

Dynamics of microbial metabolic activities during stream ecosystem succession: insights from field observations and flume experiments

Von der Fakultät für Umweltwissenschaften und Verfahrenstechnik der Brandenburgischen Technischen Universität Cottbus zur Erlangung des akademischen Grades eines Doktor der Naturwissenschaften (Dr. rer. nat.) genehmigte Dissertation
vorgelegt von

Diplom-Geographin

Linda Gerull

aus Berlin

Gutachter: PD Dr. rer. nat. habil. Michael Mutz

Gutachter: Prof. Dr. rer. nat. habil. Mark O. Gessner

Tag der mündlichen Prüfung: 11. Juli 2011

Abstract

Processes of microbial carbon transformation and accumulation during initial stream succession were investigated. Studies were carried out in the experimental watershed Chicken Creek, constructed to investigate ecosystem succession, and additionally in experimental flumes simulating sand-bed streams.

In a one year investigation, microbial respiration in soils and sediments along the hydrologic flow path of three stream corridors in the Chicken Creek watershed was measured. Contrary to expectation, respiration rates of rewetted soil and sediment from dry stream channels were similar to rates measured with sediments collected in the perennial channel sections. This suggested that permanent water availability was not a main factor determining metabolic potential in this early successional watershed.

In an outdoor flume experiment it was determined whether shallow (1cm) and deep (4cm) sediment disturbances in small sand-bed streams have similar effects on whole-stream metabolism, and whether autotrophic and heterotrophic processes and organisms respond in similar ways. Results suggested that disturbing sediments during early successional stages had no effect on whole-stream metabolism, whereas in advanced stages, deep but not shallow sediment disturbance could lead to a transitory shift towards heterotrophy.

Changes in riparian and in-stream vegetation during stream succession come along with different amounts and types of organic matter input in stream ecosystems. It was tested to determine if increasing quality and quantity of litter input changes whole-stream metabolism and activity and structure of microbial communities associated with sediments and leaves. Whole-stream metabolism was found to be similar in all treatments because sediments and leaves were constrained by oxygen and nutrient availability. There seemed to be compensation between the effect of algae fueling microbial activity in open-land treatments and microbial use of allochthonous carbon sources in the litter treatments. Fungal and bacterial activity associated with leaves was unaffected by the background litter standing stock, but the structure of communities was affected. However, leaf quality had a clear effect on microbial activity and community structure with higher activity on tree compared to grass leaves.

Zusammenfassung

In dieser Arbeit wurden Prozesse des mikrobiellen Kohlenstoffumsatzes während der initialen Gewässerentwicklung untersucht. Die Arbeiten wurden im experimentellen Wassereinzugsgebiet „Hühnerwasser“ durchgeführt, welches eigens dafür angelegt wurde, initiale Ökosystementwicklung zu untersuchen. Zusätzlich wurde eine experimentelle Fließrinnenanlage verwendet.

In drei Gewässerkorridoren des Hühnerwasser-Einzugsgebiets wurde über einen einjährigen Beprobungszeitraum die mikrobielle Respiration in Böden und Gewässersedimenten entlang von Fließpfaden gemessen. Entgegen aller Annahmen, gab es keine Unterschiede in den Respirationsraten zwischen Sedimenten aus permanent fließenden und wiedervernässten, trockenen Gewässerabschnitten oder Bodenproben. Die sedimentgebundene mikrobielle Aktivität wurde demnach nicht hauptsächlich durch dauerhafte Wasserverfügbarkeit bestimmt.

In einem Rinnenexperiment wurde getestet, ob flache (1cm) und tiefe (4cm) Sedimentumlagerungen sich ähnlich auf den Gesamtmetabolismus von Sandbächen auswirken, und ob autotrophe und heterotrophe Organismen und Prozesse ähnlich auf diese Störung reagieren. Die Ergebnisse zeigten, dass die Störung durch Sedimentumlagerung zu einem frühen Zeitpunkt keinen Einfluss auf den Gesamtmetabolismus hatte. Während des späteren Sukzessionsstadiums führte jedoch die tiefe, nicht aber die flache Sedimentumlagerung zu einem temporären Übergang des Systems in die Heterotrophie.

Während der Gewässerentwicklung führt die Sukzession der Vegetation sowohl im Gerinne als auch im Uferbereich dazu, dass zunehmend Streu in Fließgewässer eingetragen wird, zunächst vor allem Gräser, später vor allem von Bäumen stammendes Laub. Es wurde experimentell getestet, ob die zunehmende Qualität und Quantität von Streu den Gesamtmetabolismus und die an Sedimente und Laubblätter gebundene mikrobielle Aktivität und Struktur der mikrobiellen Gemeinschaften verändern. Der Gesamtmetabolismus zeigte sich vom Streueintrag unabhängig, wurde aber offenbar durch Sauerstoff und Nährstoffe limitiert. Die Ergebnisse deuten darauf hin, dass im Offenlandstadium labiler Kohlenstoff von benthischen Algen die Aktivität von bakteriellen Gemeinschaften antreibt, während in den simulierten Gehölzstadien die heterotrophe Aktivität durch den von der Streu stammenden Kohlenstoff angetrieben wird. Der Eintrag von Streu mit steigender Qualität und Quantität hatte keinen Einfluss auf die mikrobielle Aktivität am einzelnen Blatt. Die Qualität der Blätter selbst hatte hingegen starke Auswirkungen auf die Zusammensetzung der mikrobiellen Gemeinschaft, was sich durch eine erhöhte mikrobielle Aktivität am Birkenlaub im Vergleich zur Grasstreu zeigte.

Contents

List of figures	6
List of tables	7
1 General introduction	8
1.1 Ecosystem and stream succession.....	8
1.2 Factors driving carbon transformation during stream succession.....	11
1.3 Scope of the thesis	14
1.4 Methodological considerations	15
1.5 List of publications and author contributions	16
2 Variability of heterotrophic metabolism in small stream corridors of an early successional watershed	18
2.1 Abstract	18
2.2 Introduction.....	18
2.3 Material and methods.....	21
2.4 Results.....	29
2.5 Discussion	35
3 Effects of shallow and deep sediment disturbance on whole-stream metabolism in experimental sand-bed flumes	41
3.1 Abstract.....	41
3.2 Introduction.....	42
3.3 Material and methods.....	44
3.4 Results.....	48
3.5 Discussion	55
4 Remarkably stable ecosystem metabolism during simulated stream ecosystem succession	60
4.1 Abstract.....	60
4.2 Introduction.....	60
4.3 Material and methods.....	62
4.4 Results.....	70
4.5 Discussion	77
5 Leaf quality as driver of microbial metabolism and community structure during simulated stream ecosystem succession	83

5.1	Abstract	83
5.2	Introduction.....	84
5.3	Material and methods.....	87
5.4	Results.....	94
5.5	Discussion	107
6	Conclusions and outlook	112
6.1	Conclusions.....	112
6.2	Outlook	116
	Acknowledgements	118
	List of abbreviations	120
	Bibliography	122

List of figures

<i>Figure 1.1: Scheme of the theoretical concept of stream succession with three different stages.</i>	10
<i>Figure 2.1: Sampling design used for each of the three main stream corridors of the Chicken Creek watershed.</i>	25
<i>Figure 2.2: Pattern of respiration rates across 4 seasons at a total of 8 sampling sites along the hydrologic flow path in each of 3 stream corridors.</i>	30
<i>Figure 2.3: Organic matter content at 5 sites along the hydrologic flow path in the three main streams of the Chicken Creek watershed.</i>	33
<i>Figure 2.4: Respiration rates associated with sediment from the perennial sites of the three streams in the Chicken Creek watershed.</i>	34
<i>Figure 3.1: Net Community Production and chlorophyll a content during the experiment.</i>	49
<i>Figure 3.2: Whole-stream community respiration during the experiment.</i>	51
<i>Figure 3.3: Ratio of respiration and production.</i>	52
<i>Figure 3.4: Sediment associated community respiration and bacterial abundance in the early and late successional stage during the experiment.</i>	54
<i>Figure 4.1: Whole stream metabolism and sediment associated parameters.</i>	72
<i>Figure 4.2: Comparison of the oxygen profiles in the sediment of the five treatments.</i>	75
<i>Figure 4.3: Bacterial biomass, respiration, production (BSP), growth efficiency and growth rates.</i>	76
<i>Figure 4.4: Comparison of secondary production and biomass of fungi and bacteria associated to leaves of <i>Cornus sanguinea</i>.</i>	77
<i>Figure 5.1: Potential activities of five enzymes and respiration of microbial communities associated with tree and grass litter exposed in the channels stocked with different types and amounts of leaf litter.</i>	96
<i>Figure 5.2: Ratios of enzymatic activities of microbial communities associated with grass and tree litter in the five different channels stocked with different types and amounts of leaf litter.</i>	97
<i>Figure 5.3: Leaf mass loss (A) and fungal sporulation rate (B) associated with grass and tree litter in experimental channels.</i>	99
<i>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.</i>	100
<i>Figure 5.5A: NMDS ordination of bacterial community structure inferred from ARISA profiles.</i>	103
<i>Figure 5.6: NMDS ordination of fungal communities inferred from ARISA profiles</i>	105
<i>Figure 5.7: NMDS ordination of bacterial communities inferred from ARISA profiles.</i>	106

List of tables

<i>Table 2.1: Physical and chemical characteristics of the three main stream corridors in the Chicken Creek watershed.</i>	<i>24</i>
<i>Table 2.2: Results of separate nested ANOVAs of respiration, chlorophyll and organic matter data from the Chicken Creek watershed between November 2007 and 2008.</i>	<i>31</i>
<i>Table 2.3: Respiration rates in various streams comparable to those of the Chicken Creek watershed....</i>	<i>38</i>
<i>Table 3.1: Concentrations of dissolved nutrients and dissolved organic carbon (DOC) in flumes and a groundwater supply tank during the experiment.</i>	<i>45</i>
<i>Table 4.1: Physico-chemical parameters of the flume water.</i>	<i>64</i>
<i>Table 4.2: Results of calculated contrasts according to the hypotheses of the experiment.</i>	<i>71</i>
<i>Table 5.1.: Nutrient contents of the two exposed litter types and stoichiometric ratios.</i>	<i>88</i>
<i>Table 5.2. Extracellular enzymes analysed.</i>	<i>90</i>
<i>Table 5.3. Specific contrasts between the treatments according to the hypotheses.</i>	<i>94</i>

1 General introduction

1.1 Ecosystem and stream succession

Succession is one of the oldest concepts in ecology [Gleason, 1927; Clements, 1916; Pickett, 1982] and is broadly defined by the sequential replacement of species following a disturbance [Clements, 1916; White and Pickett, 1985]. Depending on the dimension of disturbance, it can be distinguished between primary succession occurring in an environment with lack of soil and devoid of vegetation (e.g. glacier retreat areas or volcanic lava beds) [e.g. Chapin *et al.*, 1994] and secondary succession, occurring on a substrate that previously supported vegetation (following floods or tsunamis) [e.g. Fisher *et al.*, 1982]. The study of this topic has generated well-known theories on mechanisms how communities develop from an initial to a climax stage or are in constant steady state [Gleason, 1927; Clements, 1936; Whittaker, 1953]. Most studies so far have been made in terrestrial ecosystems and extrapolation of these concepts to aquatic systems often led to the description of longitudinal patterns in space rather than change of functioning in time due to the overriding effect of current in lotic systems [Fisher, 1982]. The main agent of disturbance and subsequent succession is flooding, able to set mature stream ecosystems back into an earlier stage of development. Nevertheless, flooding often only changes the situation in the stream, but does not reset the development of the surrounding watershed. As stream ecosystems are highly complex systems linked to their terrestrial watershed and riparian zone, initial stream succession can only be studied in watersheds that start at point zero. Such young ecosystems are rare under natural conditions, but within the project of the Transregional Collaborative Research Centre 38, there was the opportunity to follow ecosystem development from point zero in the Chicken Creek watershed, an artificially constructed watershed in a former open cast lignite mine [Gerwin *et al.*, 2009; Schaaf *et al.*, 2011]. It was constructed in 2005 and the research, presented here covers the time from 2007 to 2010. With the opportunity to study an ecosystem from the beginning, an attempt was made to answer several questions and test a theoretical concept of stream succession (described below), that -to my knowledge- has not been tackled before.

The succession of stream ecosystems has been investigated in different landscapes as glacier retreat areas [Milner *et al.*, 2000; Milner and Gloyne-Phillips, 2005], post

mining sites [Mutz *et al.*, 2002], volcanoes [Vitousek, 2004] or in arid ecosystems after severe flood disturbances [Fisher *et al.*, 1982; Grimm, 1994]. Results of these studies show that the development seems to follow a sequence of three stages that are largely shaped by the succession of the riparian and in-stream vegetation (Figure 1.1).

The first stage of stream succession in an open landscape is characterized by the lack of vegetation in the watershed and in the stream channel and a harsh hydrologic regime with frequent flash floods going along with sediment movements of the stream bed impeding stable macrophyte establishment [Maltchik and Pedro, 2001; Riis *et al.*, 2004]. Surface runoff in such scarcely vegetated landscapes leads to rapid erosion especially of soft substrates, and formation of a network of rills and both ephemeral and perennial stream channels [Graf, 1988]. The resulting drainage network includes sites experiencing variable water availability and disturbance by scour and fill, which influence primary production and other ecosystem processes [Stanley *et al.*, 2010]. Regarding the organic matter budget, streams in the first stage have very low allochthonous input due to the lack of riparian trees with litterfall [Golladay, 1997]. Those streams are dominated by autochthonous algal production and heterotrophic microbes which benefit from algal-derived labile carbon sources [Jones, 1995; Romani and Sabater, 1999]. Thus, auto- and heterotrophic microbes in biofilms associated with benthic and hyporheic sediments are the main key player for carbon transformation processes at this stage of succession.

With increasing vegetation cover in the watershed, infiltration of terrestrial surfaces increases [Loch, 2000], causing less frequent and less severe surface runoff leading to flash floods in the stream channels [Ludwig *et al.*, 2005]. This enables the establishment of macrophytes along and within stream channels [Riis and Biggs, 2003] and leads to the second, macrophyte-dominated stage, which brings in a new source of organic matter fueling and enhancing stream ecosystem metabolism [Acuña *et al.*, 2010; Wilcock and Croker, 2004]. Besides aquatic macrophytes in the streams, often helophytes dominate the parafluvial zone, the banksides and the riparian zones of open land streams with a dominance of gramineous plants. Thus, major allochthonous input in this transition stage can be grass litter and other herbaceous plant material [Mackay *et al.*, 1992; Huryñ *et al.*, 2001; Menninger and Palmer, 2007].

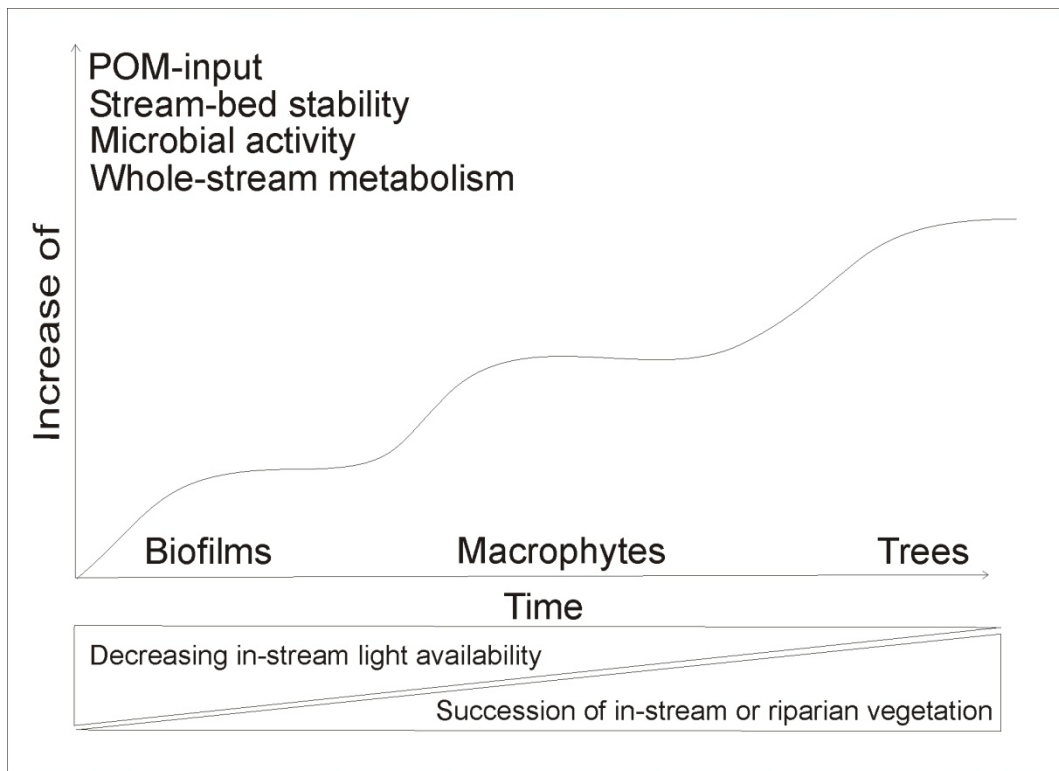


Figure 1.1:

Scheme of the theoretical concept of stream succession with three different stages. Figure was adapted from the project proposal of the SFB/TR 38, page 312 by M. Mutz and M. O. Gessner.

The third and final stage of succession is characterized by the presence of woody riparian vegetation, shading the stream and limiting primary production [Sabater *et al.*, 2000], and dead wood and tree litter in the stream channels, forming an important carbon source for stream metabolism [Fisher and Likens, 1973; Vannote *et al.*, 1980; Webster and Meyer, 1997]. In addition, change of vegetation alters the physical structures in streams as well as in the watershed with consequences for the disturbance regime and the hydraulic conditions in the hyporheic zone. These features exert significant control on the metabolic potential and carbon transformation in streams [Grimm and Fisher, 1984, Findlay, 1995, Mulholland *et al.*, 1997]. With the shift of energy sources and environmental factors during succession, it is likely that also shifts in the microbial structure and processes of carbon transformation and accumulation occur [Schaaf *et al.*, 2011]. However, to my knowledge, no data on these shifts of structure and processes exist.

1.2 Factors driving carbon transformation during stream succession

Quantity and quality of organic matter, temperature, nutrient availability, redox potential and the physical structure of soils and sediments are all important factors influencing microbial growth and activity rates of carbon transformation in soils and sediments [e.g. *Hedin*, 1990; *Koutný and Rulík*, 2007; *Arevalo et al.*, 2010; *Bond-Lamberty and Thomson*, 2010]. However, the main driver for microbial activity often is the availability of water, which has been shown in arid and semiarid climates [*Noy-Meir*, 1973; *Belnap et al.*, 2005] where occasional precipitation events create pulses of metabolic activity in soils and sediments of ephemeral stream channels similar to the situation in a very young initial open-land watershed during succession [*Sala and Lauenroth*, 1982; *Belnap et al.*, 2005; *Sponseller*, 2007]. Such increases in activity after precipitation events are considered hot moments [*Belnap et al.*, 2005; *Harms and Grimm*, 2008]. Similar to hot spots, hot moments have been defined as short periods when biogeochemical reaction rates are substantially increased compared to the intervening periods of low activity [*McClain et al.*, 2003]. They are often related to water availability. For example, growth and abundance of bacteria in sediments of ephemeral Mediterranean streams have been found to depend strongly on moisture availability and to cease instantly when sediments desiccate [*Amalfitano et al.*, 2008; *Marxsen et al.*, 2010]. Likewise, marked reductions of microbial growth and soil respiration rates have been described with decreasing soil water content [e.g. *Cook and Orchard*, 2008]. Besides water availability, carbon sources fueling microbial activity in streams, play an important role. Either dissolved organic carbon (DOC) from groundwater can be an important source for microbial activity [*Jones et al.*, 1995] as well as particulate organic matter (POM) from autochthonous or allochthonous sources from riparian or in-stream vegetation. As most studies on factors regulating microbial activity were conducted in mature ecosystems, the opportunity to tackle this subject in a less complex early successional watershed led to the following research question:

(I) What are the factors regulating microbial activity in stream corridors of an early successional watershed?

Another factor typically influencing the habitat quality of streams in open land watersheds is the harsh hydrologic regime, characterized by frequent flash floods able to

mobilize bed-sediments [Merz and Blöschl, 2003; Powell et al., 2005]. Disturbance of sediments has ecological effects on benthic stream algae and bacteria as well as on the metabolic processes driven by these microorganisms. Mechanisms include downstream flushing and abrasion of both free and attached cells resulting from increased shear stress [Francoeur and Biggs, 2006; Schwendel et al., 2010], as well as mixing into deeper sediment layers and burial [Peterson, 1996]. Many studies have contributed to the present knowledge on flood disturbances regarding major floods of bed moving force [Power and Stewart, 1987; Resh et al., 1988; Holmes et al., 1998], but very little is known about frequent but only moderate sediment disturbances due to precipitation events in open land watersheds that lead to pulse surface runoff discharging into the channel network. Based on this lack of knowledge the following research question was derived:

(II) Do shallow and deep sediment disturbances have a similar effect on whole-stream metabolism? And do these effects differ depending on what stage of community succession the disturbance occurs?

With changing environmental conditions and vegetation in the watershed, main carbon sources fueling microbial activity in stream corridors change. In open-land streams, input of allochthonous plant material into streams is low due to the overall sparse vegetation cover. In-stream establishment of macrophytes is difficult because of frequent sediment disturbance [Riis et al., 2004]. Epipsammic biofilms dominate with high production of benthic algae fueling heterotrophic organisms by exudation of labile organic compounds or by senescence of algal cells [Cole et al., 1982; Jones, 1995; Romani and Sabater, 1999]. Besides mineralization of particulate carbon sources, bacteria can also transfer DOC into the particulate fraction [Meyer, 1994], thus making it available for higher trophic levels. DOC in stream and groundwater can be the major component of stream organic matter budgets [Fisher and Likens, 1973; Webster and Meyer, 1997] and depending on its bioavailability fuel bacteria in sediment biofilms [Sobczak and Findlay, 2002, Judd et al., 2006]. Especially during pulse floods with high surface runoff, DOC from terrestrial origin can enter the streams [Dalzell et al., 2005].

With vegetation succession in the watershed as well as in the stream channels, more allochthonous sources of the particulate fraction enter the streams. With litter input,

decomposition of leaves begins where fungi, bacteria and invertebrates are involved, contributing significantly to whole stream carbon flow [Hieber and Gessner, 2002; Baldy *et al.*, 2007]. Thus, the diversity of organisms contributing to carbon transformation is also increasing with succession. As the quantity of POM on the benthic surface or mixed into the sediment is known to fuel heterotrophic activity [Hedin, 1990; Fuss and Smock, 1996; Crenshaw *et al.*, 2002], it can be assumed that streams receiving more allochthonous POM during succession show higher carbon flow rates than initially. Different stages of succession regarding the amount of litter input were experimentally simulated to answer the following research question:

(III) Does the increase of quality and quantity of litter in streams lead to higher rates of whole-stream metabolism and higher activity of leaf associated microbial communities?

