz et al., 2009). However, Ostrofsky (1997) stated that the predictive power of these simple relationships is weak and could not explain more than 50% of the variation of processing rates. As in our experiment, grass litter, with a high C:N ratio, has generally been found to decompose slowly (Menninger & Palmer, 2007; Shaftel et al., 2011). The higher potential activity of C-acquiring enzymes on grass compared to tree litter indicates that the availability of carbon was lower on grass than on tree leaves, although the absolute difference in carbon content was marginal. Within C-species, lignin is a refractory compound of leaves that is resistant to microbial decay (Gessner & Chauvet, 1994) and typically ranges between 13% and 39% in deciduous leaves (Ostrofsky, 1997) and between 3 and 6% in grasses (Rozema et
al., 1997; Hoorens et al., 2002; Griffith et al., 2009). However, a difference in lignin content could not explain the lower activity associated with grass litter in our experiment, as lignin-degrading enzyme activities, i.e. phenol oxidase and peroxidase were weak or inexistent on both litter types. This indicated that litter degradation was probably still in the initial phase, which is dominated by polysacharide decomposition (Fioretto et al., 2005; Snajdr et al., 2011). Although differences in C were not substantial between tree and grass litter, differences in the ratio of C to P and N were consequent and have presumably triggered increased activity of C-acquiring enzymes associated with grass litter.

Evidence that microbial communities on both exposed leaf types were primarily P-limited is implied by the high activity of phosphatase compared to the other measured enzymes (Taylor et al., 2003). This most probably resulted from low SRP concentration in the water supplying the channels, as it is known that nutrients from the water column are significant nutrient source for leaf associated microbial communities in addition to the leaf itself (Suberkropp & Chauvet, 1995).

C:N ratio of grass leaves was much higher than that of tree leaves pointing at lower availability of nitrogen in grass litter, especially as microbes have a greater demand for N relative to C (Sterner & Elser, 2002). Nevertheless, microbial communities colonizing grass litter were still rather limited by carbon as reflected by the C:N enzymatic ratio above 1 on that type of litter. The lower excretion of N-acquiring enzymes by the microbial communities on grass litter was probably not due to a weaker N-limitation but to metabolic limitation of these communities. The production of enzymes requires microbial investment of energy in the form of C, nutrients and particularly N. Thus, extracellular enzymes are energetically and nutritionally expensive to produce and their activity may increase with increasing availability of labile C and N (e.g. Schimel & Weintraub, 2003). As nitrogen was less available in grass litter, the investment in N-acquiring enzymes might have been too expensive for associated microbial communities, as their metabolism was already constrained by the costly acquisition of carbon as seen by the low respiration rates. Contrastingly, the presumably higher availability of carbon on tree litter and the lower C:N seem to have positively influenced microbial growth on that substrate resulting in higher fungal and bacterial biomass and respiration rates (Fig. 5.3A, B and 5.1F).
Effects of litter input and directly associated litter type on microbial community composition

The distinct dissimilarity of microbial community metabolism between the two litter types was also reflected in the fungal and bacterial community structure (Fig. 5.5). Moreover, the quality of the litter types was more relevant for microbial metabolism and community structure than the effect of the stream litter standing stock (Fig. 5.5). Substrate quality has been found to superimpose effects of environmental variation in other studies (Gulis, 2001; Mille-Lindblom et al., 2006), however, these studies focused in variation of stream water nutrient content (Harrop et al., 2009; Marks et al., 2009), which was constant among our treatments.

In our experiment, differences among channels stocked with different amounts and types of litter resulted in different microbial community structures but not in different metabolic activities, except for leucine-AP activity on tree litter. This general disconnect between microbial community composition and metabolism reinforces conclusions from Chapter 4 that microbial communities are often functionally redundant (Kominoski et al., 2010). Nevertheless, the observed differences between communities on grass and tree litter suggest that different fungal and bacterial communities were adapted to metabolizing the specific substrate they colonized. This has also been observed by Strickland et al. (2009), who found that microbial communities derived from herbaceous litter were more active on grass litter than on tree litter in spite of higher resource quality of the latter.