Besides the quantity of POM input into streams, quality of the litter also plays an important role for decomposition and activity and growth of colonizing microbial communities [Ostrofsky, 1997; Menninger *et al.*, 2007]. Differing nutrient contents of the leaves as nitrogen, phosphorus, carbon and the stoichiometric ratios of these as well as the content of lignin and phenolic compounds as tannins have been found to be correlated with decomposition rates [Gessner and Chauvet, 1994; Ostrofsky, 1997; Ardón *et al.*, 2009]. Generally, there is no clear consensus about the differences in quality between grass and tree litter and their respective importance as an allochthonous carbon source. Decomposition of tree litter has been studied far more [Webster and Benfield, 1986; Ostrofsky, 1997] than that of grass litter [Menninger and Palmer, 2007, Shaftel *et al.*, 2011] and comparative studies of tree and grass litter decomposition are rare [Scarsbrook and Townsend, 1994]. The differences in litter quality are not only likely to influence microbial activity and hence break down rates but also the composition of fungal [Gulis, 2001] and bacterial [Mc Arthur, 1985; Mille-Lindblom *et al.*, 2006] communities colonizing these leaves. However, environmental conditions were found to play an even more important role for the microbial diversity [Marks *et al.*, 2009; Harrop *et al.*, 2009]. To test differences of leaf quality of the dominant litter types present at the different stages of stream succession, and to elucidate the relative importance of leaf quality compared to background litter standing

stock on microbial activity and community composition, the following question was derived:

(IV) Is there a higher microbial activity and a more diverse microbial community on tree litter compared to grass litter? And is the leaf quality or the background litter standing stock most relevant for leaf associated microbial activity and community composition?

Answers to the preceding questions can be found in the conclusions of chapter 6.

1.3 Scope of the thesis

The work of this thesis was integrated into the Transregional Collaborative Research Centre 38 with the topic: “Structures and processes of the initial ecosystem development phase in an artificial watershed”. The overall aim of this project is to elucidate the role of structures and processes during the initial establishment of an ecosystem [Schaaf *et al.*, 2010; Gerwin *et al.*, 2009]. The aim of the studies compiled in this thesis was to better understand the effects of change of biological and physical structures during stream succession on processes of carbon transformation and carbon accumulation. Investigations were carried out in the experimental watershed Chicken Creek, the main research site of the Transregional Collaborative Research Centre 38. We used two approaches by studying processes directly in the field and by performing additional experiments in outdoor-flumes, set up at the side of the Chicken Creek watershed to simulate similar environmental conditions.

The above defined research question **(I)** was addressed by performing a one year seasonal sampling along hydrological flowpaths in three stream corridors of the initial watershed Chicken Creek assessing sediment associated respiration rates and several biotic and abiotic environmental parameters (chapter 2).

For answering research question **(II)** an experiment in outdoor flumes simulating sand-bed streams was performed. Sediment disturbances were manually applied in an early and later successional stage of the stream community. Whole-stream metabolism was measured as the main response parameter (chapter 3).

An experiment in the outdoor flumes was simulating different stages of succession by different quality and quantity of litter input. This was carried out to answer research questions **(III)** and **(IV)**. Whole-stream metabolism, respiration and production

associated with sediments and leaves (chapter 4) as well as respiration, enzyme activities and microbial community structure on grass and tree litter were measured (chapter 5).

Chapter 6 presents the conclusions of this thesis and an outlook.

1.4 Methodological considerations

Heterotrophic microbial activity in ecosystems can be assessed by measuring community respiration as an integrative process that reflects oxygen-dependent metabolism and, in aerobic environments, accounts for the bulk of CO₂ flux to the atmosphere [Young *et al.*, 2008; Martin and Bolstad, 2009]. To assess microbial activity in stream sediments, measurements of oxygen consumption in flow through chambers were applied similar to the method developed by Pusch and Schwoerbel [1994] (chapter 2, 3 and 4). Samples were measured in dark to assess heterotrophic respiration rates, integrating the respiratory activity of the entire community including algae.

Measurements performed directly in the stream determine total system metabolism. In small to midsize streams community biomass is greater in the benthos than in the water column and benthic metabolism dominates system activity [Bott, 2006]. The experimental flumes offered the opportunity to assess whole-stream metabolism similar to a huge chamber measurement, avoiding the methodological constraints of calculating reaeration in open system measurements [Odum, 1956]. Flumes were covered with airtight Perspex lids with an internal circulation of water. Oxygen optodes could measure the change in oxygen concentration in the whole flume. By measuring in light and dark conditions by covering the flumes with opaque tarpaulin, net community production and community respiration, respectively, could be assessed (chapter 3 and 4). The advantage of whole-system measurements is the non-destructive way of measurements. By sampling compartments of the whole system like sediment or leaves and measuring them separately in the lab, one may change the environmental conditions and rather assess a potential for respiration than the rates that occur in nature.

For studies dealing with litter affecting the whole system and as a response parameter, different measures suitably reflecting decomposition and microbial activity associated to leaves were assessed [Baldy *et al.*, 2002; Gulis and Suberkropp, 2006]. In this thesis leaf associated respiration rates as an index of microbial activity (chapter 4 and 5) and

leaf associated fungal and bacterial secondary production (chapter 4) and biomass were measured (chapter 4 and 5). Measurements of potential enzyme activities and microbial community structure were contributed by Aline Frossard (chapter 5).

1.5 List of publications and author contributions

The following section gives an overview of the scientific papers, which were derived from the research of this thesis and explains the respective contributions of the involved authors. As this thesis was part of a collaborative project (SFB/TRR38), the ample list of parameters measured in these studies could only be realized by the work of two PhD students. In consequence, there was a close collaboration to Aline Frossard in performing this research.

1.5.1 List of scientific papers

This thesis is based on the following papers, which were published, submitted or prepared for submission.

Chapter 2 Gerull, L., A. Frossard, M. O. Gessner, and M. Mutz (2011): Variability of heterotrophic metabolism in small stream corridors of an early successional watershed, *J. Geophys. Res.*, 116, G02012, doi:10.1029/2010JG001516.

Chapter 3 Gerull, L., A. Frossard, M. O. Gessner, and M. Mutz: Effects of shallow and deep sediment disturbance on whole-stream metabolism in experimental sand-bed flumes, accepted by *Hydrobiologia*, The final publication is available at www.springerlink.com, doi: 10.1007/s10750-011-0968-x.

Chapter 4 Gerull, L., A. Frossard, M. O. Gessner, and M. Mutz: Remarkably stable ecosystem metabolism during simulated stream ecosystem succession, manuscript.

Chapter 5 Gerull*, L., A. Frossard*, M. O. Gessner, and M. Mutz: Leaf quality as driver of microbial metabolism and community structure during simulated stream ecosystem succession, manuscript, (*The authors contributed equally to the paper).

1.5.2 Author contributions

In the following section, the respective contributions of the authors are listed. L. Gerull (LG), A. Frossard (AL), M. O. Gessner (MOG), M. Mutz (MM).

Chapter 2 MOG, MM, LG and AF designed the study; LG and AF performed the research; LG analyzed the data with contributions by MOG; and LG and MOG wrote the article with contributions by MM and AF.

Chapter 3 MM and LG designed the study; LG performed the research; AF contributed data on bacterial abundance; LG analyzed the data and LG and MM wrote the article with contributions by MOG.

Chapter 4 MOG, MM, LG and AF designed the study; LG and AF performed the research; AF contributed data on bacterial and fungal biomass; LG analyzed the data and LG and MM wrote the article with contributions by MOG and AF.

Chapter 5 MOG, MM, LG and AF designed the study; LG and AF performed the research; AF analyzed the data on enzyme activities and microbial community structure and LG and AF wrote the article with contributions by MM and MOG.

2 Variability of heterotrophic metabolism in small stream corridors of an early successional watershed¹

2.1 Abstract

Metabolic activity in stream corridors is regulated by a complex combination of factors that are difficult to disentangle in mature ecosystems. Chicken Creek in Germany, an experimentally created watershed in an early successional stage, offers the opportunity to assess the spatio-temporal variation in metabolic activity in a simplified system. We measured microbial respiration in soils and sediments along the hydrologic flow path from upland terrestrial to ephemeral to perennial sites of three stream corridors. Dry soils and sediments were rewetted before respiration measurements to mimic periods of activity during and after rainfall. Respiration rates and organic matter contents of soil and sediment were generally low. Presence of algae and accretion of vascular plant fragments in the perennial stream reaches increased respiration rates, pointing to the importance of particulate organic matter. Contrary to expectation, respiration rates of rewetted soil and sediment from dry stream channels was similar to rates measured with sediments collected in the perennial channel sections. This suggests that permanent water availability was not a main factor determining metabolic potential in the early successional Chicken Creek watershed. Carbon turnover in perennial channels was 4-8fold higher than in ephemeral channels and terrestrial sites, as water was permanently available. However, this magnitude was insufficient for perennial channels to compensate for the large surface area of terrestrial soils: extrapolated to a year and the whole watershed, stream channels contributed only 5% to total carbon turnover, 95% being due to soils during and after rainfall events.

2.2 Introduction

Landscapes are characterized by patchiness of physical and biological attributes [Winemiller *et al.*, 2010; Moore *et al.*, 2009], which influence biogeochemical processes

¹Gerull, L., A. Frossard, M. O. Gessner, and M. Mutz (2011), Variability of heterotrophic metabolism in small stream corridors of an early successional watershed, *J. Geophys. Res.*, 116, G02012, doi:10.1029/2010JG001516. Copyright [2011] American Geophysical Union.

and whole-ecosystem metabolism [Malard *et al.*, 2002]. As a result, rates of biogeochemical processes are often highly variable across landscapes, particularly at terrestrial-aquatic interfaces and along hydrologic flow paths, from upland sites to streams and longitudinally within stream networks [Larned *et al.*, 2010; Sponseller and Fisher, 2006]. Landscape patches showing disproportionately high reaction rates relative to the surrounding matrix have been termed hot spots of metabolic activity [McClain *et al.*, 2003]. They are typically found at interfaces where complementary reactants meet because different flow paths converge. Such hot spots can occur along gradients of redox potential [Mitchell and Branfireun, 2005] or in the hyporheic zone of streams where ground water and stream water mix [Mulholland, 1997; Boulton *et al.*, 2010].

Variation in metabolic activity along hydrologic flow paths in riverine landscapes is well documented. For example, high primary production and nitrate uptake rates have been described at outwelling sandbar edges where nutrient-rich groundwater and light availability enable intense photosynthetic activity [Valett *et al.*, 1994]. If enriched with dissolved organic carbon (DOC), upwelling groundwater can provide an important carbon and energy source also for heterotrophic stream microbes [Jones *et al.*, 1995; Boulton *et al.*, 2010]. Similarly, elevated hyporheic respiration rates have been observed in downwelling areas of streams, indicating that algal primary production at the surface can provide labile organic matter on which hyporheic microbes capitalize to boost their metabolism [Jones *et al.*, 1995]. Thus, both upward and downward vertical water exchange can enhance metabolic activities in stream sediments by supplying essential resources for microbial metabolism.

Besides DOC, particulate organic matter (POM) plays an important role in fuelling microbial activity in streams. Sources of POM can be allochthonous, consisting mainly of litter derived from riparian vegetation, or autochthonous, originating from in-stream vascular plants, algae or other organisms. Although litter input from riparian trees is the most important source of POM in woodland streams [Webster and Meyer, 1997], watersheds in early successional stages have scarce upland [Chapin *et al.*, 1994] and riparian vegetation [Milner *et al.*, 2010]. This leads to low POM availability and resource heterogeneity caused by isolated plant and litter patches.

Heterotrophic metabolic activity in ecosystems can be assessed by measuring community respiration as an integrative process that reflects oxygen-dependent metabolism and, in aerobic environments, accounts for the bulk of CO₂ flux to the atmosphere [Young *et al.*, 2008; Martin and Bolstad, 2009]. Quantity and quality of organic matter, temperature, nutrient availability, redox potential and the physical structure of soils and sediments are all important factors influencing rates of respiration in soils and sediments [e.g. Hedin, 1990; Koutný and Rulík, 2007; Arevalo *et al.*, 2010; Bond-Lamberty and Thomson, 2010]. However, the main driver is often water availability, especially in arid and semiarid climates [Noy-Meir, 1973; Belnap *et al.*, 2005] where occasional precipitation events create pulses of metabolic activity in soils and sediments of ephemeral stream channels [Sala and Lauenroth, 1982; Belnap *et al.*, 2005; Sponseller, 2007]. Such increases in activity after precipitation events are considered hot moments [Belnap *et al.*, 2005; Harms and Grimm, 2008]. Similar to hot spots, hot moments have been defined as short periods when biogeochemical reaction rates are substantially increased compared to the intervening periods of low activity [McClain *et al.*, 2003]. They are often related to water availability. For example, growth and abundance of bacteria in sediments of ephemeral Mediterranean streams have been found to depend strongly on moisture availability and to cease instantly when sediments desiccate [Amalfitano *et al.*, 2008; Marxsen *et al.*, 2010]. Likewise, marked reductions of microbial growth and soil respiration rates have been described with decreasing soil water content [e.g. Cook and Orchard, 2008]. This suggests elevated microbial activity in stream sediments at sites with permanent water.

Metabolic activity in mature ecosystems is regulated by a complex combination of factors that can be difficult to disentangle. However, physically and biologically simple model systems such as landscapes recently formed by volcanism [Vitousek, 2004] or glacier recession [Milner *et al.*, 2007] with low structural heterogeneity, simple food webs, low input of allochthonous organic matter, and no legacy of human use provide opportunities to assess activity patterns and their causes at the landscape scale. Alternatively, artificially created watersheds subject to natural colonization and development from an initially bare stage can be used to study processes occurring during early ecosystem succession [Gerwin *et al.*, 2009]. Surface runoff in such scarcely vegetated landscapes leads to rapid erosion especially of sandy substrates, and

formation of a network of rills and both ephemeral and perennial stream channels [Graf, 1988]. The resulting drainage network includes sites experiencing variable water availability and disturbance by scour and fill, which influence primary production and other ecosystem processes [Stanley *et al.*, 2010]. These gradients of water availability and disturbance, together with broadly homogenous substrates and scarce vegetation, make early successional watersheds suitable to distinguish places (i.e. landscape structures) and times of intense metabolic activity.

Taking advantage of an experimental watershed constructed in 2005 [Gerwin *et al.*, 2009] we investigated patterns of metabolic activity and their potential causes at the landscape scale. The objectives of the study were to determine respiration rates in soil and sediment along hydrologic flow paths within small stream corridors of this watershed in an early successional stage. We hypothesized that permanent water availability and the quantity and quality of organic matter would be the main drivers of microbial respiration. Specifically, we explored five expectations: (1) Soil from terrestrial upland sites and sediments from ephemeral channel sections will have low potential metabolic activity after rewetting compared to permanently wet or moist sediments from perennial stream sections. (2) Metabolic activity after rewetting will be higher in ephemeral stream sections than in adjacent terrestrial soil because physically sheltered and less hydrophobic sediment retains moisture longer after rainfall than upland soils. (3) Metabolic activity will decrease within perennial stream sections from up-welling to down-welling sites to sites with perched surface flow due to differences in carbon and nutrient supply. (4) Metabolic activity will be higher at permanently submerged sites than at adjacent parafluvial sites because of better vertical water exchange and nutrient delivery. And, (5) metabolic activities at places where water is permanently available will play a notable role in whole-system carbon flux to the atmosphere in spite of the small spatial extent of these places.

2.3 Material and methods

2.3.1 Study site

The study was conducted in an experimental watershed (dubbed Chicken Creek watershed) located in eastern Germany near the city of Cottbus about 150 km south-east of Berlin (51°36' N, 14°16' E). Average annual precipitation in the area is 559 mm and

average air temperature is 9.3°C (period 1971-2000; Meteorological Station Cottbus, Deutscher Wetterdienst). The Chicken Creek watershed was created in 2004 and 2005 by depositing a 2-4 m layer of Pleistocene sands on top of a 1-2 m impervious layer of Tertiary clay forming a shallow basin [Gerwin *et al.*, 2009]. The watershed of 6 ha (400 m × 150 m) has a water-storage volume (aquifer) of 117 500 m³. The total elevational difference is about 15m along the main axis, resulting in an average slope of 3.5%. Geomorphology and biological colonization of the watershed have undergone an unrestricted natural development from the initially bare stage.

Between November 2007 and October 2008, the watershed was sparsely covered by vegetation consisting primarily of forbs. The main species was Canadian horseweed (*Coryza canadensis* (L.) Cronquist), although some grasses, especially Wood small-reed (*Calamagrostis epigejos* (L.) Roth) and scattered woody plants such as Scots pine (*Pinus sylvestris* L.), Goat willow (*Salix caprea* L.) and Black locust (*Robinia pseudoacacia* L.) were also present. Within two years, the initially plane surface of the watershed had been shaped by water erosion, resulting in a well-developed network of rills and channels. The three main channels each drain separate portions of the watershed into a small pond just downstream. The upper portions of the channel network were ephemeral, while up-welling groundwater at a subterranean clay dam downstream created a short perennial section of permanent surface flow in all of the three main channels of the watershed (Table 2.1). Only part of the channel width was submerged under base-flow conditions, but the parafluvial sediment always remained water saturated, because elevational differences between the water table and the parafluvial ground surface level were minor (1-5 cm). Most precipitation events of more than 1 mm led to surface runoff and expansion of the wetted channel portions. As a result, surface flow connected the ephemeral upstream sections to the perennial downstream sections for 101 days during the sampling year, as inferred from water-level monitoring in the pond downstream.

2.3.2 Sampling procedures and field measurements

We took advantage of the three main stream corridors in the Chicken Creek watershed to analyse soils and sediments at sites with distinct characteristics along the hydrologic flow path. Specifically, eight sites differing in hydrological characteristics were selected

along each of the stream channels (Figure 2.1): (1) a terrestrial upland site; (2) an upstream ephemeral channel site adjacent to the terrestrial site and submerged only during major rain events; (3) an up-welling site of the perennial channel section downstream; (4) a parafluvial site adjacent to the up-welling site; (5) a site in the perennial channel section with perched flow; (6) a parafluvial site adjacent to the site with perched flow; (7) a down-welling site; and (8) a parafluvial site adjacent to the down-welling site. We collected all samples during one-week periods in the winter (end of November, occasional night frosts) of 2007 and in the spring (April), summer (August) and autumn (early October) of 2008. Upstream reaches with ephemeral water flow were always sampled when dry.

We took three replicate samples of surface soil or sediment (top 2 cm) at each site, resulting in a total of 288 samples (3 channels \times 8 sites \times 3 replicates \times 4 seasons). Replicate samples at each site were taken at about 10 cm distance. Each of them consisted of 8 pooled cores taken with a syringe (2.8 cm inner diameter, lower end cut off) within a circular surface area of 78.5 cm². Subsamples were taken for respiration, organic matter and carbohydrate analyses (see below). Before extracting the cores for these pooled samples, we took additional cores within the same area to determine total pore volume and chlorophyll *a*. A 10-ml syringe with the lower end cut off was used to obtain undisturbed cores (1.5 cm inner diameter, 2 cm depth) for pore-volume determinations. A 2-ml syringe (1.0 cm inner diameter) with the lower end cut off was used to collect two pooled soil or sediment samples (2 \times 1 ml, total surface area of 1.57 cm²), which were immediately placed in brown glass bottles containing 8 mL of 90% ethanol and stored in the dark for later chlorophyll analysis.

Table 2.1: Physical and chemical characteristics of the three main stream corridors in the Chicken Creek watershed. Data on the stream channels refer to the main stems of the drainage networks. Rainwater samples were collected at a single site. Note different unit for N species in rainwater. Values are means \pm 1SE with ranges in parentheses.

Parameter	Stream 1	Stream 2	Stream 3
Watershed area (m ²)	950	7473	20496
Mean discharge (L s ⁻¹)	0.02	0.13	0.37
Length of perennial stream section (m)	20	38	43
Length of ephemeral stream channel (m)	103	171	249
Permanently wetted streambed area (m ²)	4	99	68
Ephemeral streambed area (m ²)	30	390	432
Channel slope (%)	6.5	5.1	3.9
Channel width at ground level (m)	1.2 (0.6-1.9)	2.6 (1.0-5.8)	2.2 (0.2-5.4)
Surface water DON ($\mu\text{g N L}^{-1}$)	18.1 \pm 5.9	18.6 \pm 5.0	41.6 \pm 11.9
Pore water DON ($\mu\text{g N L}^{-1}$)	17.4 \pm 5.0	24.3 \pm 7.2	34.4 \pm 13.4
Rain water DON (mg N L ⁻¹)	4.0 \pm 0.8		
Surface water NH ₄ ⁺ ($\mu\text{g N L}^{-1}$)	115.6 \pm 25.5	74.4 \pm 9.5	146.4 \pm 32.7
Pore water NH ₄ ⁺ ($\mu\text{g N L}^{-1}$)	97.8 \pm 10.1	62.4 \pm 2.5	121.7 \pm 38.9
Rainwater NH ₄ ⁺ (mg N L ⁻¹)	2.2 \pm 0.3		
Surface water PO ₄ ³⁻ ($\mu\text{g P L}^{-1}$)	4.3 \pm 0.6	4.1 \pm 0.3	6.2 \pm 0.5
Pore water PO ₄ ³⁻ ($\mu\text{g P L}^{-1}$)	5.9 \pm 1.3	4.9 \pm 0.6	7.5 \pm 1.6
Rain water PO ₄ ³⁻ ($\mu\text{g P L}^{-1}$)	6.5		
Surface water DOC (mg L ⁻¹)	16.3 \pm 0.8	11.2 \pm 0.7	15.9 \pm 1.1
Pore water DOC (mg L ⁻¹)	18.3 \pm 0.9	10.8 \pm 0.6	14 \pm 0.5
Rain water DOC (mg L ⁻¹)	2.5 \pm 0.2		

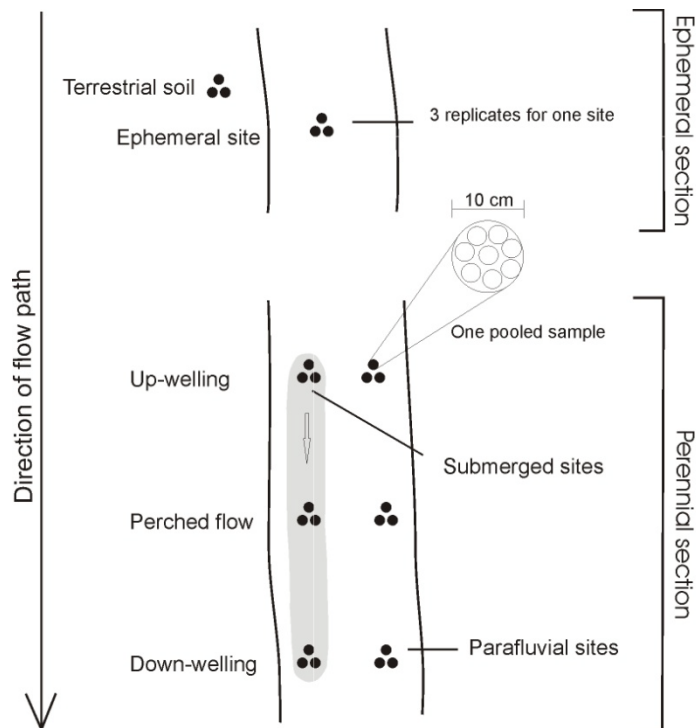


Figure 2.1:

Sampling design used for each of the three main stream corridors of the Chicken Creek watershed. A total of eight types of samples were taken in each stream corridor: upland terrestrial soil, sediment from the ephemeral stream sites, and sediment from six additional wet or moist (parafluvial) sites in the perennial stream sections.