Bacterial and fungal community structure and their relation to metabolic activities

Interestingly, most variation in the structure of fungal and bacterial communities occurred with time, i.e. between 8 and 10 weeks of exposure. The structural shift was clearer in the bacterial community than in the fungal community and was likely due to a shift of litter chemistry during decomposition as observed in other studies (Harrop et al., 2009, Snajdr et al., 2011). Ence, the shift in the microbial composition due to the litter decay stage was sometimes even larger than the difference in community composition between different leaf types (Das et al., 2007). Another possible explanation for the temporal shift in microbial community composition could have been the decline of fungal productivity seen
at decreasing sporulation rates. This might shift the community due to the
proposed interaction between fungi and bacteria (Mille-Lindblom & Tranvik,
2003; Romaní et al., 2006). However, the clear shift in the microbial community
structure with progression of decay was not reflected in the enzyme activities,
although this link was observed in another study (Snajdr et al., 2011).

Our experiment shows that the quality of the leaves directly colonized is
more important than the quality and quantity of litter input in stream for fungal
and bacterial community structure and activity. The input of new allochthonous
organic matter throughout stream succession had only a partial effect on the
bacterial community structure, a weak effect on the fungal community structure,
and no effect on microbial metabolic activities associated to a single leaf. Thus,
metabolic activities of the microbial communities reflected the specific
limitations in carbon and nutrients which varied among leaf types and showed
the prime importance of leaf quality for microbial metabolism, independently of
background environmental conditions. We conclude that microbial diversity and
functions are expected to augment with the associated increase of the quality of
the litter source along the succession from open-land streams with low quality
green grass litter to forested streams with higher quality tree litter.

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Chapter 6

Conclusions and Outlook

6.1. General Conclusions
Various aspects of the complex relationships between microbial community structure and functions were investigated in the context of stream ecosystem succession. The reported results provides new insights into how changes in organic matter sources during stream succession influence microbial community structure and function relate to carbon transformations. Such data were nonexistent so far (Schaaf et al., 2011). Here I summarize the major findings and interpretations of the different aspects of microbial community structure and function explored in this thesis.

Microbial community structure and activities and their relation to environmental variation
Investigations in the early successional catchment of Chicken Creek allowed me to discern patterns of microbial structure and activities in the early stage of ecosystem development. Most notably, I found a disconnect between bacterial community structure and microbial metabolic activities (Chapter 2). Additionally, there was a strong seasonal effect on microbial functions assessed as microbial potential enzyme activities, and remarkable variation among different potential activities. These results contrast with the unresponsiveness of spatial pattern within the stream corridors of the Chicken Creek catchment, which were observed despite the highly differing water availability. Furthermore, neither water availability nor the direction of the vertical water exchange in the stream sediment was important in regulating microbial potential enzyme activities. More than by temperature, the temporal pattern of C- and P-acquiring potential enzyme activities were possibly influenced by a priming effect of algae, which might be important for heterotrophic microbial communities in Chicken Creek since the available DOC was highly recalcitrant (Gerull et al., 2011). Notably, the
patterns of microbial activities were not related to bacterial community structure. The stochastic distribution of bacterial taxa might reflect a high microbial dispersal ability, which could override the influence of spatial heterogeneity. Moreover, functional redundancy among taxa might explain the observed disconnect between bacterial community structure and potential enzyme activities.

**Importance of the microbial inoculum for stream metabolism**

Manipulation of the initial microbial inoculum present in bare channels simulating early successional streams revealed the importance of fungi in the experimental streams despite a paucity of particulate organic matter. Composition of the microbial community at the beginning of the succession was very important for stream metabolisms in the early successional stage (Chapter 3). Moreover, the study demonstrates that the role of fungi in stream metabolism can go well beyond a direct contribution to leaf litter decomposition. This conclusion is supported by the observation that an increased fungal biomass in leaf litter correlated both with microbial respiration rate in the leaf litter and with whole-system metabolism in the channels inoculated with fungi. Another important finding of this experiment was the capacity of fungi to colonize extensively the few leaves present in the channels. This colonization was not only observed in the channels receiving a fungal suspension from forested streams, but also those inoculated with a fungal community originating from the early-successional catchment of the Chicken Creek. Moreover, the development of fungal communities indirectly affected the structure of the bacterial communities associated with both leaves and sediments, microbial respiratory activity in sediments, algal biomass, and whole-stream metabolism.