2.3.3 Community respiration, organic matter, carbohydrates and chlorophyll *a*

Community respiration captured all organisms associated with the sand grains in soil and sediment. It was measured as oxygen consumption in a flow-through laboratory system at constant temperature (10°C in winter, 15°C in spring and autumn, 18°C in summer) within 12 hours after sampling. The flow rate was normally set at 0.1-0.2 mL

min^{-1} to mimic flow conditions in the field. When oxygen consumption was high, flow rate was occasionally increased to up to 0.36 mL min^{-1} . Prior trials showed that the relationship between flow rate and oxygen consumption was linear under these conditions. Samples from perennial reaches were percolated with surface water from the respective channel. Rainwater obtained from rain samplers in the Chicken Creek watershed was used for sediments from the ephemeral reaches and upland soil to simulate the effect of precipitation events on respiratory activity at these sites. Six respiration chambers filled with sediment were simultaneously monitored together with an additional control chamber containing either stream or rainwater only. The chambers used for measuring respiration were glass syringes (10 or 20ml volume) sealed at the top end by a rubber stopper in which an oxygen optode microsensor (Microx TX3, PreSens GmbH, Regensburg, Germany) was inserted. Chambers were connected to a peristaltic pump (ISMATEC Ecoline, Glattbrugg, Switzerland) controlling flow rate and placed in a water bath for temperature control. Measurements were started after water in the sediment chamber had once been completely replaced and flow rate and oxygen consumption rates were stable. This took between 20 and 60 min, depending on the flow rate and syringe volume. Five measurements were then taken per sample every 3 min and the mean was used for further analysis. After the respiration measurements, samples were dried at 105°C to constant weight and combusted in a muffle furnace (3h at 430°C) to determine total dry mass (DM) and organic matter (OM) content.

Effects of dissolved nutrients and carbon sources on community respiration were determined at one occasion in August 2008 when plant biomass was highest in the watershed. Sediment was collected from all three streams at the sites with perched flow. Respiration was measured in subsamples of this sediment percolated either with stream water from the watershed containing natural DOC or with a mineral nutrient solution in deionized water (1.62 mM NaNO_3 and $0.106 \text{ mM KH}_2\text{PO}_4$). Particulate organic matter (POM) that was loosely associated with sediment [*Pusch and Schwoerbel, 1994*] was removed from a third sediment subsample by thoroughly rinsing with 400 mL of water. Subsequently, this subsample was also percolated with mineral nutrient solution.

We measured total carbohydrate content in sediments as a proxy for extracellular polymeric substances (EPS), which are indicative of microbial biofilm development. Samples for carbohydrate analysis were frozen in liquid nitrogen within 8 hours after

sampling and stored at -20 °C. Carbohydrate content was later estimated in thawed samples by the phenol-sulphuric acid method [Dubois *et al.*, 1956] with slight modifications. Briefly, 2 ml of deionized water and 50 µl of aqueous phenol (80%) were added to 60-80 mg fresh weight of soil or sediment before mixing the solution by rapid addition of 5 mL of sulphuric acid. The samples were then incubated for 20 min in a water bath at 25°C before measuring absorbance of the supernatant at 485 nm (Perkin Elmer Lambda 2 spectrophotometer, Waltham, USA). Blank samples were prepared as above but without sediment added. Standard curves were established with glucose solutions ranging in concentration from 10 to 150 mg L⁻¹. Dry weight of the sample was estimated from replicate samples that were dried and weighed.

Chlorophyll *a* served as a proxy for algal biomass. Chlorophyll 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 the maximum power (Elma Transsonic Digital T790/H, Singen, Germany). The extracts were filtered through two paper filters (MN 619, Macherey-Nagel, Düren, Germany) and absorbance measured at 665 and 750 nm according to standard procedures. No attempt was made to correct for phaeophytin content.

2.3.4 Physico-chemical parameters

A syringe was used to collect surface and sediment pore water in each sampling period and site of the permanently flowing channels. The water was immediately filtered through prewashed 0.45µm pore size cellulose acetate filters, frozen and later analyzed for nutrients (PO₄³⁻, NH₄⁺, NO₂⁻ + NO₃⁻ = NO_x⁻) according to standard procedures. DOC was measured with a TOC-5000 (Total Organic Carbon Analyzer; Shimadzu, Duisburg, Germany). Influence of freezing on nutrient and DOC concentrations was tested in preliminary trials and found to be negligible at the concentrations measured in the present study. Rainwater collected by a rain sampler in the watershed during each sampling period was analyzed directly after sampling.

To provide information on oxygen availability in the sampled surface sediment, sediment oxygen profiles were measured *in situ* once in summer 2008 at the six wet and moist sites in each of the three streams. Oxygen profiles were determined with an optode microsensors (0.9 mm diameter; Microx TX3, Presens GmbH, Regensburg, Germany) protected inside a 20-cm long steel tube with a 1.7-mm² opening at the tip.

The tube was lowered into the sediment to defined depths and pore water was drawn into the tube with a syringe connected to the upper end of the tube. Volumes of 50-100 μL were sufficient to cover the probe tip and avoid mixing of two successive samples. Temperature profiles needed to correct the oxygen data were measured immediately after each oxygen profile by inserting an electronic temperature sensor directly in the sediment and taking readings at 1-cm depth intervals.

Sediment grain size distribution was determined by passing combusted soil or sediment (3 analytical replicates per sample) through a series of 11 nested sieves (mesh sizes ranging from 0.032 to 6.3 mm) on a sieve shaker. Total pore volume and water content were determined by drying and weighing an undisturbed core volume (top 2cm, 4 mL).

2.3.5 Carbon turnover at the watershed scale

To gain insight into the importance of soils and sediments for energy flow integrated at the landscape scale, we calculated carbon turnover at each site and extrapolated the values to a whole year and the entire watershed. As a rough approximation, carbon turnover rates (k) were calculated as the ratio of temperature-corrected community respiration rates (R) to organic matter (OM) standing stocks [Minshall *et al.*, 1983]. Temperature corrections of respiration rates were based on an exponential relationship between temperature and respiratory activity using a Q_{10} value of 2 [Davidson and Janssens, 2006]. Respiration rates from seasonal sampling were extrapolated to whole seasons using daily temperature records from the weather station in the watershed. Oxygen consumption rates were converted to carbon units by assuming a respiratory quotient (RQ) of 0.85 [Hargrave, 1973], and soil and sediment organic matter (OM) was assumed to contain 40% carbon [Cahill and Autrey, 1987]. Extrapolation to a year (15 November 2007 to 14 November 2008) was based on continuous records of surface runoff in the watershed. Spatial extrapolation was based on GIS data derived from aerial photographs. For soil and sediments of ephemeral channels, respiration on dry days was assumed to be 30fold lower than respiration measured after rewetting. This approximation is based on the observation that activity has been found in arid environments to increase up to 30fold after heavy rainfall [Sponseller, 2007; Thomas and Hoon, 2010]. Results differed little from those of an alternative scenario where respiration at terrestrial sites and ephemeral channel sites was assumed to be nil on days

without surface runoff. Microbes at the sites within the perennial stream section were assumed to be permanently active.

2.3.6 Data analysis

Results of community respiration were expressed per soil or sediment dry mass (DM), organic matter (AFDM) and surface area based on a soil or sediment depth of 2 cm. Both measured and temperature-corrected respiration rates based on a Q_{10} of 2 [Davidson and Janssens, 2006] are presented. Analysis of variance (ANOVA) was used to test for differences in respiration and other variables among seasons, streams and sites within streams. Season was considered a fixed factor and stream and site nested within stream as random factors. Samples taken in different seasons were considered independent because the study site is highly dynamic and samples were taken several months apart. Since some measurements were missing from different sites in different seasons, use of repeated-measures ANOVA would have meant discarding a disproportionately large fraction of the data. When QQ-plots, frequency histograms and Wilk-Shapiro's test indicated that residuals did not meet assumptions required for parametric tests, variables (x) were transformed according to $\ln(x+c)$, where $c = q_{0.25} x^2 / q_{0.75} x$ and q stands for quantile [Seminar for Statistics, ETH Zurich, pers. comm.]. Linear regressions were used to explore potential drivers of respiration rates. All analyses were performed with SPSS 15.0. Values in the text are reported as ranges or as means with standard errors.

2.4 Results

2.4.1 Metabolic activity

Metabolic activity in soil and sediments measured as oxygen consumption varied from undetectable to $2.3 \mu\text{g O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$ (Figure 2.2) and, when expressed per surface area, from undetectable to $3.2 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$. Permanent water availability did not have a major influence on metabolic activity; after rewetting, terrestrial soil and sediments in the ephemeral section had similar respiration rates as sediments in the perennial section. Significant differences were observed along the flow path from soils to the downwelling sites in single seasons and streams. However, there was no consistent temporal pattern (Figure 2.2). This was reflected in significant interactions of 'season x stream' and

‘season x site nested in stream’ in the ANOVA (Table 2.2). Significance of the ‘season x stream’ interaction disappeared when respiration rates were temperature-corrected, but the interaction ‘season x site within streams’ remained significant, indicating that temperature variation was not the only reason for seasonal differences (Table 2.2).

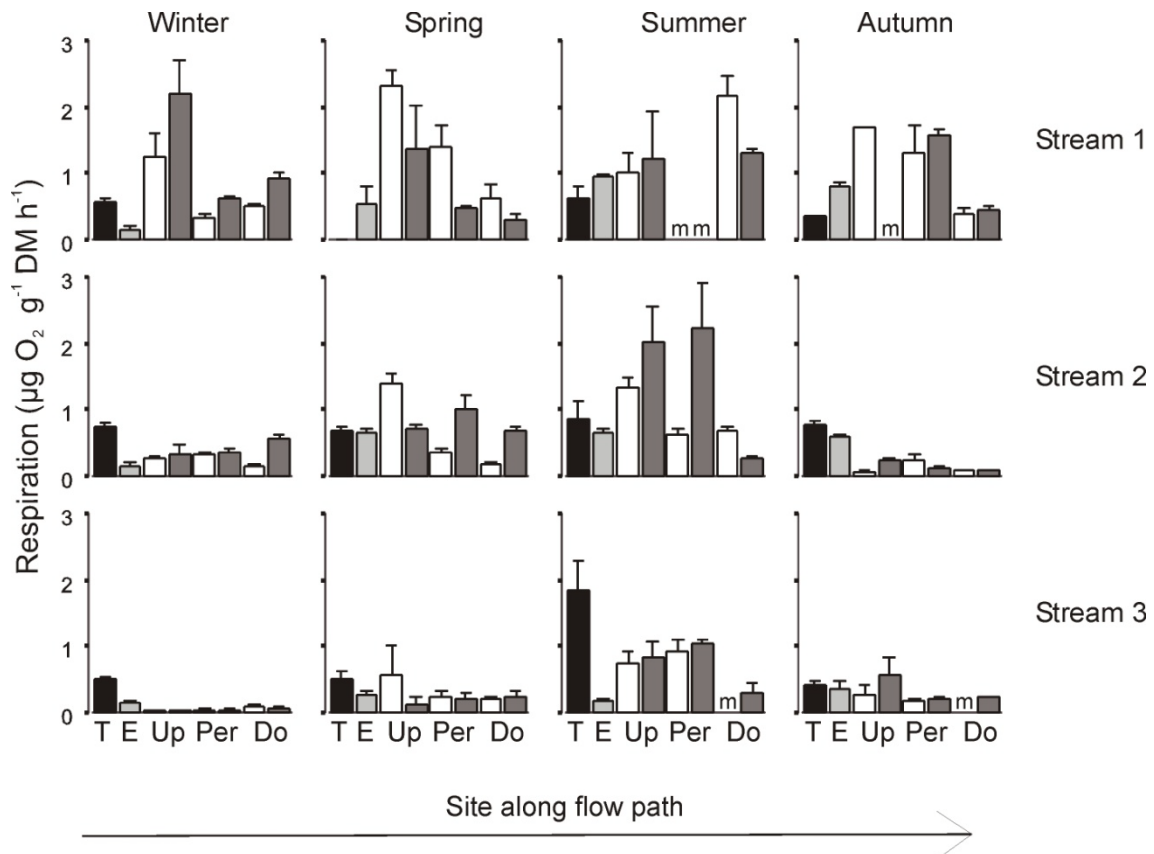


Figure 2.2:

Pattern of respiration rates (means \pm 1SE, $n = 3$) across 4 seasons at a total of 8 sampling sites along the hydrologic flow path in each of 3 stream corridors. T = terrestrial (black bars), Ephemeral (light gray bars), Up = upwelling, Per = perched surface flow and Do = downwelling sites. White bars refer to permanently submerged sites within the perennial stream sections and dark gray bars denote parafluvial sites. m = missing value.

Table 2.2: Results of separate nested ANOVAs of respiration, chlorophyll and organic matter data from the Chicken Creek watershed between November 2007 and 2008.

Variable	df	MS	F	P
Respiration ($\mu\text{g O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$)				
Intercept	1	136.6	7.02	0.118
Season	3	13.1	3.31	0.099
Site(stream)	21	1.9	1.84	0.035
Stream	2	19.6	4.06	0.059
Season \times stream	6	4.0	3.84	0.003
Season \times site(stream)	58	1.1	4.64	<0.001
Respiration at 10 °C ($\mu\text{g O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$)				
Intercept	1	54.6	12.54	0.071
Season	3	0.5	1.21	0.383
Site(stream)	21	0.5	1.93	0.026
Stream	2	4.4	6.62	0.014
Season \times stream	6	0.4	1.57	0.171
Season \times site(stream)	58	0.3	4.77	<0.001
Chlorophyll <i>a</i> (mg m^{-2})				
Intercept	1	84.3	2.91	0.230
Season	3	6.5	0.96	0.469
Site(stream)	21	1.4	1.38	0.169
Stream	2	29.0	4.06	0.071
Season \times stream	6	6.7	6.72	<0.001
Season \times site(stream)	59	1.0	4.69	<0.001
Organic matter ($\text{mg AFDM g}^{-1} \text{ DM}$)				
Intercept	1	234.9	194.13	0.005
Season	3	0.6	4.41	0.058
Site(stream)	21	1.0	7.90	<0.001
Stream	2	1.2	1.21	0.319
Season \times stream	6	0.1	1.01	0.428
Season \times site(stream)	60	0.1	2.49	<0.001

2.4.2 Environmental characteristics

Soil and sediment structure was similar across all sampled sites and seasons, with fine to medium sand as the main fraction ($D_{50} = 201 \pm 4 \mu\text{m}$, $n = 93$). However, silt was about 3-4 times less abundant in sediments ($1.3 \pm 0.1\%$) than soil ($5.1 \pm 0.8\%$), reflecting preferential downstream transport of the finest particles in the stream channels. Total sediment pore volume did not differ among sampling sites. Water content increased from terrestrial soils ($4.9 \pm 0.4\%$, $n = 36$) and the ephemeral channel sites ($5.9 \pm 0.6\%$, $n = 36$) to fully saturated in sediments of the perennial sections ($15.2 \pm 0.1\%$, $n = 213$). Surface-water concentrations of DOC and nutrients (PO_4^{3-} , NH_4^+ , $\text{NO}_2^- + \text{NO}_3^- = \text{NO}_x^-$) varied across streams, seasons and along the flow path but did not show any relation to respiratory activity. PO_4^{3-} and NO_x^- concentrations were low in the permanently flowing stream sections, whereas NH_4^+ and especially DOC concentrations were rather high (Table 2.1). Pore-water concentrations of dissolved nutrients and DOC were in the same range as surface-water concentrations. Ammonium concentrations of both pore and surface water generally declined along the flow path in the perennial stream section from 125 to 76 $\mu\text{g L}^{-1}$, whereas NO_x^- concentrations increased from 15 to 29 $\mu\text{g L}^{-1}$, suggesting that nitrification occurred in the bed sediments. However, this pattern was not consistent across all streams and seasons. NO_x^- and NH_4^+ concentrations in rain water, which was used for percolating dry sediments and soil during respiration measurements, were much higher than in stream water, whereas the concentration of PO_4^{3-} was similar and DOC concentrations were lower (Table 2.1).

Vertical oxygen profiles taken in September 2008 in sediments of the perennial stream sections showed a consistent decline with depth. Oxygen penetrated deeper at the downwelling sites (8 -18 cm) than at the upwelling sites (0.5 - 6 cm), but there was generally sufficient oxygen at all sites to ensure aerobic microbial metabolism in the upper 2 cm of sediment that were sampled.

Soil organic matter content ranged from 2.1 to 15.1 mg AFDM g^{-1} DM and exceeded the organic matter content of sediments (0.63 - 5.84 mg AFDM g^{-1} DM) in all seasons (Figure 2.3). In the perennial stream sections, concentrations of POM that was loosely associated with sediments differed greatly among streams at the time of maximum plant biomass in summer (Figure 2.4). In contrast, concentrations of POM that was strongly associated with sediments varied little among streams. Loosely associated POM was

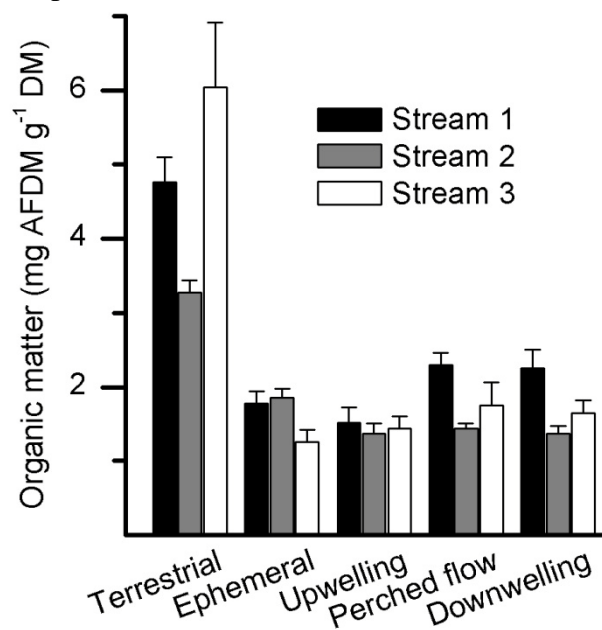


Figure 2.3:

Organic matter content at 5 sites along the hydrologic flow path in the three main streams of the Chicken Creek watershed. Histograms show pooled values over 4 seasons (mean +1SE, $n=12$). Data from submerged and parafluvial sites in the perennial stream sections were also pooled ($n=24$).

from undetectable to 150 mg m^{-2} , but also varied among stream corridors (Stream 1: 37.0 ± 0.3 ; Stream 2: 23.8 ± 0.2 ; Stream 3: $10.2 \pm 0.1 \text{ mg chl } a \text{ m}^{-2}$). There was no clear pattern along the flow path or among streams (Table 2). However, parafluvial sediments had higher chlorophyll *a* than submerged sediments (paired *t*-test, $t=4.68$, $p<0.001$, $n=70$).

2.4.3 Organic carbon sources for community respiration

Regression analysis indicated that respiration rate did not co-vary with soil and sediment organic matter ($R^2=0.04$, $p<0.001$, $n=267$) or carbohydrate content ($R^2=0.09$, $p<0.001$, $n=198$). However, respiration rate in stream sediments showed a positive relationship with chlorophyll-*a* content, although this relationship was not very strong ($R^2=0.42$, $p<0.001$, $n=233$). Adding other environmental factors (e.g. NO_x^- , NH_4^+ or PO_4^{3-} concentration) to a sequential regression model did not increase its explanatory power.

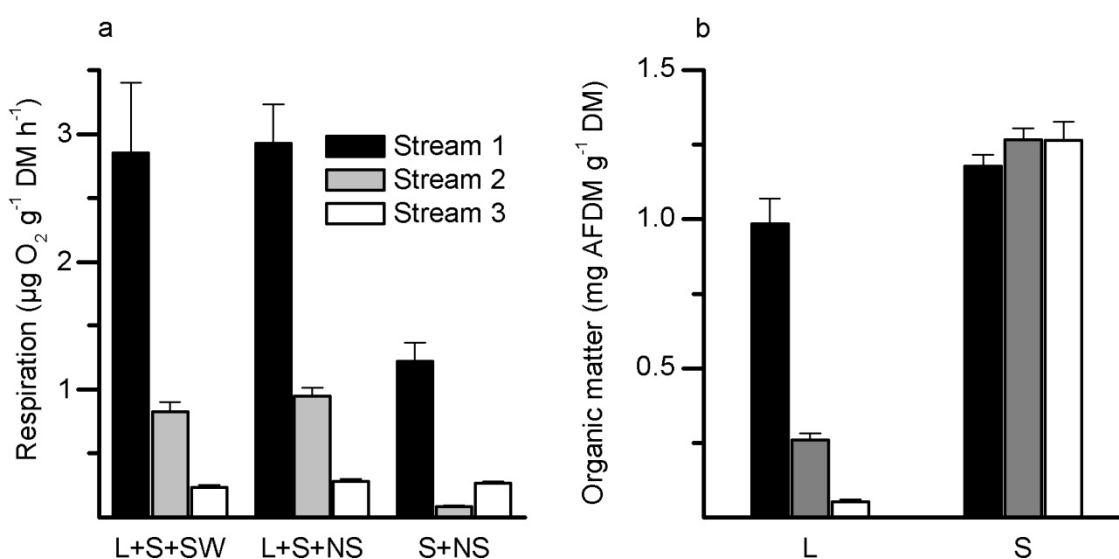


Figure 2.4:

Respiration rates associated with sediment from the perennial sites of the three streams in the Chicken Creek watershed. (a) Respiration rates (means +SE, $n=6$) measured in three different conditions to assess the contribution of different carbon sources for sustaining respiration. L= loosely associated POM; S= strongly associated POM; SW= stream water; NS= nutrient solution. (b) Contents of strongly (S) and loosely (L) associated POM in sediments (mean +1SE, $n=12$).

Respiratory activity of sediments from the perennial section measured at one occasion in August 2008 was very similar in sediments percolated with either a mineral nutrient solution or stream water (t -test, $p=0.83$, $n=36$). Differences in respiration rates of sediments with and without loosely associated POM suggest that this organic matter fraction supported 58 and 91 % of the total respiration per g sediment DM in streams 1 and 2, respectively, but only 5% in stream 3 (panel a in Figure 2.4). As concentrations of POM strongly associated with sediment were similar in the 3 streams, this POM fraction could not account for the observed differences in respiratory activity (panel b in Figure 2.4).

Carbon turnover rates (k) in the perennial stream sections were 4-8fold higher than at ephemeral and terrestrial sites, ranging from 9.5 ± 1.7 and 12.3 ± 1.8 % per year at the terrestrial and ephemeral sites, respectively, to 80 ± 23 % at perennial sites ($n= 3$ sub-watersheds). When extrapolated to the entire watershed and whole year, the higher rates in the perennial stream sections were insufficient to compensate for the large surface

area of terrestrial soils, which accounted for 95% of the total annual carbon turnover in the Chicken Creek watershed. The perennial and ephemeral stream area each accounted for about half of the remaining 5%.