**Effect of changing quality and quantity of organic matter input for stream-bed microbial communities during simulated succession of riparian vegetation**

Riparian plant development and associated changes in the quality of particulate organic matter supplies for heterotrophic microbial communities were found to affect stream microbial communities in my experimental stream channels mimicking five successional stages (Chapter 4, 5). Moreover, the simulated changes in riparian vegetation induced gradual shifts both in bacterial
community structure and microbial potential enzyme activities in the stream bed sediment. These shifts appeared to be primarily driven by nutrient acquisition rather than by carbon demand as suggested by the observed variation in the N- and P-acquiring enzymes but not in the C-acquiring enzymes activities. Potential activities of nutrient-acquiring enzymes were also higher than those of carbon-acquiring enzymes, suggesting nutrient limitation of sediment-associated microbes throughout the experimentally simulated stream succession. Algae showed the same pattern as the nutrient-acquiring enzymes, implying that they could have affected potential enzymes activities and sediment bacterial communities, although this was not clearly apparent.

The quality of leaves colonized by microbes was more important for fungal and bacterial community structure and activity than the quality and quantity of the litter used to stock the experimental stream channels (Chapter 5). Moreover, metabolic activities (i.e. potential enzyme activities) of the microbial communities appeared to reflect specific limitations in carbon and nutrients. Their variation among leaf types revealed the prime importance of litter quality for microbial metabolism, independent of background environmental conditions. In contrast, microbial potential enzyme activity on tree and grass litter was unaffected by the quantity and type of litter used to stock the experimental channels, whereas major differences were apparent between grass and tree litter within the same channel. However, bacterial and fungal community structure varied not only between the litter types that were colonized but also among channels stocked with different litter types and quantities. This indicates functional redundancy among the various communities establishing at different stages of stream ecosystem succession.

The significance of microbes in the study of ecosystem succession!

In conclusion, changes in the physical and biological structure of streams during ecosystem succession (Chapter 1) were of great importance in shaping bacterial and fungal community structure and regulating microbial activities. During stream succession, the input of fresh leaf litter influenced both specific functions and the structure of the microbial communities (Chapters 4 and 5). Moreover, the quality of the litter colonized by microbes had a greater influence than the stream environment (Chapter 5). In contrast, bacterial community structure and microbial potential enzyme activities appeared to be little affected by variation of
important environmental factors, such as water availability, in the early successional catchments of the Chicken Creek (Chapter 2). Finally, composition of the initial microbial community was found also to affect whole-stream metabolism during the early stage of succession (Chapter 3). Taken together, these patterns of microbial community structure and function in early-successional streams clearly affect the trajectories of ecosystem succession (Walker & del Moral, 2003). Thus, responses of microorganisms to changing environments during the early stages of succession clearly influenced the development of stream ecosystem, such as the establishment of a complete food web or biogeochemical cycles.

Long-term studies on primary succession in terrestrial ecosystems have shown that during the first 5 years of succession (e.g. after ice-sheet retreat), ecosystems are largely dominated by physical processes (Milner et al., 2007). Dominance of physically processes in early stages of ecosystem succession was also one of the hypotheses of the overall project focused on the development of the Chicken Creek catchment (Gerwin et al., 2009; Schaaf et al., 2011). However, evidences presented in this thesis (Chapter 2-5) indicates that biological processes were important from the first stages of succession (<5 years). For example, the development of autotrophic organisms was clearly important in the first stages of stream ecosystem development, with the appearance of algae being related to potential microbial potential enzyme activities (Chapter 2 and 4), microbial community structure (Chapter 4), and also whole-ecosystem metabolism (Chapter 3).