2.5 Discussion

2.5.1 Variation of metabolic activity along flow paths

A remarkable result of our respiration measurements along the flow path in early successional stream corridors was that potential metabolic activity of rewetted upland soil and sediment from dry stream channels was strikingly similar to rates measured in permanently wet or moist sediments downstream. It appears, therefore, that microbial communities in the young soils and sediments of the Chicken Creek watershed can take rapid advantage of pulses in water availability, raising their metabolism almost instantaneously to full capacity, as has been observed in other circumstances where water availability is low and typically occurs in pulses [e.g., *Cable and Huxman*, 2004; *Kuehn et al.*, 2004]. This observation suggests that permanent water availability is not a main factor determining heterotrophic metabolic potential of microbes in these substrates.

Responses of soil and sediment microbes experiencing mesic climate conditions in the Chicken Creek watershed thus resembles those in semiarid and arid regions, where the short-term stimulating effect of precipitation on microbial activity in soils and ephemeral stream channels is well documented [*Romani and Sabater*, 1997; *Austin et al.*, 2004; *Belnap et al.*, 2005; *Sponseller*, 2007]. Minor differences in potential microbial activity, measured as Electron Transport System (ETS) activity, were also observed across dry and wet sites in an Australian braided river with sediments mainly composed of shifting sand [*Claret and Boulton*, 2003]. However, full metabolic capacity after rewetting has not been observed in all cases. Often, responses of microbes to sediment rewetting rather appear to depend on the length of the preceding dry period. Rates of both oxygen consumption and ETS activity decreased exponentially for a week as a function of time after sediments fell dry in an ephemeral river in New Zealand [*Larned et al.*, 2007], and bacterial biomass production in a temporary Mediterranean river declined similarly with decreasing water content of sediment submerged for the measurements [*Amalfitano et al.*, 2008]. Thus, full metabolic activity as observed after

rewetting of dry soil and sediment in our and other investigations is not a universal phenomenon.

The reasons for the discrepancies among studies are not clear but they might be related to varying degrees of desiccation stress. Differences in soil and sediment structure (e.g. organic matter concentration, grain size) leading to variable protection of enzymes and microbes from desiccation in the mucilaginous matrix of biofilms [Decho, 2000] might account for this effect. Different adaptations of microbial communities to drying-rewetting stress frequency [Fierer *et al.*, 2003] and physiological condition of microbes might also have contributed. Consistent with these explanations, microbial biomass production in rewetted stream sediments was more strongly reduced, and recovered more slowly, in a Mediterranean river where desiccation was more severe, and changes in microbial community structure more profound, than in a small temperate stream of Central Europe [Marxsen *et al.*, 2010].

Although potential metabolic activity did not show systematic differences along the flow path in the Chicken Creek watershed, carbon turnover rates were considerably higher in perennial sections of the stream channels. Permanent water availability in the perennial sections facilitated constant microbial activity and, exacerbated by low organic matter contents in the channels, resulted in an overall higher carbon turnover at the aquatic sites. The elevated activity at these metabolic hot spots was insufficient, however, for perennial stream channels to have a strong influence on total carbon flux at the watershed scale. The large spatial extent of terrestrial soils ensured that the annual carbon flux in the whole watershed was still dominated by soil microbial respiration during the hot moments lasting for about 100 days per year when water was available during and shortly after rainfall events.

2.5.2 Overall level of respiration activity

Sediment respiration per unit surface area in the Chicken Creek watershed was low in comparison to natural sand-bed streams (Table 2.3). This limited activity is not surprising given the scarcity of soil and sediment organic matter in the watershed. It is unlikely to be due to methodological bias because measurements in disturbed soil or sediment samples tend to stimulate rather than curb respiratory activity [Grimm and Fisher, 1984]. The low organic matter content (mean of 1.8 with a range of 0.6 - 5.8 mg

AFDM g^{-1} DM) recorded across all sites and seasons is at the low end of values reported even from streams in arid regions [2-12 mg AFDM g^{-1} DM; *Grimm and Fisher, 1984; Claret and Boulton, 2003*] and reflects the initial successional state of the Chicken Creek landscape characterized by sparse vegetation [*Gerwin et al., 2009*] and limited terrestrial and in-stream primary production. When expressed per soil or sediment organic matter, metabolic activities in the watershed (0-2.9 with a mean of 0.36 mg O_2 g^{-1} AFDM h^{-1}) compare more favorably with respiration rates measured, for example, across a large number of Piedmont streams in the eastern USA (0-0.62 with a mean of 0.41 mg O_2 g^{-1} AFDM h^{-1} ; *Hill et al., 2002*). However, the rates reported by *Hill et al.* [2002] are also at the low end of published values in streams and have been surmised to be underestimates resulting from respiration measurements in closed centrifuge tubes without advection [*Bott et al., 2006*]. Thus, low quantity of organic matter is a likely factor limiting respiration in stream sediments and soils of the early successional Chicken Creek watershed.

Low organic matter quality or nutrient limitation are additional potential factors contributing to the low overall respiration activity in the Chicken Creek watershed. In view of relatively low PO_4^{3-} concentrations and high N:P ratios in both stream and rain water, the most likely limiting nutrient would be phosphorus, which *Mulholland et al.* [2001] found to be correlated with whole-stream respiration across a number of different biomes of North America. However, PO_4^{3-} concentrations of stream water and rain water used to rewet dry sediment and soil from the Chicken Creek watershed showed only minor differences, suggesting that nutrient availability did not account for spatial differences in respiration activity. Furthermore, respiration rates were similar in sediments percolated with either stream water or a mineral nutrient solution. Consequently, it is unlikely that nutrients were a major factor limiting soil and sediment respiration in the Chicken Creek watershed.

Table 2.3: Respiration rates in various streams comparable to those of the Chicken Creek watershed. Mean values are reported with ranges in parantheses.

Stream name	Stream characteristics and location	Respiration rate (g O ₂ m ⁻² d ⁻¹)	Reference
Chicken Creek	Sand bed, early successional	0.56 (0.0-3.2)	This study
Fort River	Sand bed, temperate	4.08 ^a	Fisher [1977]
Sycamore Creek	Sand bed, Sonoran desert	4.1	Grimm and Fisher [1984]
Hassayampa Creek	Sand bed, Sonoran desert	(1.33-1.5)	Uehlinger et al. [2002]
Rattlesnake Springs	Sand bed, cold desert	7.8 ^a	Cushing and Wolf [1984]
Creightons Creek	Sand bed, warm temperate, south-east Australia	(0.6-3.7)	Atkinson et al. [2008]
Various (n=64)		16 (2.44-68.1) ^a	Battin et al. [2008]

2.5.3 Carbon Sources fuelling respiration

Several distinct carbon sources were available to fuel metabolism of heterotrophic microorganisms in the stream corridors of the Chicken Creek watershed. These include algal exudates and biomass [Cole, 1982], dissolved organic carbon (DOC), and root exudates and litter from the vascular plants that became established in some of the stream channels. Chlorophyll measurements in the Chicken Creek streams (0-150 mg m⁻² with a mean of 24 mg m⁻²) indicated that algal biomass was at the low end of values reported from oligotrophic open-canopy [Mulholland *et al.*, 2001] and forest streams [Veraart *et al.*, 2008]. However, positive relationships between respiration rate and chlorophyll *a* suggests that at some sites and times algae accounted for a portion of the total community respiration (measurements in the dark), or provided high-quality

organic matter that stimulated heterotrophic microbial activity. Similar relationships have been reported from other streams [Jones *et al.*, 1995; Romání and Sabater, 1998; Fischer *et al.*, 2002].

In the perennial stream sections, a potentially more important carbon source than algae was DOC in upwelling ground water, which had high concentrations in all streams (Table 1). However, although DOC was abundant, respiration rates of pore water without sediment were exceedingly low, indicating poor bioavailability of this DOC component. This result is consistent with our observation that respiration rates were similar in sediments percolated with DOC-free mineral nutrient solution or DOC-containing stream water (panel A in Figure 2.4). Furthermore, dark incubation of pore water in bottles inoculated with a microbial cell suspension and shaken daily for 70 days did not lead to a decline in DOC concentration (L. Gerull, unpublished data). This reinforces our conclusion that DOC in upwelling ground water in the Chicken Creek watershed was poorly bioavailable and contributed little to overall respiration.

The third important carbon fraction available to heterotrophic microbes in both soils and sediments is particulate organic matter (POM). Similar to other studies [Findlay and Sobczak, 2000, $R^2=0.15$; Uehlinger *et al.*, 2003, $R^2=0.11$], strong relationships between respiration rate and total POM was not found in the present study, implying that total POM was not a major source of variation in microbial metabolic activity in the Chicken Creek watershed. However, the loosely attached POM fraction, which likely represents small organic particles in sediments [Pusch and Schwoerbel, 1994], was strongly related to respiration rate in the perennial stream sections with relatively high plant cover during summer ($R^2 = 0.96$, $p < 0.001$, $n = 18$). Loosely associated POM at these sites mainly consisted of dead roots and fragments of herbaceous plants, indicating that, similar to woodland streams [Fischer *et al.*, 1996], POM derived from recent plant production was of prime importance for microbial metabolic activity in the early successional landscape of the Chicken Creek watershed.

2.5.4 Variation of metabolic activity among streams

The notable variation in respiration and other response variables (e.g. chlorophyll) that we observed among streams in the Chicken Creek watershed was unexpected. This is because the three studied sub-watersheds had formed from the same substrate and were

subject to very similar environmental influences during the two years of their existence prior to the present study. One explanation for the discrepancies among the streams might be high stochasticity in the early successional landscape that we investigated. Alternatively, the discrepancies could be due to differences in habitat characteristics among the sub-watersheds. The most obvious of these was size. Given the flashy surface runoff and discharge regime in the watershed [Gerwin *et al.*, 2009], such size differences could have caused differences in sediment mobility among streams, with the largest stream experiencing the greatest disturbance. Sediment movement during high flow impacts benthic algae [Cushing and Wolf, 1984; Holmes *et al.*, 1998; Atkinson, 2008]. It also affects macrophyte establishment and persistence [Riis *et al.*, 2004; Heffernan, 2008]. This observation is corroborated by a rapid vegetation survey we conducted in the summer of 2008. It showed that vascular plants established more successfully in the smallest than in the largest channel, with the channel of intermediate size (stream 2) in between (L. Gerull, unpublished data). In contrast to algae and plants, heterotrophic microbial communities appear to show only a minor direct influence of sediment disturbance [Grimm and Fisher, 1984]. However, since algae (and vascular plants) can supply significant quantities of dissolved and particulate organic matter supporting heterotrophic respiration in streams [Jones *et al.*, 1995; Uehlinger *et al.*, 2002], flow-related sediment disturbance could indirectly affect heterotrophic microbial communities as well.

3 Effects of shallow and deep sediment disturbance on whole-stream metabolism in experimental sand-bed flumes²

3.1 Abstract

Sandy sediments are common in many lowland streams. They are easily mobilized, relocated and re-deposited when discharge fluctuates, with the depth of sediment scour varying with shear stress. While sediment movement during major floods is a major disturbance for benthic algae and bacteria, effects on stream metabolism are not well known, and impacts of minor disturbance events, which are frequent in sand-bed streams, are especially poorly documented. Our objective was to determine whether shallow and deep sediment disturbances in small sand-bed streams cause similar impacts on whole-ecosystem metabolism, and whether autotrophic and heterotrophic processes and organisms respond in similar ways. We used 16 experimental outdoor flumes that were filled with sand and subjected to repeated manual sediment disturbance to two different depths (1 and 4 cm) during an early and advanced stage of stream community succession. To separate effects on heterotrophic and autotrophic metabolism, half of the flumes were permanently covered. At the early successional stage, sediment disturbance did not affect net community production, while sediment mixing reduced production independent of the disturbance depth in the advanced stage. Microbial respiration, in contrast, was significantly stimulated when sediment was mixed to greater depth. These results suggest that disturbing sediments during early successional stages has no effect on whole-stream metabolism, whereas in advanced stages, deep sediment disturbance can lead to a transitory shift towards heterotrophy. The recovery time of net community production from perturbation was independent of disturbance depth, with the similar trajectories observed after deep and shallow sediment disturbance indicating that delayed recovery was not simply due to mixing algae into deeper sediment layers but primarily a result of disrupting the fine structure of sediment at the surface.

² Gerull, L., A. Frossard, M. O. Gessner, and M. Mutz (2011): Effects of shallow and deep sediment disturbance on whole-stream metabolism in experimental sand-bed flumes. *Hydrobiologia*, doi: 10.1007/s10750-011-0968-x. The final publication is available at www.springerlink.com.

3.2 Introduction

Sandy sediments are common in many lowland streams, and become increasingly widespread as unsustainable land management fosters soil erosion and sediment loading to stream channels [Wallbrink, 2004; Rabeni *et al.*, 2005; Shields, 2009]. Small grain sizes and low cohesion make sand a highly mobile substratum [Morisawa, 1968], to the extent that changes in stream flow caused by even minor precipitation events are often sufficient to mobilize the sand grains. As a result, sediment movement can be rather frequent in sandy streams, with average recurrence intervals as short as one week [Luce *et al.* 2010]. In some cases, the sediment can even be in constant motion [Biggs, 1996; Fischer and Pusch, 2001; Uehlinger *et al.* 2002; Atkinson *et al.* 2008].

The entrainment of sediment (scour) and the downstream transport and subsequent deposition (fill) in sand-bed streams results in vertical mixing of the upper sediment layers [Powell *et al.*, 2006], although the streambed level is maintained in steady state [Powell *et al.* 2007]. Apart from sediment grain size, depth of mixing (i.e. scour and fill) depends on shear stress. It is mainly a function of stream power and is thus positively related to discharge and channel slope, and inversely related to stream size [Powell *et al.*, 2007]. Mixing depth in a sand-bed stream at low discharge ($0.05 \text{ m}^3 \text{ s}^{-1}$) was $< 2 \text{ cm}$ on two thirds of the stream bed, and about 5 cm on 75 % of the stream area at a discharge of $1 \text{ m}^3 \text{ s}^{-1}$ [Powell *et al.*, 2005]. Similar mean scour depths (0.9 and 4.7 cm) and small net changes in stream-bed level (0 and 1.3 cm incision) were observed over a full year in a first-order lowland stream, except during a major flood caused by a hurricane [Metzler and Smock, 1990]. This suggests that sediment disturbance to a depth of several cm is typical of small sand-bed streams [Baas, 1999; Rennie and Millar, 2000; Powell *et al.*, 2005].

Disturbance of sediments has ecological effects on benthic stream algae and bacteria as well as on the metabolic processes driven by these microorganisms. Mechanisms include downstream flushing and abrasion of both free and attached cells resulting from increased shear stress [Francoeur and Biggs, 2006; Schwendel *et al.*, 2010], as well as mixing into deeper sediment layers and burial [Peterson, 1996]. Resistance of stream algae to flood disturbance varied considerably with the successional state of the algal communities in experimental flumes (i.e. 9, 18, 24 and 33 days after an initial simulated flood) [Peterson and Stevenson, 1992]. Moreover, communities that had been

acclimated to low stream flow proved less resistant than algae acclimated to high flow conditions [*Peterson and Stevenson, 1992*]. Thus, early successional taxa were small and highly resistant to experimental flow disturbances, whereas taxa dominating in advanced successional stages were larger in size and less resistant [*McCormick and Stevenson, 1991; Peterson and Stevenson, 1992*]. To recover to pre-disturbance levels, algal biomass and community structure required 3 to 9 days in that experiment [*Peterson and Stevenson, 1992*], but recovery can also take 2 to 3 weeks as in desert streams after flash floods [*Fisher et al., 1982; Holmes et al., 1998*].

Compared to algae, bacteria recovered even more slowly, taking 60 days to reach pre-flood abundances after a major flood had scoured the streambed to 30 cm depth and re-deposited a 10 cm layer of sand [*Holmes et al., 1998*]. Bacteria in the deposited sediments were immediately active, however, as shown by counts of respiring cells, indicating resistance to disturbance caused by sediment scour, abrasion during transport and sediment re-deposition.

Bed-moving floods in a gravel-bed stream have been found to lead to distinct declines also in metabolic activity, with community respiration being more resistant to disturbance than net community production [*Uehlinger and Naegeli, 1998*]. Difference in the response of autotrophic and heterotrophic metabolism was attributed to more severe impacts on the algal community at the streambed surface [*Biggs, 1996*], compared to the community of heterotrophic bacteria. This is in accordance with the delayed recovery of algal primary production in a Prairie stream with sandy sediments, which took two weeks after a severe flood [*Dodds et al., 1996*]. In contrast to algae, heterotrophic bacteria can maintain activity also in deeper sediment layers where they might profit from high-quality carbon supplied by algal cells when they die as a result of mechanical damage or deprivation of light [*Cole, 1982; Romani and Sabater, 2000*]. However, whether and how such interactions between algae and bacteria affect resistance of metabolic activity to, and recovery from, sediment disturbance in streams is not well understood.

While effects of large bed-moving floods on autotrophic and heterotrophic microbes and processes in streams have previously been documented [*Power and Stewart, 1987; Grimm and Fisher, 1989*], responses to discharge fluctuations that are small and yet powerful enough to give rise to shallow scour and fill in sand-bed streams are virtually

unknown. Effects of such moderate sediment mixing on whole-stream metabolism is especially poorly understood [Uehlinger, 2000; Houser *et al.*, 2005; Atkinson *et al.*, 2008]. Thus the objectives of the present study were to determine (i) whether shallow sediment disturbance has a similar impact on whole-stream metabolism and benthic algae and bacteria in sand-bed streams as large bed-moving floods, (ii) whether autotrophic and heterotrophic organisms and metabolism are similarly affected, and (iii) whether disturbance effects differ between early and advanced successional stages of community succession. To test for these effects, we conducted a disturbance experiment in outdoor experimental flumes designed to mimic small lowlands streams with sandy sediment and an open canopy.

3.3 Material and methods

3.3.1 Study site and experimental set-up

The experiment was conducted in 16 outdoor flumes (0.12 x 0.12 x 4 m) set up in an experimental catchment, referred to as Chicken Creek catchment, which was located about 150 km south-east of Berlin, Germany (51°36' N, 14°16' E). The flumes were filled with a 4-cm layer of sediment collected from a dry ephemeral stream reach in the catchment. Ground water to supply the flumes also originated from the catchment. Nutrient concentrations of the water were low, whereas concentrations of dissolved organic carbon were high (Table 3.1), although poorly bioavailable [Gerull *et al.*, 2011]. The collected ground water was stored in a subterranean tank with a head space. It was well oxygenated when pumped into the flumes with a peristaltic pump (520-SN, Watson Marlow, Cheltenham, UK) delivering enough water to replace the volume in the flumes every six hours. Current velocity of the partially re-circulating water was $1.7 \pm 0.3 \text{ cm s}^{-1}$. Water depth was 1 cm. Half of the flumes were permanently covered with an opaque tarpaulin to prevent primary production and thus separate heterotrophic and autotrophic activities.

The experiment ran for 52 days, from late April to late June 2009. Sediment in the flumes was disturbed after 11 days (early successional state) and again after 31 days (advanced successional state). Disturbance consisted of thoroughly mixing the top 1 cm (shallow disturbance) or upper 4 cm (deep sediment disturbance) of sediment with a

spoon. Measurements and samples were taken at the beginning and end of the experiment (days 0 and 52), and directly before disturbing the sediment (days 11 and 31). One exception was on day 14, when sediment-associated respiration was measured 3 days after the first disturbance event. Non-destructive whole-stream metabolism was measured at 5 additional time points.

Table 3.1: Concentrations of dissolved nutrients and dissolved organic carbon (DOC) in flumes and a groundwater supply tank during the experiment. Means \pm 1SE of three sampling dates (days 14,31 and 52). Water samples were immediately filtered through pre-washed cellulose acetate filters (0.45 μ m pore size), frozen and later analyzed. Dissolved organic carbon (DOC) was measured with a Total Organic Carbon Analyzer (TOC-5000, Shimadzu, Duisburg, Germany). Asterisks (*) denote significant differences between light-exposed and dark flumes ($p < 0.05$).

In- and outflow	SRP (μ g/l)	NO _x ⁻ (μ g/l)	NH ₄ ⁺ (μ g/l)	SiO ₂ -Si (mg/l)	DOC (mg/l)
Dark shallow	2.9 \pm 0.3	76.5 \pm 5.4	49.0 \pm 2.4	2.15 \pm 0.04	10.4 \pm 0.3
Dark deep	3.3 \pm 0.3	72.5 \pm 5.7	47.4 \pm 2.9	2.43 \pm 0.03	11.1 \pm 0.5
Light shallow	2.8 \pm 0.2	33.7 \pm 4.3*	46.2 \pm 2.4*	2.70 \pm 0.01	11.3 \pm 0.2
Light deep	3.2 \pm 0.3	25.7 \pm 3.0*	45.7 \pm 2.1*	3.48 \pm 0.06	11.3 \pm 0.2
Tank	3.1 \pm 0.3	85.9 \pm 5.6	42.4 \pm 4.1	3.72 \pm 0.01	10.2 \pm 0.3

3.3.2 Whole-stream metabolism

Whole-stream metabolism was measured by sealing the flumes with Perspex lids and measuring the change in oxygen concentration with a 10-channel Minisensor Oxygen Meter (Oxy 10 mini, PreSens GmbH, Regensburg, Germany). To measure community respiration (CR), all flumes were covered with opaque tarpaulin and the decline in oxygen concentrations was recorded. To determine net community production (NCP) the tarpaulin was subsequently removed and the change in oxygen concentration measured under light conditions. Temperature in the flumes was recorded at the beginning and end of the measurements. Changes in oxygen concentrations in an air-tight glass bottle filled with ground water were determined simultaneously to estimate respiration rates in the flume water.

Gross primary production (GPP) was calculated as the sum of NCP during the daylight period (16 hours) and the sum of CR during the day (16 hours). The measured CR was extrapolated to 24 hours based on the assumption that daytime respiration equalled respiration at night to calculate the P/R as the ratio of GPP to CR [Odum, 1969]. This approach initially overestimated daytime CR but the error decreased as algal biomass increased. Based on an assumed 20 % error in daytime CR [Uehlinger and Naegeli, 1998] the uncertainty in estimating GPP and P/R was 1 to 15 % and 2 to 15 %, respectively. NCP was also expressed per unit chlorophyll *a* as a proxy for algal biomass (see below). Since chlorophyll *a* had to be sampled destructively, no samples were taken during periods between the two disturbance events. Therefore, cover slips exposed on the sediment surface at the start of the experiment (day 0) and again after both disturbance events were sampled for chlorophyll *a* more frequently (i.e. on days 1, 3, 7, 21, 26, 31, 37, 39, 46 and 52). Chlorophyll *a* on cover slips and in sediment were tightly correlated ($r=0.82$, $n=60$).

Total global radiation (300-3000 nm) was measured by a weather station at about 50 m distance from the flumes. Photosynthetically active radiation (PAR: 400-700 nm) was assumed to be 45 % of the total global radiation [Behrendt and Nixdorf, 1993]. For 3 days during the experiment, PAR was also directly measured with a cosine sensor (Universal Light Meter 500, Heinz Walz GmbH, Effeltrich, Germany) positioned at the sediment surface of the flumes. Results matched the estimate from the data derived from the weather station ($r=0.92$, $n=256$). At one occasion, the cosine sensor was used to determine light penetration depth in the sediment.

3.3.3 Community respiration, chlorophyll *a* and bacterial abundance in sediment

To assess community respiration (CR) in sediment, sediment cores were taken with 50-ml syringe barrels with the lower end cut off. The cores extended to 4 cm depth and were sliced in a top layer (0 to 2 cm) and a bottom layer (2 to 4 cm). Three grams of each layer was preserved in 10 ml of 2 % formalin containing 0.1 % pyrophosphate, and stored at 4°C. CR in the sediment was measured with oxygen optodes (Microx TX3, PreSens GmbH, Regensburg, Germany) in a flow-through laboratory system (see section 2.3.3). Measurements were taken within 12 hours after sampling at situ

temperature (14°C on day 14, 17°C on days 31 and 52). After the respiration measurements, the sediments were dried at 105°C to constant weight, weighed, combusted in a muffle furnace (3 h at 430°C), and reweighed to determine total sediment dry mass (DM) and organic matter (OM) content.

Chlorophyll *a* in sediment and on cover slips was used as a proxy for algal biomass. For details of the method see section 2.3.3.

Bacterial abundance was determined by flow cytometry after detaching the bacterial cells from the sediment for 3 x 20 s using a Branson Digital Sonifier 250 (Danbury, USA; flat tip, actual output of 38 W; [Buesing and Gessner, 2002]). A 1-ml subsample of the cell suspension was placed on top of 0.5 ml of Histodenz[®] solution in Eppendorf tubes (1.3 g ml⁻¹, Sigma-Aldrich, #2158, Buchs, Switzerland) and the tubes centrifuged (17,135 × *g*, 4°C, 90 min) to separate bacterial cells from other particles [Caracciolo *et al.*, 2005]. Subsequently, the upper Histodenz[®] layer containing the bacteria was quantitatively removed, the suspended bacterial cells stained with a solution of 0.1 μl ml⁻¹ SYBR[®]Green I in anhydrous dimethylsulfoxide (DMSO) and incubated in the dark for 15 min. Samples were diluted 10 or 100fold with filtered (0.22 μm Millex[®]-GP, Millipore, Wohlen, Switzerland) mineral water (Evian, France), to ensure that cell concentrations did not exceed 10⁶ ml⁻¹.

Bacteria were counted on a CyFlow[®] space Flow Cytometer System (Partec, Görlitz, Germany) equipped with a 200-mW solid-state laser emitting light at 488 nm and volumetric counting hardware [Hammes and Egli, 2005]. Green and red fluorescence was collected in channel FL1 (520 nm) and FL3 (630 nm), respectively. The flow cytometer was set as follows: gain FL1 = 495, gain FL3 = 650, speed = 4 (implying an event rate never exceeding 1000 events per s). Data were processed with Flowmax software (Partec, Görlitz, Germany), using electronic gating to separate the desired particles. All samples were collected as logarithmic signals and were triggered on the green fluorescence channel (FL1). Collection of the data as FL1/FL3 dot plots ensured optimal distinction between stained intact microbial cells and instrument noise or sample background.

3.3.4 Oxygen

Oxygen depth profiles in the sediments were measured with an optode microsensor (0.9 mm diameter; Microx TX 3, Presens GmbH, Regensburg, Germany) protected inside a 20-cm steel tube with a 1.7-mm² opening at the tip. The tube was lowered into the sediment to a defined depth and pore water (50 to 100 µl) was drawn into the tube with a syringe connected to the upper end of the tube. The small volumes of pore water sampled were sufficient to cover the probe tip and avoid mixing of two successive samples. Two measurements were taken at each of three spots evenly distributed along each flume. Temperature was simultaneously measured with a temperature sensor fixed to the probe tip.

3.3.5 Data analysis

NCP and CR are expressed per square meter of sediment surface and were standardized to 17°C based on the assumption that relationships between temperature and metabolic rate were exponential according to a Q_{10} of 2 [Davidson and Janssens, 2006]. All values are reported as means with ranges in parentheses. Repeated-measures analysis of variance (rmANOVA) was used to test for effects of sediment disturbance depth and, where pertinent, light availability and the interaction of disturbance depth and light availability, which were both treated as fixed factors. Paired *t*-tests were used to test for differences before and after sediment disturbance, and to disentangle differences between single sampling days. Univariate ANOVA was used if single treatment effects were tested for single days. All analyses were performed with SPSS 17.0.

3.4 Results

3.4.1 Net community production and algal biomass

No difference was detected in the response of net community production (NCP) between shallow and deep sediment disturbance regimes in either the early (rmANOVA, $n=28$, $p=0.78$) or advanced successional stage (rmANOVA, $n=35$, $p=0.96$) (panel a in Figure 3.1). Sediment disturbance on day 11 did not appear to affect NCP, whereas strong declines were observed following the second disturbance on day 31 (paired *t*-test between days 31 and 32, $n=8$, $p=0.007$). Maximum NCP was reached

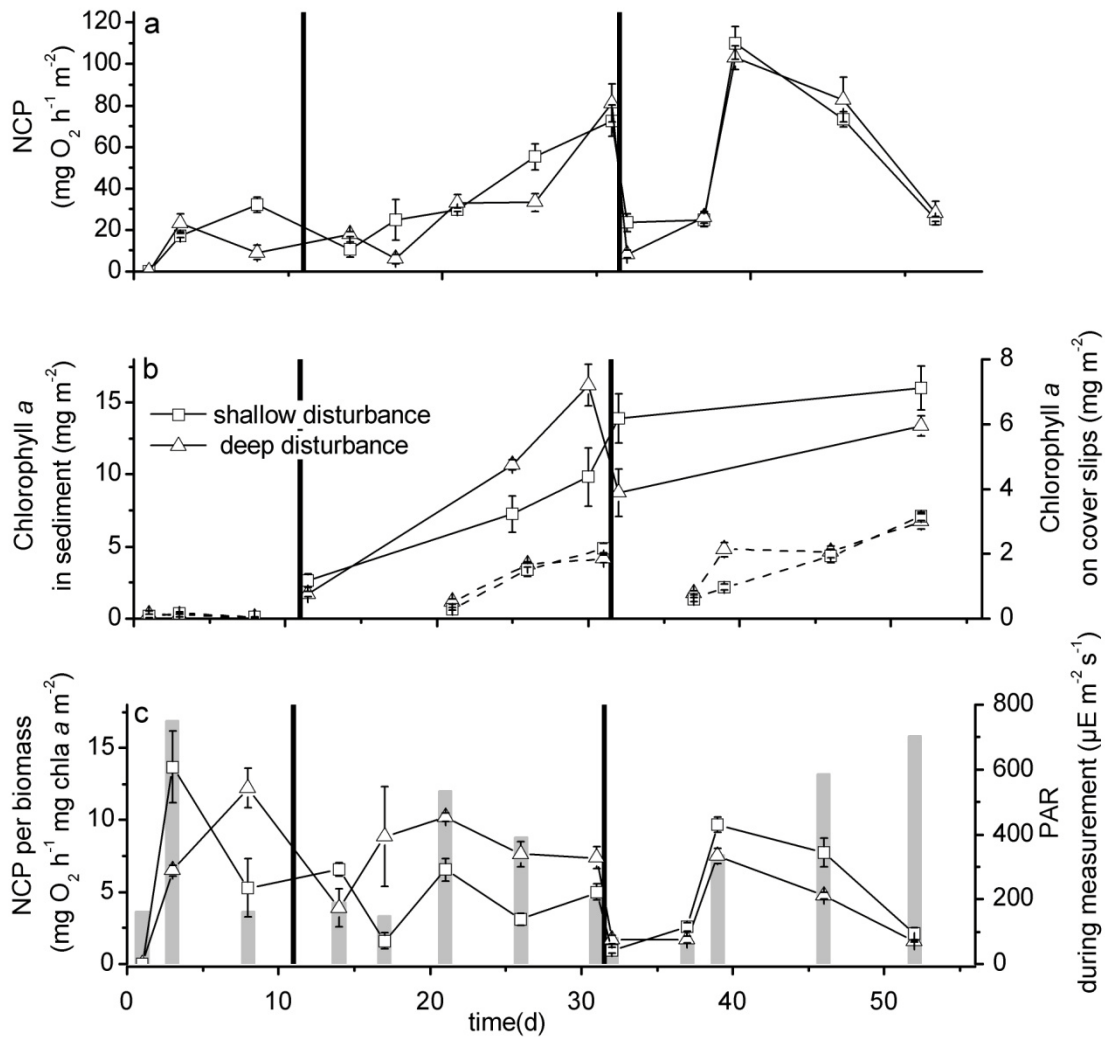


Figure 3.1:

Net Community Production (temperature standardized) and chlorophyll *a* content during the experiment (mean \pm SE, $n=4$), black bars mark sediment disturbances. Panel A: NCP (Net Community Production) Panel B: Chlorophyll *a* content in sediment and on exposed cover slips. Straight lines refer to the left axis and show sediment bound chlorophyll *a* whereas the dashed lines refer to the right axis and show data from cover slips exposed on the sediment surface. Exposure of the cover slips was on day 0 after filling and on day 11 and 31 after disturbances were applied. Panel C: NCP referring to biomass (chlorophyll *a*) and PAR averaged over the 1 hour measurements (grey columns).

on day 39 and was followed by a gradual decline to low values despite high levels of algal biomass towards the end of the experiment.

Algal biomass estimated as chlorophyll *a* in the top sediment layer (to 1 cm depth) increased throughout the experiment. Chlorophyll *a* was higher in flumes subjected to shallow compared to deep sediment disturbance immediately after the first disturbance event (day 11) (ANOVA, $n=8$, $p=0.048$). However, during the following days, more chlorophyll *a* was found in deeply disturbed sediments (rmANOVA on days 26 and 31, $n=18$, $p<0.001$) (panel b in Figure 3.1). In the advanced successional stage, deep sediment disturbance led to a decrease in chlorophyll *a* (paired *t*-test between days 31 and 32, $n=8$, $p=0.03$). Shallow sediment disturbance had no evident effect at this stage. Chlorophyll *a* was similar under both disturbance regimes following the last mixing of sediment on day 31 (rmANOVA, $n=16$, $p=0.12$).

NCP per algal biomass was relatively constant over time. The two exceptions were a peak on day 3 of the experiment, shortly after sediments in the flumes were wetted, and a sharp decline after the second disturbance (paired *t*-test, $n=8$, $p<0.001$) (panel c in Figure 3.1). Overall, however, NCP per unit algal biomass did not differ between the shallow and deep disturbance regime in either the early (rmANOVA, $n=28$, $p=0.16$) or advanced successional stage (rmANOVA, $n=35$, $p=0.96$). PAR was never limiting during the experiment. The penetration depth of PAR into the sediment (values above zero) measured at one occasion was 0.3 ± 0.2 cm.

3.4.2 Community respiration

Community respiration (CR) varied from undetectable to $98.7 \text{ mg O}_2 \text{ h}^{-1} \text{ m}^{-2}$ (Figure 3.2). It was relatively high initially before any disturbances occurred. In dark and light-exposed flumes, no difference was observed between disturbance regimes after day 11 (rmANOVA, days 14 to 26, $n=56$, $p=0.44$), while the later disturbance on day 31 increased respiration in the flumes with deeply disturbed sediments until the end of the experiment (rmANOVA, days 32 to 52, $n=70$, $p=0.002$), indicating that deep mixing stimulated microbial respiration. Higher respiration in the light-exposed compared to dark flumes was limited to the period after the first disturbance event (rmANOVA, days 14 to 26, $n=56$, $p<0.001$). Although NCP and algal biomass remained high after the disturbance in the advanced successional stage, respiration rates in light-exposed and

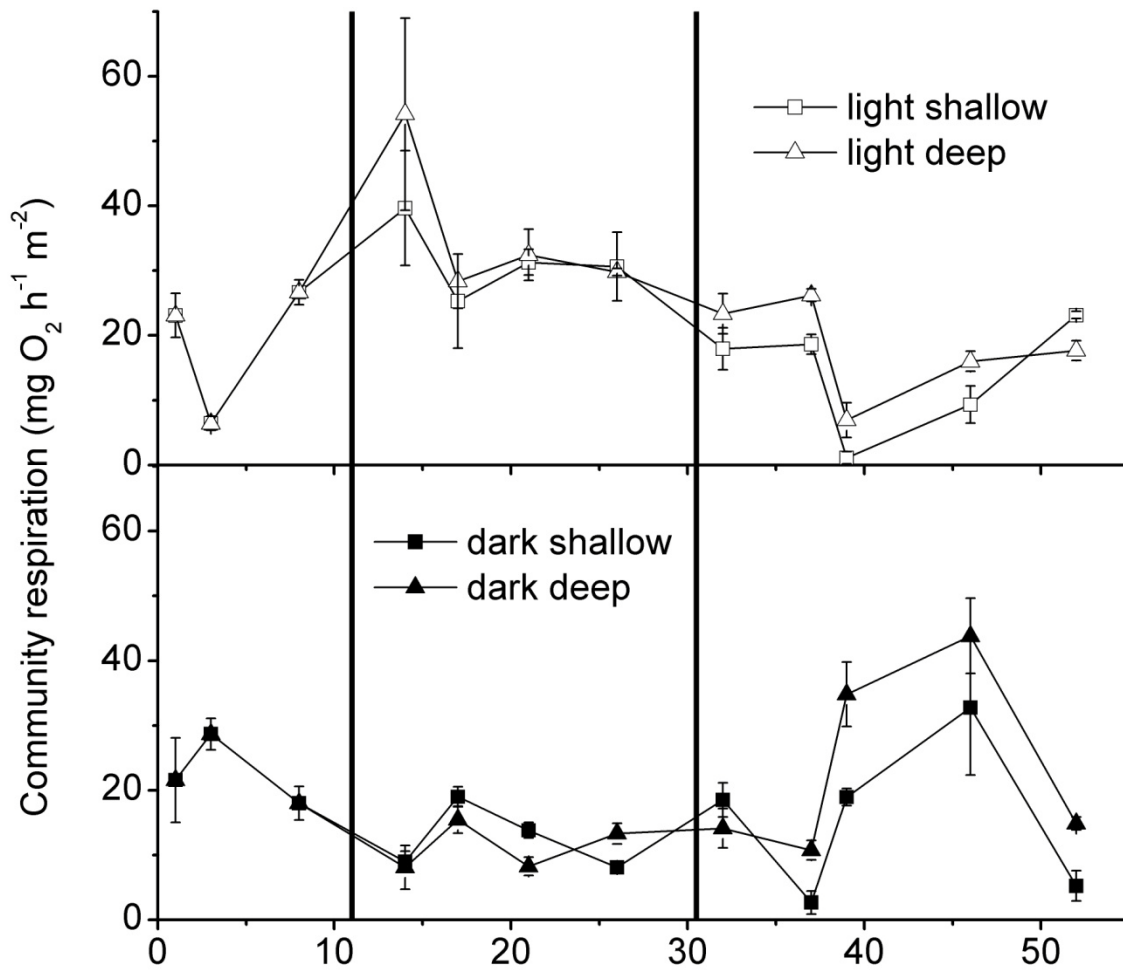


Figure 3.2:

Whole-stream community respiration (temperature standardized) during the experiment (mean \pm SE, $n=4$), black bars mark sediment disturbances.

dark flumes were similar (rmANOVA, days 32 to 52, $n=70$, $p=0.073$). Disturbance had a stronger effect on CR during that period than light availability.

3.4.3 P/R ratio

The P/R ratio in the light-exposed flumes increased from an initially low value < 1 to a maximum on day 3 (Figure 3.3). After this early peak, the ratio decreased again to values < 1 . With algal biomass increasing over time, shallow sediment disturbance shifted the P/R ratio to > 1 after day 17, and the deep sediment disturbance resulted in a

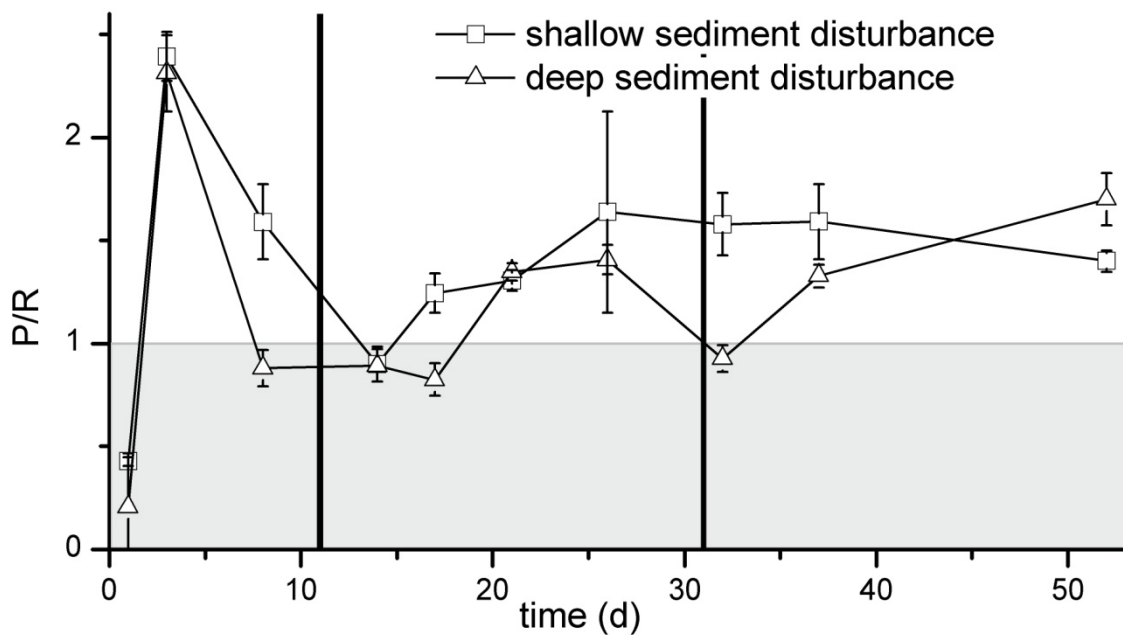


Figure 3.3:
Ratio of respiration and production (mean \pm SE, $n=4$), black bars mark sediment disturbances.

P/R ratio > 1 on day 20. In general, the flumes remained autotrophic (i.e. $P/R > 1$) until the end of the experiment. An exception was the effect of deep sediment disturbance in the advanced successional stage on day 31. Flumes with deeply disturbed sediments were reset to the heterotrophic state ($P/R < 1$) the next day, whereas the shallow sediment disturbance did not change the P/R ratio.

3.4.4 Community respiration in sediment

Community respiration in the sediments was affected by deep but not shallow sediment disturbance (Figure 3.4) in dark and light-exposed flumes and caused higher respiration after deep sediment disturbance in both sediment layers (0 to 2 cm) and (2 to 4 cm) (rmANOVA, day 14 and 31, $n=48$, $p<0.001$). On day 52, deep sediment disturbance also increased the respiration rate compared to shallow disturbance (ANOVA, day 52, $n=32$, $p<0.001$). Light availability also had an effect, with higher respiration rates recorded in light-exposed flumes (ANOVA, day 52, $n=32$, $p<0.001$). Separate analyses

for the two depth layers revealed that the surface layer did not show differences between disturbance regimes (ANOVA, $n=16$, $p=0.19$) whereas differences in light availability had an effect (ANOVA, $n=16$, $p=0.001$). The bottom layer (2 to 4 cm) showed higher respiration rates in the deep sediment disturbance regime (ANOVA, $n=16$, $p=0.001$) but only in the light-exposed flumes (ANOVA, interaction disturbance regime \times light, $p=0.038$). These results on community respiration in the sediment partly matched the response of CR measured at the whole-flume level, which also was higher after deep sediment disturbance in the advanced successional stage.

3.4.5 Sediment organic matter and bacterial abundance

Total organic matter content (OM) of sediments showed no differences between disturbance or light regimes when measured on days 31 or 52 (rmANOVA, $n=24$, $p=0.21$).

Bacterial abundance in the sediments ranged from $0.3 - 28.2 \times 10^6$ cells g^{-1} DM, with a grand mean of 11.3×10^6 cells g^{-1} DM, and decreased during the experiment only in the dark flumes (rmANOVA, $n=33$, $p<0.001$) (Figure 3.4). No difference between disturbance regimes was observed for either the early or later disturbance event (rmANOVA, $n=33$, $p=0.71$).

3.4.6 Oxygen

Although surface water in the flumes was well oxygenated, oxygen concentration in the sediment pore water strongly declined with depth. On day 31, where data is only available for the light-exposed flumes, only the top 1 cm of sediment was well oxygenated (>2 mg l^{-1}). There was no difference in pore-water oxygen concentrations between disturbance regimes at this time (ANOVA, $n=24$ $p=0.78$). On day 52, 21 days after the second disturbance, the pore water was well oxygenated at all depths in the light-exposed flumes subjected to the shallow disturbance, whereas sediments of all other treatments were hypoxic (< 2 mg l^{-1}) below 2 cm depth. Differences between disturbance treatments were just not significant (ANOVA, $n=40$, $p=0.052$).

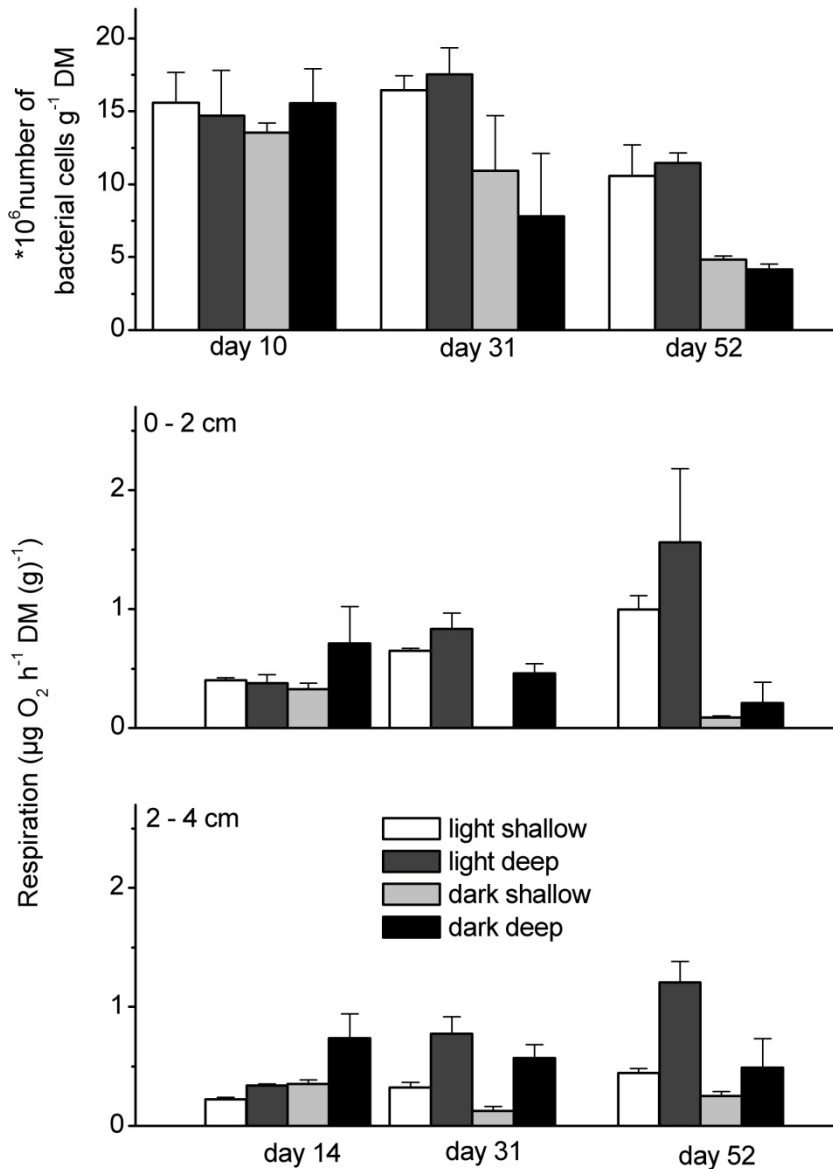


Figure 3.4:

Sediment associated community respiration and bacterial abundance in the early and late successional stage during the experiment. Bacterial abundance and sediment associated community respiration were measured in two vertical depth layers which were aggregated for bacterial abundance due to similarity. First values of bacterial abundance are of day 10 before the first sediment disturbance, first measurements of sediment associated community respiration are of day 14, shortly after the first sediment disturbance.