However, biological processes are closely linked to physical and chemical processes during all steps of stream succession. This also applies to effects following physical disturbance of the stream bed (Gerull 2011, Chapter 3) or the availability to stream-bed microbial communities of carbon (Chapter 2) and nutrients (Chapter 4). Carbon and nutrient concentrations in stream water did not change during the experiment simulating riparian vegetation development (Chapters 4 and 5). In studies on succession of terrestrial ecosystems, changes in carbon and nutrient pools are first expected in a later stage of development when the forested vegetation establishes (Milner et al., 2007). Thus, changes in carbon and nutrients in stream water, and consequent effects on microbial potential enzyme activities and community structure (e.g. Crenshaw et al., 2002; Findlay et
al., 2003; Artigas et al., 2008a), might be expected in later stages of succession after full development of riparian tree vegetation.

6. 2. Outlook
The combination of surveys in the Chicken Creek catchment and experiments in artificial outdoor channels was efficient in revealing patterns of microbial communities during stream ecosystem succession. The artificially created Chicken Creek catchment provides a rare opportunity to follow ecosystem development in real time at the ecosystem scale. This makes the approach suitable for long-term investigations. However, comparison of patterns in the Chicken Creek catchment with other ecosystems undergoing succession (e.g. volcanic areas, recently-retreated glaciers, other post-mining sites) is needed to generalize the findings presented in this thesis. Following the development of streams in the long run should give valuable information about ecosystem theory in general, including the relative role of deterministic and stochastic pathways throughout the ecosystem succession (Milner & Robertson, 2009). In view of the results presented above, future investigations in three directions appear to be particularly promising: 1) priming effects of algae on heterotrophic microbial communities, 2) the level of functional redundancy in microbial communities, and 3) the activity and structure of microbial communities along vertical environmental gradients in the hyporheic zone of streams.

Autotrophic organisms appear to be important during stream ecosystem succession. Although exploring different questions, all projects (Chapters 2-5) point to a large influence of algae on microbial community structure and activities. These effects of algae on microbial communities in early successional streams contrast with recent observations reporting the importance of heterotrophic organisms at the beginning of ecosystem succession (Hodkinson et al., 2002; Peter, 2003; Bardgett et al., 2007; Fierer et al., 2010; Sattin et al., 2010). Priming effects of algae, as the enhancement of recalcitrant organic matter degradation by heterotrophic microorganisms through the input of labile organic matter by autotrophic organisms, has been well recognized in the soil environment but a very limited number of studies have investigated the phenomenon in aquatic ecosystems (Guenet et al., 2010). A positive effect of algae on potential enzyme activities of heterotrophic microbes has been repeatedly demonstrated in streams (e.g. Jones et al., 1995; Romaní & Sabater, 2000b; Rier et
al., 2007), but the nature of the relation is still difficult to disentangle. Therefore, there is high demand for experiments investigating both the heterotrophic and autotrophic community structure and activities in streams, not only in the context of stream succession.

Functional redundancy also seems to be important in microbial communities in early-successional streams. It could explain the disconnect between microbial potential enzyme activities and bacterial community structure observed in the Chicken Creek catchment (Chapter 2). During simulated stream succession, microbial potential activities of C-acquiring enzymes and bacterial and fungal community structure in the stream bed and associated with leaf litter showed no direct links (Chapters 4 and 5). However, potential activities of N- and P-acquiring enzymes (Chapter 4) or of N-acquiring enzymes only paralleled changes in the structure of bacterial and fungal communities. This suggests that only specific functions might be linked to community structure, whereas broad metabolic capacities tend to be redundant in microbial communities (Chapter 4). One reason why little is known about microbial functional redundancy is that detailed knowledge about the microbial populations performing a specific process is not available (Allison & Martiny, 2008). Moreover, functional redundancy has been found even in simple microbial communities (e.g. Setälä & McLean, 2004; Wohl et al., 2004; Langenheder et al., 2010). Increased functional abilities were related to greater microbial diversity only in experimental conditions with communities containing less than one hundred taxa (Bell et al., 2009). Thus, relationships between microbial diversity and ecosystem functioning in natural environments, where microbial communities are much more diverse, are difficult to disentangle. High-throughput pyrosequencing might be a mean to provide new insights into these relationships. Sequencing of specific functional genes and linking them to enzyme activities might be an especially useful approach.

The hyporheic zone is a highly dynamic compartment of stream ecosystems. It supports a high bacterial diversity (Feris et al., 2003). Although microbial community structure and potential enzyme activities did not differ along vertical water flowpaths (e.g. between groundwater upwelling and downwelling of surface water) in the hyporheic zone in early successional streams (Chapter 2), other factors such as carbon supply (Findlay et al., 2003) or oxygen depletion (Gerull, 2011; Chapter 4) can shape microbial communities
within hyporheic sediments. The vertical distribution of hyporheic microbial populations and associated functions was not investigated in this thesis. However, microbial metabolism and the structure of microbial communities are likely to vary with sediment depth, since oxygen concentrations sharply declined in the channels. Again, pyrosequencing could provide insight into the “black box” of microbial communities in hyporheic sediments.
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Bibliography


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Date of Birth: 15 December 1982
Nationality: Swiss
Languages: French (native), English (very good), German (very good), Spanish (basic)

EDUCATION AND RESEARCH EXPERIENCE:

2011 PhD at Eawag/ETH Zurich. Title of thesis: Microbial dynamics during stream ecosystem succession: community structure and enzyme activities. Advisors: Prof. Mark Gessner, Eawag/ETH and PD Dr. Michael Mutz, BTU Cottbus (Germany). PhD project carried out as part of an integrative project (SFB TRR 38; http://www.tu-cottbus.de/sfb_trr/eng) funded by the German Research Council (DFG).

2006 M.Sc. in Biogeosciences, University of Neuchâtel, Switzerland. Title of thesis: Structure and activities of rhizosphere microbial communities along a recent glacier forefield in the Swiss Alps. Advisors: Prof. Michel Aragno, Dr. Jakob Zopfi and Dr Sonia Tarnawski.

2004 B.Sc. degree in Biology, University of Neuchâtel, Switzerland
2001 Swiss Federal maturity diploma, La Chaux-de-Fonds, Switzerland

PUBLICATIONS


Frossard A., Gerull L., Mutz M. and Gessner M. O. Fungal importance for stream metabolism: an experimental test in early successional stream channels. (unpublished manuscript)

Frossard A., Gerull L., Mutz M. and Gessner M. O. Microbial community structure and function during experimental stream succession. (unpublished manuscript)

Frossard A. and Gerull L., Mutz M. and Gessner M. O. Leaf quality as driver of microbial metabolism and community structure during simulated stream ecosystem succession. (unpublished manuscript)

REVIEWING EXPERIENCE
Microbial Ecology
Freshwater Biology

TEACHING EXPERIENCE
2009 (April-June) Supervision of Alison Ling (Master student, USA) during an internship at Eawag, Switzerland.
2006 (Spring semester) Practical class “Fundamentals of Microbiology”, Department of Biology, University of Neuchâtel, Switzerland.