3.5 Discussion

3.5.1 Effects of sediment disturbance on net community production

Declines in NCP after burial of algal cells as observed here have been noted previously [Power, 1990], and our results reveal that this lack of resistance of NCP to disturbance was independent of sediment mixing depth. This was expected because both shallow and deep mixing deprives algae from light, which in our study penetrated to an average sediment depth of 0.3 cm only. Times of recovery following shallow and deep sediment disturbance should differ, however, if they are determined by algal movement back to the lit surface. Pennate diatoms, the dominant algae in our experimental flumes (unpublished data), can actively move to the surface of sandy sediments [Stevenson, 1996]. Gliding speeds in the range of 60 to 600 $\mu\text{m min}^{-1}$ have been measured [Cohn and Weitzell, 1996; Gupta and Agrawal, 2007; Du et al., 2010]. If one assumes an extreme scenario in which a cell moves upwards from 4 cm depth along a path defined by a sphere packing of idealized sand grains with a diameter of 200 μm , then a motile alga would need between 2 and 18 hours to return to the surface. This rapid recovery predicted on the basis of algal movement speeds contrasts with the recovery times of > 6 days observed in the experiment. Furthermore, recovery times after shallow and deep disturbance events did not differ. This suggests that algae were less affected by disconnecting them from light supply as a result of dislocation to deeper sediment layers. Alternative mechanism such as disruption of the fine structure of the top sediment layer thus are more likely as the main cause of the perturbation.

Although NCP declined in response to sediment disturbance, algal biomass in the upper 1 cm of sediment was unaffected by the shallow disturbance regime. This observation reinforces the above conclusion that the loss of fine structure at the sediment surface is sufficient to reduce algal NCP, independent of changes in biomass. Algae and bacteria associated with sand grains and other surfaces form biofilms by secreting a cohesive matrix of exopolymeric substances (EPS) [Gerbersdorf, 2009]. As a result, they bind fine-grained sediments and stabilize sediment fine structure to the extent even that sediment erosion can be reduced [Gerbersdorf, 2008]. The proximity of algae and bacteria in such a matrix improves the transfer of nutrients to the algae [Lock et al., 1984; Blenkinsopp and Lock, 1994; Arnon et al., 2010]. Therefore, disruption of the

matrix and biofilm architecture by sediment mixing can have large negative effects on algal NCP as observed here.

Recovery of algal communities from disturbance has been found to depend on successional stage and biomass; young communities with low biomass recover rapidly, whereas well-developed communities at advanced stages of community succession take longer [Biggs and Close, 1989; Peterson and Stevenson, 1992; Peterson, 1996]. The response of NCP to disturbance in the present study is in accordance with these findings, since sediment mixing had no consequences for NCP on day 11 but produced a strong effect on day 31 when algal biomass was substantially higher.

The decline of NCP after recovery from disturbance in the advanced successional stage during our experiment, but not after recovery from the first disturbance, was similar to observations made in laboratory flumes where the same pattern was attributed to nutrient limitation [Mulholland *et al.*, 1991]. These results are in accordance with the notion that sustaining well established algal communities requires higher nutrient levels than are needed to build up algal biomass in the first place [Bothwell, 1989]. This might reflect an intrinsically triggered period of biomass loss following one of biomass accrual, which often alternate during algal biofilm dynamics [Biggs, 1996] and might be related to ageing and diffusion limitation of algal growth at the base of thick biofilms [Biggs and Close, 1989].

3.5.2 Effects on community respiration

Unlike NCP, community respiration (CR) showed a clear response to disturbance depth in the advanced successional stage. The observed rise in respiration rate after deep sediment mixing, particularly in the deeper sediment layer, points to an enhanced supply of microbial communities with oxygen, organic carbon and possibly other resources from the surface. The sediment was hypoxic ($< 2\text{mg l}^{-1}$) below a sediment depth of 1 cm at day 31 even in the light-exposed flumes, reflecting faster oxygen consumption than supply from the surface. Clogging of interstitial pores by biofilms may have exacerbated the oxygen deficiency by further constraining vertical water transport [Baveye *et al.*, 1998; Battin and Sengschmitt, 1999]. Our experimental disturbance mixed oxygen rich surface water into the sediment and also broke up any biofilm aggregates that clogged the interstitial pores, thus increasing vertical

connectivity and oxygen supply. In addition, it mixed POM into the sediment, which could have further stimulated heterotrophic microbial activity [Naegeli *et al.*, 1995; Jones *et al.*, 1995; Fischer *et al.*, 2002]. Although strong positive relationships between respiration rate and POM content in sediments are not always evident [Rees *et al.*, 2005], algal-derived organic matter, both biomass and exudates, tends to be readily available to heterotrophic microbes [Romani and Sabater, 1999]. Such high-quality organic matter of algal origin was available in our experimental flumes and possibly important in enhancing CR after disturbance of the deeper sediment layer.

Higher respiration in light-exposed than permanently dark flumes revealed that, independent of any disturbance events, algae increased CR either directly by respiring during oxygen measurements in the dark, or indirectly by providing senescent or dead biomass and possibly exudates as carbon and/or nutrient source for heterotrophic microbes [Cole, 1982; Haack and McFeters, 1982; Romani and Sabater, 2000; Holmes *et al.*, 1998]. Sobzak and Burton 1996] proposed that such positive linkages between bacteria and algae only occur in mature biofilms. However, in line with observations by Carr *et al.* [2005], constant bacterial numbers in the light-exposed flumes during the experiment versus strong declines in the dark flumes, support the idea of a positive algal-bacterial linkage also in biofilms during early stages of community succession.

3.5.3 Effects on the P/R ratio

Gradual shifts of the flumes from predominantly heterotrophic to autotrophic metabolism (i.e. from $P/R < 1$ to $P/R > 1$) as community succession proceeded was reflected in rising algal biomass during the experiment. Sediment disturbance disrupted this trajectory and temporarily reset the flumes to heterotrophy both by stimulating CR (after deep sediment disturbance only) and by decreasing NCP (after both shallow and deep sediment disturbances). This disturbance-induced shift of community metabolism from autotrophy to heterotrophy matches findings from other streams, where high frequencies of sediment disturbance kept open-land streams heterotrophic even when high light availability favoured autotrophy [Uehlinger *et al.*, 2002; Atkinson *et al.*, 2008; Cronin *et al.*, 2007).

3.5.4 Level and variability of metabolic activity in experimental flumes

Overall, metabolic activities in the flumes used in our experiment were low, but still broadly comparable to those of natural streams. Specifically, GPP ($0.25 - 2.5$ with a mean of $1.05 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$) was in the lower range of values compiled by *Battin et al.*, [2008] for numerous natural streams ($0.13 - 48$ with a mean of $6.2 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$, $n=62$; data converted from $\text{g C m}^{-2} \text{ d}^{-1}$ based on a photosynthetic quotient of 1.2) and similar to GPP measured in a sand-bed stream with frequently disturbed sediments ($0.0 - 0.5 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$; [Atkinson et al., 2008]). Likewise, in the advanced successional stage of our experiment, rates of NCP before and after sediment disturbance were similar to those measured before ($60 \text{ mg O}_2 \text{ m}^{-2} \text{ h}^{-1}$) and after ($17 \text{ mg O}_2 \text{ m}^{-2} \text{ h}^{-1}$) a flood in a prairie stream [Dodds et al., 1996]. CR in the flumes ($0.00 - 1.92$ with a mean of $0.63 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$) was also at the lower end of values compiled by *Battin et al.* [2008] (values converted from $\text{g C m}^{-2} \text{ d}^{-1}$ based on a respiratory quotient of 0.85; [Hargrave, 1973]) for natural sand-bed streams ($2.44-68.1$ with a mean of $16.1 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$). These relatively low metabolic activities in our experiment, both autotrophic and heterotrophic, are not surprising in view of the early successional stage of the Chicken Creek Catchment [Gerwin et al., 2010, Gerull et al., 2011], which provided sediment and groundwater for the experimental flumes, and rather low nutrient concentrations in the flume water (Table 2.1). Silicon concentrations were well above the limiting level for diatoms (0.1 mg l^{-1} ; Schelske and Stoermer, 1971), the most abundant algal group in our flumes (unpublished data), and a molar Si:P ratio of 1460 along with an N:P ratio of 110 indicate that phosphorus was the limiting element for algal growth [Brzezinski, 1985, Hillebrand and Sommer, 1999, Klausmeier et al., 2004].

As the ranges given above indicate, there was considerable temporal variability of metabolism independent of the applied sediment disturbance. The observed high initial activities might reflect the sudden mobilization of resources after rewetting of desiccated sediments [Baldwin and Mitchell, 2000; Collins et al., 2008; Romani and Sabater, 1997; Sponseller, 2007; Gerull et al., 2011]. However, overall variability in our flume study over 52 days (50 % for GPP and 68 % for CR) was not unusual for natural streams. For example, GPP varied seasonally between 48 and 90 % and CR between 33 and 68 % in five woodland streams of southern New Zealand [Young and Huryn, 1999]. High day-to-day and storm-related variability in CR and GPP was also

found by *Roberts et al.* [2007] in a first-order woodland stream when metabolism was continuously recorded over two years.

3.5.5 Conclusions and implications

Our flume experiment was designed to assess the response of stream metabolism to a moderate short-term sediment disturbance and, in doing so, to separate the effects of (i) vertical sediment mixing and (ii) disrupting the sediment fine structure at the surface. These goals were achieved as indicated by (i) the responses of NCP and CR to, and rapid recoveries from, the imposed experimental disturbance events, and (ii) a sharp decrease of algal biomass in surface sediments after deep sediment disturbance on day 31. A key finding emerging from this experiment is that sediment scour and fill can reduce NCP for several days even when a disturbance event is limited to 1 cm depth. This delayed recovery of NCP and the concomitant stimulation of CR have implications particularly for sand-bed streams experiencing frequent sediment movement. Typical scour depths reported from such streams are 1 to 2 cm [*Powell et al.*, 2005] and current-induced ripples of sandy sediments also result in mixing of sediments to the same depth [*Baas*, 1999]. If such shallow sediment movement is frequent, metabolism could shift from autotrophy to heterotrophy, even in the absence of a riparian canopy. This points to the importance of even moderate sediment disturbance for ecosystem metabolism in sand-bed streams.

4 Remarkably stable ecosystem metabolism during simulated stream ecosystem succession

4.1 Abstract

Changes in in-stream and riparian vegetation during stream corridor succession come along with different quantity and quality of organic matter available in the stream ecosystem. We hypothesized that during different stages of succession with increasing quality and quantity of litter input, whole-stream metabolism would increase by the fueling effect of the new organic matter source. Fifteen outdoor experimental stream channels were stocked 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 woodland stage with a mix of grass and tree litter (*Betula pendula*), 4) a young woodland stage with tree litter, and 5) a mature woodland stage with a high quantity of tree litter. Whole-stream metabolism was similar in all treatments and unaffected by litter input. There seemed to be a trade-off between a strong positive linkage of bacteria to algae by the use of autochthonous sources in the open-land treatments and a microbial use of allochthonous organic matter in the litter treatments. Hypoxic conditions in the bed, which were less pronounced in the open-land treatments due to algal oxygen production, decreased the contribution of sediment associated respiration to whole-stream metabolism. Litter associated microbial biomass, production and litter decomposition showed no treatment effects, and leaf associated activity in dense leaf packs also seemed to be constrained by oxygen and nutrient availability. Fungal biomass exceeded bacterial biomass associated with leaves, but growth rates of fungi were lower indicating that the main contribution to carbon flux was due to bacteria. Changing quality and increasing quantity of allochthonous litter with stream succession could be driving factors for increasing stream metabolism, if not constrained by oxygen and nutrient availability in leaf packs and hyporheic sediments.

4.2 Introduction

Stream succession in initial landscapes has been observed in glacier retreat areas [Milner and Gloyne-Phillips, 2005], in post mining sites [Mutz et al., 2002], after break

out of volcanoes [Vitousek, 2004], and in arid ecosystems after severe floods [Fisher et al., 1982; Grimm, 1994]. According to these studies initial stream succession follows a sequence of three stages largely characterized by vegetation and availability of allochthonous organic matter. The initial stage of stream succession in open landscape is characterized by the lack of riparian vascular plants, the lack of allochthonous coarse particulate organic matter (CPOM) [Golladay, 1997], and the dominance of benthic biofilms [von Schiller, 2007; Gerull et al., 2011]. Establishment of macrophytes helophytes and grasses in the stream and the riparian corridor leads to the second macrophyte-stage, and introduces allochthonous organic matter additionally fueling stream metabolism [Huryn et al., 2001; Wilcock and Croker, 2004; Menninger and Palmer, 2007; Acuña et al., 2010]. The third and final stage of succession is characterized by riparian trees. They shade the stream, limiting in-stream primary production [Sabater et al., 2000] and deliver leaf litter and dead wood for stream metabolism [Fisher and Likens, 1973; Vannote et al., 1980; Webster and Meyer, 1997]. The increasing quantity and new quality of the organic matter with each of these three successional stages are likely to result in major differences in whole-stream metabolism. In open-land streams high benthic primary production and algal growth can supply heterotrophic organisms in the benthic and hyporheic zone with algal exudates. This is a carbon source of good quality as it is labile and easy accessible for heterotrophic microbes [Jones, 1995, Romani and Sabater, 1999]. With litter input, processing of leaves by fungi, bacteria, and invertebrates contributes significantly to carbon transformation [Hieber and Gessner, 2002; Baldy et al., 2007]. Leaves on the surface or mixed into the sediment is known to fuel heterotrophic activity [Hedin, 1990; Fuss and Smock, 1996; Crenshaw et al., 2002] largely contributing to whole stream respiration [Grimm and Fisher, 1984; Pusch, 1996; Naegeli and Uehlinger, 1997]. Hence, increased respiration rates with increasing amount of allochthonous carbon were assumed.

While poor litter quality, especially that of grasses, is known to slow down decomposition [Ostrofsky, 1997, Tank et al., 2010; Shaftel et al., 2011], the importance of litter quantity for the decomposition processes is poorly understood. Leaf decomposition rates were not different in leave-augmented or depleted stream reaches, independent of the presence of invertebrates [Reice, 1991; Eggert and Wallace, 2003;

Tiegs et al., 2008]. Nevertheless, abundant benthic litter was proposed to promote microbial decomposition as a consequence of the life-cycle of aquatic fungi, the key components of decomposer assemblages on leaf litter in streams [Bärlocher and Kendrick, 1974]. Aquatic hyphomycetes show rapid sporulation following initial establishment and growth on litter [Gessner and Chauvet, 1994]. Accordingly, an increase of dispersed spores has been found following autumnal peak litter input (Bärlocher, 2000) and experimentally enhanced litter retention (Laitung et al. 2002). Considering the positive correlation of spore concentration and fungal colonisation of leaves (Treton et al. 2004), increased colonization and faster decomposition [Suberkropp, 1995] can be expected when quantity of benthic litter is high.

The experiment in this study assessed the effects of litter input of increasing quality and quantity during landscape and stream succession on whole-stream metabolism, microbial litter decomposition, and leaf associated microbes. In experimental flumes we simulated the following five treatments: (1) the biofilm stage without litter, (2) the macrophyte stage with grass litter, (3) the transition between macrophyte and woodland stage with a mix of grass and tree litter, (4) the young woodland stage with tree litter and (5) the mature woodland stage with a higher quantity of tree litter. Comparing various combinations of these treatments we tested the following hypotheses: i) supply of allochthonous CPOM increases the microbial activity in the stream (contrasting treatment 1 with 2, 3, 4, and 5); ii) the intensity of microbial activity increases with succession, with lower activity in open-land treatments (no litter and grass litter) compared to treatments with tree litter input (contrasting treatment 1, 2 with 3, 4 and 5); iii) leaf associated microbial activity will be higher and leaf decomposition will be faster in treatments with tree compared to grass litter (contrasting treatment 2 and 4); and iv) microbial activity increases with CPOM quantity (contrasting treatment 4 and 5).

4.3 Material and methods

4.3.1 Study site and experimental set-up

The experiment was conducted in autumn 2009 in 15 outdoor flumes (0.12 x 0.12 x 4 m) set up in an experimental catchment, referred to as Chicken Creek catchment [Gerwin et al., 2009], which was located about 150 km south-east of Berlin, Germany

(51°36' N, 14°16' E). The flumes were filled with 4 cm of sediment, collected from a dry ephemeral stream reach in the catchment. Ground water from the catchment was supplying the flumes. Nutrient concentrations of the ground water were low, whereas concentrations of dissolved organic carbon were high (Table 4.1), although poorly bioavailable [Gerull *et al.*, 2011]. The ground water was stored in a subterranean tank with a head space. Hence, it was well oxygenated when pumped into the flumes with a peristaltic pump (520-SN, Watson Marlow, Cheltenham, UK) at a constant rate to replace the volume in the flumes every six hours. The water column in the flumes was 1 cm deep and recirculated by pumps at $1.7 \pm 0.3 \text{ cm s}^{-1}$ (Laing DDC, ITT Lowara GmbH, Weinstadt, Germany).

We simulated successional variation of plant litter input by the following treatments (each replicated 3 times): 1) no litter addition, 2) 100 g dry mass (DM) m^{-2} of grass litter, 3) mix of 50 g DM m^{-2} of grass litter and 50 g DM m^{-2} of tree litter, 4) 100 g DM m^{-2} of tree litter and 5) 250 g DM m^{-2} of tree litter. 20% of the litter was buried in the sediment to simulate the natural distribution of CPOM in sand-bed streams [Fuss and Smock, 1996]. Tree litter was Silver birch (*Betula pendula* (L.) Roth) grass litter was Wood small-reed (*Calamagrostis epigejos* (L.) Roth). The leaves were collected from one stand for each species in autumn 2007. For the assessment of leaf associated microbial activity, biomass and production and leaf decomposition, a model leaf species, common Dogwood (*Cornus sanguineus*) was used. Discs (1 cm diameter) of this fast decomposing tree species were exposed in 1 mm mesh bags in all treatments.

Two inocula of microbes and fungi from open-land and woodland streams were added to each flume. To generate the inocula submerged grass and tree leaf litter were collected from three streams (open-land and forested stream) in the Brandenburg region (Germany). 12 g DM of the litter was gently washed to exclude attached fine particulate organic matter, exposed 48 h in 8 l ground water from the Chicken Creek catchment, and aerated for fungal sporulation. The bacterial inoculum was generated by sonification (3 min, 35 W) of 12 g DM in 8 l of ground water from the Chicken Creek Catchment. 500 ml of each suspension were gently poured into the water column of each flume, and flumes were then operated in close-circulation mode for 24 h to allow sedimentation of spores and bacteria [Dang *et al.*, 2005]. Stream-bed sediment, leaf litter and water samples were collected randomly in each flume after 6, 8 and 10 weeks.

Table 4.1: Physico-chemical parameters of the flume water, mean \pm 1SE of 3 sampling dates. Dissolved organic carbon (DOC), DON=NO₂⁻ plus NO₃⁻, dissolved nitrogen (DN), soluble reactive phosphorus (SRP) and dissolved phosphorus (DP).

DOC mg l ⁻¹	NO _x -N μg l ⁻¹	NH ₄ -N μg l ⁻¹	DN mg l ⁻¹	SRP μg l ⁻¹	DP μg l ⁻¹	pH	Conductivity μS cm ⁻¹
9.5 \pm 1.5	47.4 \pm 7.7	12.0 \pm 1.8	0.2 \pm 0.1	2.0 \pm 0.04	3.9 \pm 0.8	7.4 \pm 0.02	752.4 \pm 96.0

4.3.2 Whole stream metabolism measurements

Whole-stream metabolism was assessed while the flumes were sealed with air tight Perspex lids and operated in close circulation mode. Change in oxygen concentration was measured in the recirculating water column. For details of the method see section 3.3.2.

4.3.3 Sediment and litter associated community respiration, total carbohydrates and chlorophyll *a*

To assess community respiration (CR) associated to the sediment, sediment cores (4 cm) were taken with 50-ml syringe barrels with the lower end cut off. Three grams of each sample was preserved in 10 ml of 2 % formalin containing 0.1 % pyrophosphate, and stored at 4°C for later determination of bacterial abundance. CR in the sediment was measured with oxygen optodes (Microx TX3, PreSens GmbH, Regensburg, Germany) in a flow-through laboratory system (for details see section 2.3.3). Measurements were taken within 12 hours after sampling close to *in situ* temperature (10°C). Data was referred to DM (105°C) and organic matter content (ash free dry mass, (AFDM), 4h at 430°C).

Community respiration associated with the leaf litter was measured from six of the exposed leaf discs in closed glass bottles with 50 ml of flume water. The bottles were gently shaken to prevent gradients of oxygen. Oxygen depletion was recorded through an overnight period at constant 10°C with a 10 channel Oxygen optode (Oxy 10 mini, PreSens GmbH, Regensburg, Germany). At the end of the measurement, the leaves were freeze-dried to determine dry mass (DM).

For carbohydrate analysis subsamples were taken from the sediment used for the measurement of the hyporheic respiration and frozen in liquid nitrogen within 8 hours after sampling and stored at -20°C . Carbohydrate content was later estimated in thawed samples by the phenol-sulphuric acid method (see section 2.3.3).

Chlorophyll *a* served as a proxy for algal biomass. Samples were taken from the top 1 cm of sediment with a syringe with a cut off end covering 1.57 cm^2 . Chlorophyll *a* was extracted following the method described in section 2.3.3.