PRESENTATIONS AT CONFERENCES AND WORKSHOPS
2011 (July) Enzymes in the environment: activity, ecology and applications, Bad Nauheim, Germany. **Oral presentation**: Microbial enzyme activities in an early successional stream network.
2011 (June) Symposium for European Freshwater Sciences (SEFS), Girona, Spain. **Oral presentation**: Effects of litter quality and quantity on microbial community structure, biomass and enzyme activities during stream succession.
2011 (January) 4th Swiss Microbial Ecology meeting (SME), Engelberg, Switzerland. **Oral presentation**: Importance of fungi for stream metabolism: a test in experimental streams.
2010 (September) Meeting of young researchers in earth science (MYRES), Cottbus, Germany. **Oral presentation**: Fungal vs bacterial significance for stream metabolism: a test in experimental streams.
2010 (September) 1st international meeting on “Structure and processes of the initial ecosystem development”, Cottbus, Germany. **Oral presentation**: Fluctuating patterns of microbial enzyme activities in an early successional stream network.
2010 (August) 13th meeting of the International Society of Microbial Ecology (ISME), Seattle, USA. **Poster presentation**: Fungal vs bacterial significance for stream metabolism: a test in experimental streams.
2010 (June) ASLO-NABS joint meeting, Santa Fe, NM, USA. **Oral presentation**: Fluctuating patterns of microbial enzyme activities in an early successional stream network.
2009 (February) Biology 2009, Bern, Switzerland. **Poster presentation**: Microbial enzyme activities in newly formed streams: evidence for metabolic hot spots?
2009 (January) 3rd Swiss Microbial Ecology meeting (SME), Einsiedeln, Switzerland. **Oral presentation**: Microbial enzyme activities as indicators of metabolic hot spots in newly formed streams.
2008 (September) German Society of Limnology (DGL), Constance, Germany. **Poster presentation**: Microbial enzyme activities as indicators of metabolic hot spots in newly formed streams.
2006 (September) 2nd Swiss Microbial Ecology meeting (SME), Bellinzona, Switzerland. **Poster presentation**: Genetic and metabolic structure of rhizosphere microbial communities along a recent glacier foreland in the Swiss Alps.
GRANTS AND AWARDS

2011 FEMS (Federation of European Microbiological societies, EUR 400) to attend the conference “Enzymes in the Environment: Activity, Ecology, Applications”, 17-21 July, Bad Nauheim, Germany

2010 MYRES travel fund (EUR 400) to attend the MYRES conference in Cottbus, Germany

2010 ETH travel fund (CHF 700) for the ASLO-NABS and ISME conferences (Santa Fe and Seattle, USA)

2009 Eawag Mobility support (CHF 2800) to conduct PhD research at a foreign laboratory (Department of Biology, University of New Mexico, NM, U.S.A.; local advisor: Prof. Robert L. Sinsabaugh)

2009 ETH travel fund (CHF 500) for attending the Advanced Course “Recent topics in stream ecology”, Coimbra, Portugal

2006 Award “Jean-Luc Crélerot” (CHF 1000) for an outstanding Master thesis, University of Neuchâtel, Switzerland

2006 Award “Jean Landry” (CHF 1000) for completing a Master degree with a grade higher than 5.5, University of Neuchâtel, Switzerland

2002 Award from the “Schweizer Jugend Forscht” for an excellent Maturity thesis (CHF 1500), St-Gallen, Switzerland

2001 Award “Maurice Ditisheim” for an excellent Maturity thesis (CHF 300), Lycée Blaise-Cendrars, La Chaux-de-Fonds, Switzerland

WORKSHOP ORGANIZATION

2010 (January 31–February 5) ETH Zurich Winterschool “Ecosystem Genesis”, Centro Stefano Franscini, Ascona, Switzerland

TECHNICAL SKILLS

**Microbiology:** Molecular microbiology (DNA extraction, PCR, DGGE, ARISA, qPCR), flow cytometry, bacterial and fungal abundance and biomass, bacterial and fungal production, microbial potential enzyme activities, culture-based methods

**Ecology:** community respiration and production, cartography, field methods (limnolgy, geology, soil science), physicochemical soil properties, phytosociology

**Microscopy:** Epifluorescence microscopy (EM), environmental scanning electron microscopy (ESEM).

**Software:** Molecular biology (Gene mapper, GelCompar, ImageJ), geographic information system (ArcGIS), statistics (SPSS, R).

CERTIFICATES

Driving licence (2001), Switzerland

Diving certificates: “PADI Rescue Diver” and “PADI Advanced Open Water Diver” (2004, St-Eustatius, Netherlands Antilles, “PADI open water” (2003, Neuchâtel, Switzerland)

Rescue swimming certificates: “Brevet 1” and “ABC 1” (1997), “CPR” (2003), Swiss Rescue Society (SLRG-SSS)