4.3.4 Bacterial Secondary Production and Bacterial Abundance

Bacterial production was estimated as leucine incorporation into bacterial protein [Kirchman *et al.*, 1985]. A specifically adapted protocol involving ultrasonication and NaOH extraction ensured high protein extraction efficiencies and a good signal-to-noise ratio (generally 15:1 for litter and only 3:1 for sediment due to very low production rates) [Buesing and Gessner, 2003]. Basic assumptions underlying the method, such as linearity of leucine incorporation, substrate saturation, and isotope dilution were analogue to other studies [Buesing and Gessner, 2003, 2006]. We followed the protocol of Buesing and Gessner [2005]. We used [^3H]leucine with a specific activity of 56mCi mol^{-1} for the first sampling and leucine with a specific activity of 160mCi mol^{-1} for the second and third sampling. Leave discs and sediment were collected in the field and subsamples were put into 20 ml econo glass vials for measuring incorporation rates of leucine into bacterial production, control samples where microbes had been killed as well as subsamples for determining the dry mass of the material. 2.95 ml filtered and autoclaved stream water was added to the samples. Control samples were then treated with trichloroacetic acid (TCA) to a final concentration of 5%. 50 μl of leucine solution as a mixture of radioactive and non-radioactive leucine were added to each sample at timed intervals for a final concentration of 4 μmol leucine and incubated for 30 min at in situ temperature (final specific activity sampling 1: 46.3GBq mmol^{-1} ; sampling 2 and 3: for leaves 6.1GBq mmol^{-1} , for sediment 43.2GBq mmol^{-1}). To stop leucine incorporation, TCA was added to a final concentration of 5% to each sample at timed intervals. Purification started with cooling on ice and sonification of 1 min (Labsonic 2000U, B.Braun Melsungen AG, Germany). After another 15 min of cooling, samples were transferred to a filter manifold equipped with a $0.2\text{ }\mu\text{m}$ polycarbonate filter

(GTTP[®], ISOPORE, Millipore, Ireland) backed by a cellulose filter (HA, MF[®], Millipore, Ireland). Several washing steps followed, first with TCA, then with non-radio-active 40 mM leucine solution, after that with 1 ml of ethanol and at last with 1 ml nanopure water. After that filter and litter or sediment were transferred to 2 ml screw cap microcentrifuge tubes and extraction started by adding 1 ml of an alkaline extraction solution (0.5M NaOH, 25mM EDTA, 0.1% SDS). Samples were extracted over 60 min in a 95°C dry block heater to dissolve proteins and afterwards cooled down to ambient temperature. Samples were centrifuged at 14000 g at 4°C for 10 min and 100 µl of the supernatant were mixed with 10 ml of scintillation fluid (Hionic Fluor; Packard). Radioactive decays were measured in a liquid scintillation analyzer (TRI-CARB 2100TR, Canberra-Packard, Schwadorf, Austria). DM and AFDM were determined from replicate samples. Bacterial carbon production was calculated from rates of incorporation of leucine into protein by multiplying rates of leucine incorporation by the formula weight of leucine (131.2) and the ratio of cellular carbon to protein (0.86) and dividing by the fraction of leucine in protein (0.073) [Buesing and Gessner, 2004].

Bacterial abundance was measured by flow cytometry as described in section 3.3.3. For leaf litter samples, the actual output of the sonifier probe was 20 W, for sediment it was 40 W, for an optimum detachment of bacterial cells on this type of substrate (unpublished tests). The conversion factor used to calculate bacterial carbon biomass was 20 fg C cell⁻¹ [Norland, 1993]. Growth rates were calculated as P/B ratios, where P is the bacterial production rate and B is the bacterial biomass.

Bacterial growth efficiency (BGE) was calculated by the ratio of bacterial production (BP) and the sum of BP and bacterial respiration (BR) [del Gioglio and Cole, 1998]. For sediments, measured microbial respiration was assumed to be largely due to bacteria although included algal respiration can lead to an error, which was assumed to be small. For respiration measured on leaves, fungal and bacterial respiration could not be separated, but calculating BGE with total microbial respiration would rather underestimate BGE, which has to be considered. For leaves microbial growth efficiency (MGE) was calculated by the sum of fungal and bacterial production divided by microbial respiration.

4.3.5 Fungal secondary production and fungal biomass

Fungal growth rates and production were measured as acetate incorporation into ergosterol, following the method of *Subberkropp and Gessner* [2005]. Briefly, samples of 0.5 ml sediment and three leaf discs were filled into glass vials and 1.95 ml of filtered stream water were added. 50 μ l of radiolabelled acetate solution were added at timed intervals and samples were incubated on a shaker at in situ temperature (10°C) for 5 hours. For each sampling, control samples with 200 μ l of 37% formalin, which was added before the acetate solution, were also incubated. Incorporation of acetate was stopped by adding 200 μ l of 37% formalin into all samples. Thereafter samples were filtrated on GF/F filter and rinsed with nanopure water. Filters were put into plastic vials and stores at -20°C until determination of ergosterol [*Gessner et al.*, 2005]. Extract of ergosterol with scintillation cocktail was measured in a scintillation counter.

Fungal biomass was determined by extracting and quantifying ergosterol [*Gessner and 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 and Schmitt*, 1996]). The extraction efficiency was routinely monitored with external ergosterol standards. The extracts eluting from the SPE cartridges were purified and ergosterol 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 \times 25 cm; Merck Inc., Darmstadt, Germany), and a Jasco MD 2010 Plus multiwavelength detector set at 282 nm. The column temperature was 33 °C. The conversion factor used to calculate the biomass was 5.5 mg of ergosterol g⁻¹ fungal biomass [*Gessner and Chauvet*, 1993]. Fungal carbon content was assumed to be 43 % of dry mass [*Baldy and Gessner*, 1997]. Growth rates were calculated as P/B ratios, as for bacteria.

4.3.6 Fungal sporulation, mass loss and leaf toughness

Sporulation of aquatic hyphomycetes was induced by submerging six leaf discs in 60 ml filtered (5 μ m membrane filters, Sartorius, Goettingen, Germany) Chicken Creek water kept at 10 °C with constant shaking [*Gessner et al.*, 2003]. Two ml of 37 % formalin was added after 48 h to preserve the samples. 1-3 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 \times . Sporulation rates were converted to conidial production based on conidial dry mass determined for individual species (*Bärlocher and Schweizer, 1983; Gessner and Chauvet, 1994; Chauvet and Suberkropp, 1998*).

Leaf mass loss was determined as difference of DM (at 105 °C) before and after the exposition. A penetrometer was constructed according to the design of *Graça and Zimmer [2005]*. The device consisted of a 3.2 mm diameter plunger that pierced a leaf surface when adequate pressure was applied. Water was trickled into a beaker pressing on the plunger until it penetrated the sample at so defined weight.

4.3.7 Physico-chemical parameters

Water from the water column was collected in each flume, filtered through prewashed 0.45 μm pore size cellulose acetate filters, frozen and later analyzed for nutrients (soluble reactive phosphorus=SRP, dissolved oxidized nitrogen=DON= NO_2^- plus NO_3^- , NH_4^+) according to standard procedures.

Depth profiles of oxygen in the sediments were measured three times at three sites in each flume following the method described in section 3.3.4.

Water samples for methane analyses were taken with PEWA-injection solution bottles (VWR International AG, Dietikon, Switzerland) below the water surface. Five to six pellets of potassium hydroxide (KOH) were added to stop biological activity. The bottles were closed with butyl-elastomer stoppers (Maagtechnik, Dübendorf, Switzerland), ensuring no air bubbles were trapped in the sampling bottle. The samples were returned to the laboratory and stored at 4 °C until CH_4 concentrations were analyzed by gas chromatography. Water samples for CH_4 analyses were prepared by creating a 25-ml headspace in the sample bottles with N_2 . The bottles were treated in an ultrasonic bath for 15 min to equilibrate gas concentrations between the headspace and water before withdrawing a gas sample from the headspace for CH_4 quantification on an Agilent 6890N gas chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA).

The chromatograph was equipped with a JasValve auto-sampler (Joint Analytical Systems Benelux BV, Eindhoven, The Netherlands; 500 μl sample loop), a Carboxen 1010 Plot column (length: 30 m, inner diameter: 0.53 mm; Supelco, Bellefonte, PA, USA), and a flame ionization detector. To analyze CH_4 stripped from the water samples, temperature was kept constant at 40 $^{\circ}\text{C}$ for 5 min and then raised to 120 $^{\circ}\text{C}$ at a rate of 10 $^{\circ}\text{C min}^{-1}$. For gas samples from the funnel traps and the atmospheric samples, the temperature was kept constant at 100 $^{\circ}\text{C}$ for 4 min and then raised to 130 $^{\circ}\text{C}$ at a rate of 10 $^{\circ}\text{C min}^{-1}$. Hydrogen gas (H_2 5.0, PanGas, Dagmersellen, Switzerland) was used as carrier gas at a flow rate of 9 ml min^{-1} . The CH_4 retention time was 7.5 min for the method used for water samples, and 4.4 min for the method used for gas samples from the funnel traps. The instrument was calibrated with standard CH_4 at concentrations ranging from 100 to 10,100 ppm.

4.3.8 Data analysis

NCP and CR are expressed per square meter and were standardized to 10 $^{\circ}\text{C}$ based on the assumption that relationships between temperature and metabolic rate were exponential according to a Q_{10} of 2 [Davidson and Janssens, 2006]. Transformation from oxygen to carbon was performed by using a respiratory quotient of 0.85 [Hargrave, 1973]. Leave surface area was calculated on the basis of 2 cm^2 per leaf disc multiplied with the factor 2 for both sides of the leaf. Sediment grain surfaces were calculated following the calculation of Leichtfried [1985]. All values in the text are reported as means \pm 1SE. 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 during the sampling dates. Treatment and time were treated as fixed effects with the time variable centred on sampling date 3 so that estimated intercepts represent the situation at the end of the experiment when the biggest effects were expected. If the time effect was significant, the analysis was additionally repeated only for the last sampling after 10 weeks to see if treatments effect only appeared after a longer time of exposition. A random intercept effect was included for replicates to account for repeated measures on the same flumes. QQ-plots and frequency histograms indicated that residuals did not meet assumptions required for parametric tests. Thus variables (x) were transformed

according to $\ln(x+c)$, where $c = q_{0.25} x^2 / q_{0.75} x$ and q stands for quantile [Seminar for Statistics, ETH Zurich, pers. comm.]. Contrast tests were performed to distinguish differences between the five treatments. We applied contrasts related to our hypotheses, comparing treatments 1 vs. 2/3/4/5, 1/2 vs. 3/4/5, 2 vs. 4, and 4 vs. 5. To test for differences between sediment and leaves, univariate ANOVA was used on observations from the last sampling point after 10 weeks of exposition, with treatment and substrate as fixed factors.

4.4 Results

4.4.1 Whole-stream metabolism

Whole stream community respiration (CR) did not vary between treatments or in time with a mean respiration rate of $6.8 \pm 0.64 \text{ mg C m}^{-2} \text{ h}^{-1}$ (Table 4.2, panel a in Figure 4.1). Net community production (NCP) ranged from -11.4 to $7.7 \text{ mg C m}^{-2} \text{ h}^{-1}$ with a mean of $-1.76 \pm 1.3 \text{ mg C m}^{-2} \text{ h}^{-1}$. However, it was variable between treatments and time and the tested contrast did not show a pattern regarding simulated successional stages (Table 4.2, panel c in Figure 4.1). Heterotrophic processes constantly dominated as shown by $P/R < 1$. When referring community respiration to carbon standing stock in the flumes, the treatment without litter showed higher respiration rates compared to all other treatments (Table 4.2, panel b in Figure 4.1).

Table 4.2: Results of calculated contrasts according to the hypotheses of the experiment, p-values. Net community production (NCP), community respiration (CR), organic matter standing stock (OM), bacterial secondary production (BSP), bacterial growth efficiency (BGE), microbial growth efficiency (MGE), bold numbers mark significant differences according to the applied contrasts

Hypotheses/ contrasts		no litter vs. litter	No and grass litter vs. mix and tree litter	grass vs. leaf litter	low vs. high tree litter quantity
Parameter	n	1 vs. 2/3/4/5	1/2 vs. 3/4/5	2 vs. 4	4 vs. 5
Whole System					
NCP	45	0.906	0.471	0.719	0.399
CR	45	0.505	0.526	0.683	0.505
CR referring to OM					
stand. stock	45	0.017	0.256	0.825	0.825
Methane_s3	15	0.007	0.007	0.391	0.798
Oxygen 0.5cm	15	0.068	0.047	0.711	0.103
Oxygen 1 cm	15	0.201	0.257	0.737	0.523
Oxygen 2 cm	15	1.000	0.832	1.000	1.000
Oxygen 3 cm	15	1.000	0.921	1.000	1.000
Sediment					
Chl a	45	0.001	<0.001	0.019	0.439
Carbohydrates	45	0.005	0.005	0.586	0.586
Organic matter	45	0.820	0.421	0.820	0.820
Bacterial biomass	45	0.737	0.218	0.820	0.820
Respiration_s3	15	0.212	0.041	0.337	0.017
BSP	45	0.011	0.001	0.050	0.893
BGE_s3	45	0.040	0.006	0.156	0.156
Leaves					
Leaf toughness	45	0.586	0.746	1.000	1.000
Mass loss	45	0.392	1.000	0.131	0.483
Bacterial biomass	45	0.351	0.137	0.354	0.527
Respiration	45	0.926	1.000	0.859	0.926
BSP	45	0.432	1.000	0.432	0.437
Fungal biomass	45	0.725	0.364	0.317	0.651
Fungal production	45	1.000	0.817	0.739	1.000
Sporulation rate	15	0.815	0.347	0.815	0.638
MGE	45	1.000	1.000	1.000	1.000

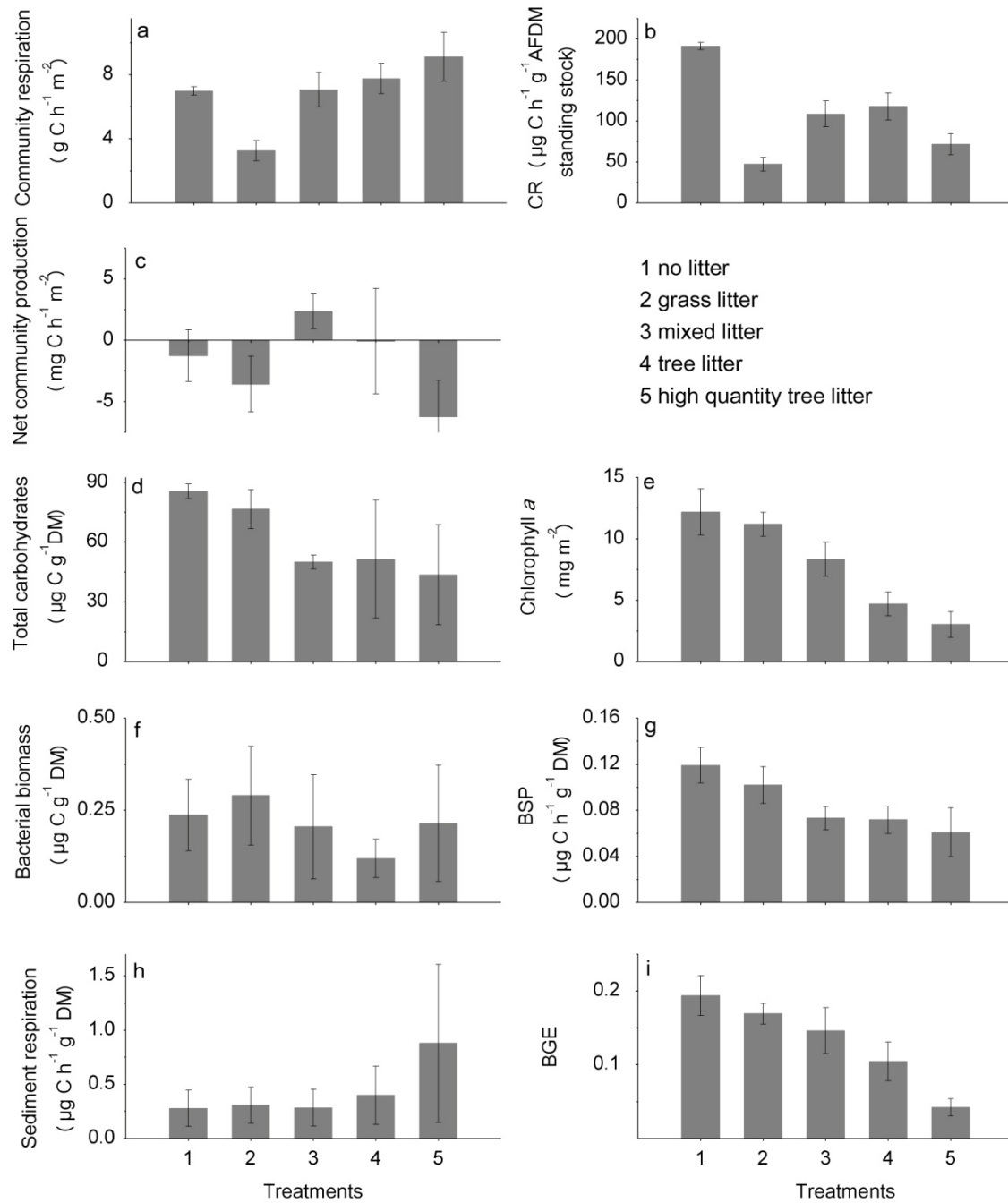


Figure 4.1: Whole stream metabolism and sediment associated parameters means $\pm 1\text{SE}$ of 3 sampling dates ($n=12$). Sediment associated respiration and bacterial growth efficiency (BGE) show data only of sampling date 3, means $\pm 1\text{SE}$ ($n=4$) after 10 weeks of the experiment

4.4.2 Sediment associated parameter

Neglecting the buried litter, the quantity of organic matter in the sediments was $0.09 \pm 0.003\%$, did not vary between treatments and in time. Whereas organic matter quality, indicated by the amount of total carbohydrates, was higher in the treatment without litter compared to all other treatments and higher in the two open-land treatments (no litter and grass litter) compared to the treatments with tree litter addition (panel d in Figure 4.1, Table 4.2).

Bacterial biomass in the sediment varied among treatments as well as in time (Table 4.2, panel e in Figure 4.1) still showing a trend to higher bacterial biomass in the open-land treatments (no litter and grass litter) compared to the leaf litter treatments. Benthic chlorophyll *a* and bacterial secondary production showed a similar but significant pattern (Table 4.2), with higher values in the treatment without litter compared to all other treatments. Furthermore values were higher in the two open-land compared to the all treatments containing tree litter and in the grass compared to the tree litter treatment (panels e, g in Figure 4.1).

Microbial respiration associated with sediments increased in time and was similar for the treatments when integrating over all sampling dates. However, after 10 weeks, sediment associated respiration was higher in the tree litter treatments compared to the open-land treatments without any litter or just grass-litter. Hence the pattern of sediment respiration was reverse to the other sediment parameters (panel h in Figure 4.1, Table 4.2). Bacterial growth efficiency in the sediment showed a dynamic pattern, but as the sediment associated respiration it showed treatment differences after 10 weeks similar to chlorophyll *a* and bacterial secondary production, with a higher bacterial growth efficiency in the open-land treatments (0.18 ± 0.01) compared to the tree litter treatments (0.1 ± 0.02) (panel i in Figure 4.1, Table 4.2).

Oxygen concentration in the sediments showed a decrease in depth and time in all treatments (Figure 4.2) and after 10 weeks just the upper 1 cm and in treatment 1 to 4 and only the upper 0.5 cm in treatment 5 was well oxygenated ($>2 \text{ mg l}^{-1}$). Comparing this sediment layer (0.5 cm, week 10) oxygen was higher in the open-land treatments than in the tree litter treatments (Table 4.2). Methane concentration of the water showed the reverse pattern, with less methane in the open-land compared to the tree litter treatments (Table 4.2).

4.4.3 Leaf associated parameter

None of the leaf associated parameters showed any differences between the treatments (Table 4.2). There was just a trend in time with leaf toughness of *Cornus sanguinea* showing lower toughness in treatments 4 and 5 compared to treatments 1, 2 and 3. However, decomposition measured as mass loss was similar among all treatments with a remaining weight of 48 ± 2 % after 10 weeks (Table 4.2).

Fungal biomass accounted for 94 % of total carbon biomass on *Cornus* (panel b in Figure 4.4). Referring to the surface area, bacterial biomass was 400 times higher on the tree leaf litter than on sediment surfaces (ANOVA, $n = 30$, $p < 0.001$, panel a in Figure 4.3).

Bacterial production rates were stable in time at $1123.6 \pm 91.2 \mu\text{g C h}^{-1} \text{g}^{-1} \text{AFDM}$, whereas fungal production rates declined from $24.4 \pm 3.3 \mu\text{g C h}^{-1} \text{g}^{-1} \text{AFDM}$ measured in week 6 to $5.4 \pm 2.7 \mu\text{g C h}^{-1} \text{g}^{-1} \text{AFDM}$ in week 8 and $1.7 \pm 0.2 \mu\text{g C h}^{-1} \text{g}^{-1} \text{AFDM}$ in week 10 (panel a in Figure 4.4). Low production rates and high biomass of fungi resulted in growth rates (expressed as P/B) of $0.003 \pm 0.001 \text{ h}^{-1}$ in week 6, which decreased thereafter to $0.0001 \pm 6 \cdot 10^{-5} \text{ h}^{-1}$. Bacterial growth rates of $0.3 \pm 0.03 \text{ h}^{-1}$ were 100 times higher. Bacterial production per surface area was higher on leaves compared to sediment (ANOVA, $n=30$, $p<0.001$) but again due to the higher bacterial abundance on leaves, P/B ratios were about 10 times lower on leaves than on sediment grain surfaces (panel c, e in Figure 4.3). Sporulation rates of fungi did not differ between the treatments (Table 4.2). Data for week 6 was not available, however, with $0.21 \pm 0.04 \text{ ng spores h}^{-1} \text{mg}^{-1} \text{DM}$ in week 8 and $0.04 \pm 0.01 \text{ ng spores h}^{-1} \text{mg}^{-1} \text{DM}$ in week 10, sporulation declined similar to fungal growth rates.

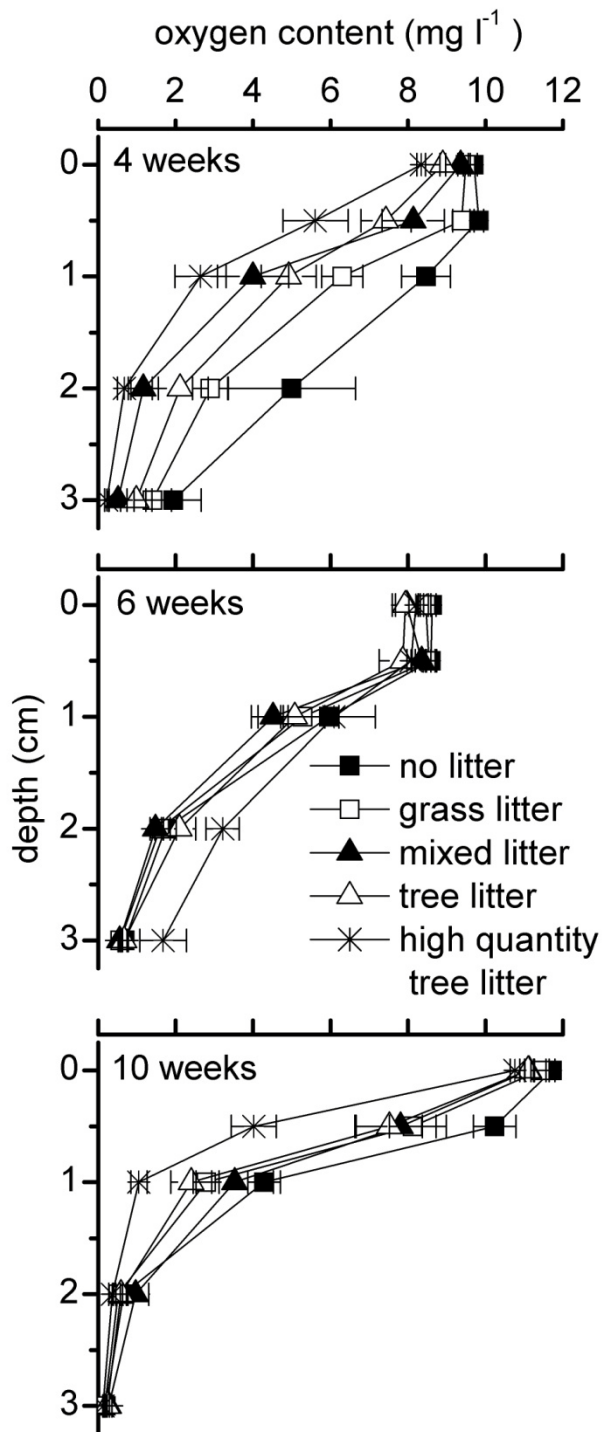


Figure 4.2: Comparison of the oxygen profiles in the sediment of the five treatments after 4 ($n=6$), 6 ($n=3$) and 10 ($n=9$) weeks of the experiment.

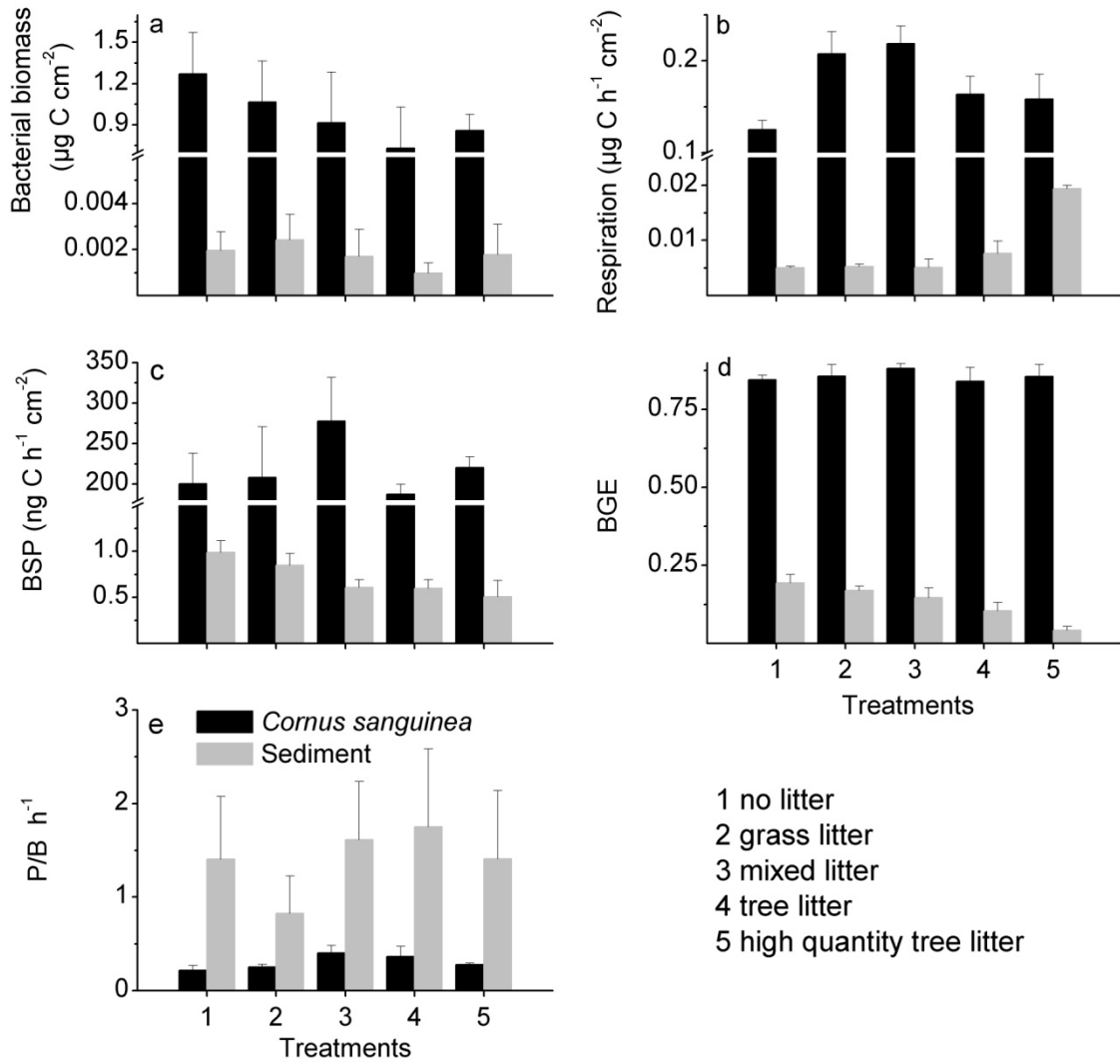


Figure 4.3: Bacterial biomass, respiration, production (BSP), growth efficiency and growth rates expressed as P/B ratio on an areal basis on leaf and sediment grains surfaces. Data shows means + 1SE of 3 sampling dates ($n=12$). Bacterial growth efficiency BGE and respiration panels only show data of the last sampling after 10 weeks ($n=4$).

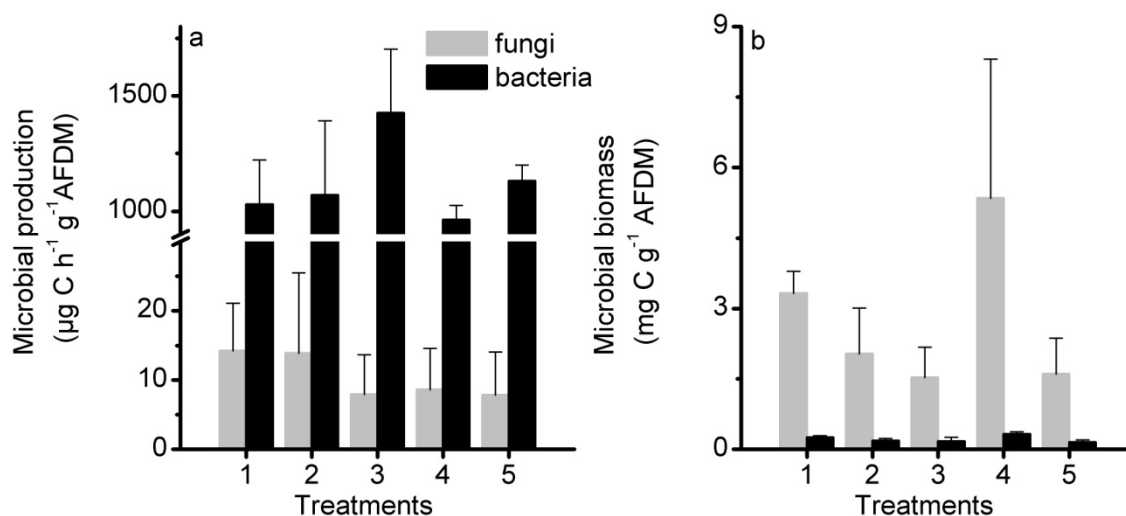


Figure 4.4:

Comparison of secondary production and biomass of fungi and bacteria associated to leaves of *Cornus sanguinea*. Data shows means+1SE ($n=12$) of 3 sampling dates.

Respiration per leaf surface area of *Cornus* was 80 times higher than on sediment surfaces ($n = 30$, $p < 0.001$, panel b in Figure 4.3) and hence differed less between the two substrates than bacterial biomass and production. Microbial growth efficiency for the total bacterial and fungal community associated to leaves showed no differences between the treatments at 0.8 ± 0.008 (Table 4.2). Bacterial growth efficiency was higher on leaf surfaces compared to sediment grain surfaces (panel d in Figure 4.3).

4.5 Discussion

4.5.1 Curb of whole system response by oxygen and nutrients

Remarkably, the treatments with variable quantity and quality of CPOM had similar whole stream metabolism. The leaves, which showed substantial respiration potential when measured for *Cornus* in the laboratory, did obviously not contribute this activity to whole stream respiration in the flumes. Even if we assume a lower respiratory activity associated to *Betula* than to *Cornus*, still an increase in whole-stream respiration would have been expected due to litter input. Microbial communities associated to litter depend on stream water as source of oxygen and nutrients [Suberkropp and Chauvet, 1995]. Hence, there might have been an over estimation of potential leaf respiration

measured in the well mixed laboratory microcosms where availability of nutrients from the water was better than for leaves densely packed on the stream bed. In consequence, microbial communities associated with leaves in the flumes were not able to fully utilize their respiratory potential to contribute to whole-stream respiration. In an inter-biome comparison, *Mulholland et al.* [2001] found phosphorus and the size of the hyporheic zone to control stream respiration, which was unrelated to the quantity of CPOM (leaves and wood) and fine particulate organic matter.

Whole stream respiration is often dominated by the respiration of the hyporheic sediments [*Grimm and Fisher*, 1984; *Pusch*, 1996; *Naegeli and Uehlinger*, 1997; *Marxsen*, 2006] and hyporheic particulate organic matter like buried leaves fuels the sediment activity [*Hedin*, 1990; *Jones et al.*, 1995; *Metzler and Smock*, 1990]. The sediment respiration measured in week 10 mirrored the litter input supporting the fuelling effect of litter on sediment associated respiration. However, this response was measured in sediment columns in the laboratory which were well percolated by oxygenated water. Furthermore, the sediment fine structure was disturbed by the sampling method which can additionally stimulate respiratory activity in sediments [see chapter 2]. In the flumes, the oxygen supply by the surface-subsurface water exchange was obviously insufficient to make the full metabolic potential of the microbes and the organic matter in the sediments accessible. This was corroborated by the hypoxia in the deeper sediment layers and the methanogenesis [*Baker et al.*, 2000]. Hypoxia in the sediments is result of the activity of microbial communities but at the same time reduces their potential to contribute to whole-stream respiration [*Dahm*, 1991]. The correlation of leaf litter input with oxygen deficiency and methanogenesis was also found in the hyporheic zone of a first-order Appalachian stream [*Crenshaw et al.*, 2002]. Hypoxia is a common phenomenon in small grained bed sediments of lowland streams with poor morphological diversity of the stream bed as in the experimental flumes [*Wood and Armitage*, 1997, *Malcolm et al.*, 2010].

In the open-land treatments, the hypoxia was probably alleviated by the oxygen production of algae in the upper sediments, as could be seen in the greater depth of oxygenation after 10 weeks. Although sediment respiration rates measured in the laboratory indicated a higher activity in the tree litter treatments, extrapolation to the flumes regarding the size of the oxygenated hyporheic zone revealed a similar

contribution to whole-stream respiration for all treatments. This corroborates the compensatory effect of algae in the open-land compared to the tree litter treatments also seen in the bacterial secondary production. Bacterial production correlated with chlorophyll *a*, with higher rates in the open land treatments compared to the treatments with tree litter input. The fuelling of bacteria with labile organic matter (see below) and the oxygenation of hyporheic sediments by primary production were probably the main factors compensating the lack of allochthonous CPOM in the open-land treatments.

Similar to community respiration, net community production did not show any response to the litter input. Regarding the sediment associated biomass of algae measured as chlorophyll *a*, higher primary production could be expected in the open-land treatments. Input of litter covered the stream bed with subsequent shading effects for the sediment surface. This effect resulted in lower algal biomass at the sediment surface but had no effect on NCP. Litter surfaces were obviously also colonized by algae [Neely, 1994; Neely and Wetzel, 1997] compensating the shading effects on the sediment surface. Over time, leaves in the flumes were moved in the flumes and probably turned upside down, which caused changes in light availability on their surfaces and might have been the reason for the great variability of NCP in time.

4.5.2 Autochthonous versus allochthonous fueling of sediments

Interestingly, the major response to litter input was seen in sediment associated parameters, which showed opposite values to increasing litter quality and quantity. Increasing the amount of litter limited algal growth by shading effects. In light exposed biofilms, algal-derived organic matter, both biomass and exudates, tends to be readily available to heterotrophic microbes [Jones *et al.*, 1995; Romani and Sabater, 1999], fuelling bacterial activity and growth in a tight coupling [Ylla *et al.*, 2009]. This linkage was shown by the identical pattern of chlorophyll *a*, carbohydrate content, bacterial biomass and production in the treatments.

Sediment respiration on the contrary showed the opposite pattern indicating the use of allochthonous carbon from litter input by bacteria. The resulting bacterial growth efficiency was higher in open-land compared to the tree litter treatments indicating better bacterial growth conditions in the open-land treatments. Although there is no consensus on the regulation of bacterial growth efficiency in aquatic systems, the

availability and quality of organic carbon have been suggested as controlling factors [delGiorgio and Cole, 1998], pointing at a better quality of carbon sources in the open-land treatments. As *Kritzberg et al.* [2005] showed for lentic systems, bacteria prefer the use of autochthonous compared to allochthonous carbon and show higher growth efficiency on autochthonous carbon. That may explain why bacterial production in the benthic sediments was coupled to algal derived carbon sources in the open-land treatments. However, high respiratory activity was found to be positively correlated with the quantity of allochthonous carbon [McCallister and delGiorgio, 2008], which matches the higher respiration rates assessed in the tree litter treatments.

4.5.3 Substrate quality of sediments and leaf litter and the role of fungi for stream succession

Comparing leaf and sediment grain surfaces regarding their substrate quality for colonizing microbial communities, leaves are clearly the substrate with higher energy resources as seen by the 300 to 400 times higher bacterial biomass and production associated to leaf compared to sediment grain surfaces. Nevertheless, bacterial growth rates on sediment grains exceeded those on leaves, pointing again at the good accessibility of labile organic matter produced by algae in biofilms associated to sediments. This supports the findings of *Fazi et al.*, [2005], who found surface sediment to be a better substrate for bacteria than leaves, if algal presence is dominant. However, bacterial growth efficiency associated to sediments was low (0.1) compared to the range observed for freshwater sediments, from 0.1 to 0.4 [Bell and Ahlgren, 1987; Törnblom, 1996; Goedkoop et al., 1997; Bastviken et al., 2003]. This low growth efficiency is consistent with the proposed limitation of oxygen and nutrients curbing bacterial activity and growth in our experimental flume system, especially in sediments.

Litter input during stream ecosystem succession not only delivers additional carbon and nutrient sources to the stream [Webster and Meyer, 1997], it also is a new type of substrate suitable for fungi which rapidly establishes as a microbial key decomposer on submerged litter [Bärlocher and Kendrick, 1974]. Consequently, we expected an increase in energy transfer along with litter input mediated by this microbial group. The fungal dominance of more than 90 % of the microbial biomass in all treatments was consistent with that expectation and the findings of other studies

[*Findlay et al.*, 2002; *Newell et al.*, 1989; *Kominkova et al.*, 2000]. But in contrast to many studies on litter break down in streams [*Gulis and Suberkropp*, 2003; *Baldy et al.*, 2002; *Pascoal and Cassio*, 2004], bacteria dominated over fungi in productivity in our experiment. It must be taken into account that we sampled in the late phase of leaf processing after at least 6 weeks. Fungi are known to be fast colonizers with highest productivity within 2 to 8 weeks after colonization [*Gessner and Chauvet*, 1994], so their productivity may have had already declined at that point of experiment. Nevertheless, bacteria seemed to be the major mediators for carbon flow associated to leaves [*Findlay and Arsuffi*, 1989; *Baldy and Gessner*, 1997; *Buesing and Gessner*, 2006], indicating that fungi did not play the expected role in the successional change of carbon metabolism with increasing litter input. Invertebrates, also substantially involved in litter decomposition, might have changed the results but were not present in this experiment.

4.5.4 Conclusion

Besides increasing whole-stream metabolism by additional leaf-associated microbial activity, a fuelling effect of the hyporheic zone was expected consequence of litter input. However, there seems to be a trade-off between autochthonous carbon produced by algae being a main and high quality carbon source in open-land streams and allochthonous carbon from litter fuelling sediment respiration in woodland streams. As observed in this experiment, the contribution of litter input to whole-stream metabolism has not to be extensive. The availability of oxygen and nutrients controlling microbial activity can constrain the activity in dense leave packs. Similar the full potential of sediment respiration potentially fuelled by allochthonous carbon can be constrained by low oxygen resupply at least in sandy sediments. These sediments offer large surfaces for microbes but the vertical water exchange and resupply of oxygen and nutrients from the surface stream is poor, often leading to depletion of oxygen with sediment depth [*Findlay*, 1995; *Brunke and Gonser*, 1997; *Baker et al.*, 2000].

We conclude that changing quality and increasing quantity of allochthonous litter with stream ecosystem succession can be driving factor for increasing stream metabolism, but there are several constraints, which can limit the fuelling effect, mainly oxygen and nutrient availability. It must be considered that with landscape and stream succession

not only the quality and quantity of allochthonous carbon change, but also the sediment structure. With ecosystem succession and increasing vegetation cover in the watershed, less fine sediments enter the streams and grain size-sorting and transport processes lead to a trend to coarser sediments [*Smith and Ferguson, 1995*]. This can favour an increase of vertical water exchange [*Packman and Salehin, 2003*] additionally enhanced by in-stream wood [*Mutz et al., 2007*]. With high vertical water flux, buried litter was already found to have no effect on the oxygen concentration in the hyporheic zone [*Boulton and Foster, 1998*]. Thus, further research on the consequences of litter input during landscape and stream succession should also consider the change in sediments and vertical water flux not assessed in this study.

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

5.1 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.

5.2 Introduction

Various investigations in early successional ecosystems of newly formed landscapes such as volcanic lava fields (Vitousek et al., 2004), glacier forefields (Milner and 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 vegetation succession and in particular by the quality and quantity of allochthonous coarse particulate organic matter (CPOM). Open-land streams receive litter from in-stream macrophytes and grasses establishing in the riparian zone [Acuña et al., 2010; Wilcock and Croker, 2004, Mackay et al., 1992; Huryn et al., 2001; Menninger and Palmer, 2007]. With emerging woody riparian vegetation, tree litter and woody debris are additionally delivered to the stream [Fisher and Likens, 1973; Vannote et al., 1980; Webster and Meyer, 1997]. The dominance of either grass or tree litter is likely to change the environmental conditions within the stream, and affect activity and structure of leaf associated microbial communities.

There is a contradiction of two opposing theories regarding microbial activity and diversity associated with leaves during decomposition in aquatic environments. Some studies state the importance of leaf quality for determining the activity and structure of the bacterial [McArthur, 1985 Mille-Lindblom et al., 2006] and fungal [Gulis, 2001] communities. Others suggest that environmental conditions as e.g. water chemistry and stream morphology prevail over variability in leaf quality [Marks et al., 2009, Harrop et al., 2009]. However, in those studies, several environmental parameters differed between the sites where litter was exposed, obscuring the main cause for those results.

The effect of the shift in input and standing stock of litter during stream ecosystem succession, with an initial dominance of grass litter and a transition to dominance of tree litter, is unclear. It is probable, that quality and quantity of background litter standing stock in a stream is one major factor of influence for leaf associated microbial activity and community structure. Thus, following one theory, background litter standing stock could be of no relevance if leaves of different quality are exposed. The contrasting result could also prove to be right showing a stronger effect of background litter on microbial activity and community structure associated with leaves of different quality.

Different decomposition rates have been shown for tree leaf species differing in nutrient contents (nitrogen, phosphorus, carbon) and lignin and phenolic compounds (e.g.

tannins) [Gessner and Chauvet, 1994; Ostrofsky, 1997; Ardón *et al.*, 2009; Tank *et al.*, 2010]. However, there is no clear consensus about the differences in decomposition rates between grass and tree leaves. Decomposition of tree leaves has been far more studied [Webster and Benfield, 1986; Ostrofsky, 1997] than that of grass leaves [Scarsbrook and Townsend, 1994; Menninger and Palmer, 2007, Shaftel *et al.*, 2011]. Vendrameni *et al.* [2000] documented relationships between leaf nutrient content and leaf traits of different plant functional types. They found the group of deciduous woody plants to be high in N and P and with lowest C:N and C:P ratios resulting in fast decomposition rates whereas graminoids showed the opposite characteristics. However, monocotyledon plants are not generally of low quality as shown in a comparative study of Griffith *et al.* [2009], who observed faster decomposition rates and higher microbial activity associated with maize than with maple leaves. Differences in leaf quality have been shown to influence leaf associated microbial community composition in both, fungal and bacterial communities. The distinct composition differences between tree and grass litter point to the specific ability to colonize these different substrates [Gulis, 2001] and use grass or tree litter leachates [Mc Arthur, 1985]. Enzymatic activities of microbial communities colonizing leaf litter species have shown to vary due to differences in the leaf chemical composition [Griffin, 1994]. Similarly, shift in the chemical composition during decomposition of one leaf species were also observed to induce changes in the microbial community composition and enzyme activities [Snajdr *et al.*, 2010].

Streams surrounded by sparse woody riparian vegetation receive less litter input than mature forested streams [Schade and Fisher, 1997]. Few studies have tested the effect of litter quantity on decomposition processes. However, with higher quantity of background litter, a greater inoculum of aquatic hyphomycetes is expected [Laitung *et al.*, 2002]. Aquatic hyphomycetes are known as key players in microbial litter decomposition [Gessner *et al.*, 2007] and are characterized by rapid sporulation following initial establishment and growth on leaves [Gessner and Chauvet, 1994]. Abundance of hyphomycete spores peaked after litter fall [Bärlocher, 2000] and was observed to be directly related to the quantity of litter in the stream [Laitung *et al.*, 2002]. Considering the correlation of spore concentration and fungal colonisation [Treton *et al.* 2004], faster colonization and decomposition of leaves would be expected

when quantity of benthic background litter is high. However, microbial leaf decomposition in litter-augmented or depleted stream reaches showed no differences in a study by *Tiegs et al.* [2008].

During transition from open-land to forested streams, there will be a mix of litter entering the streams with unclear effects on structure and diversity of microbial communities associated with grass or tree litter. Studies testing decomposition of mixed and single species litter have shown contrasting effects: additive, which means that responses in mixes are predictable from component species decaying alone [e.g. *Taylor et al.*, 2007] and non-additive with higher [e.g. *Kominoski et al.*, 2007] and lower decomposition rates [e.g. *Swan and Palmer*, 2004] in mixes compared to the decaying of component species alone. The effects of mixed litter on microbial diversity are also unclear. Increase of microbial diversity was found in mixed litter compared to single litter species on a forest floor [*Chapman and Newman*, 2010], but no effect of mixed litter on the diversity of fungal communities was found by *Taylor et al.* [2007] in streams.

5.2.1 Objectives and hypotheses

In this experiment, we aimed to elucidate the relative importance of leaf quality compared to the effect of background litter standing stock and its shift during stream succession. Input of typical litter entering the streams during successional stages of stream ecosystem development was simulated in fifteen experimental outdoor channels; (1) the biofilm stage without litter input, (2) the macrophyte stage with input of grass litter, (3) the transition between macrophyte and forested stage with a mix of grass and tree litter input, (4) the young forested stage with a low quantity of tree litter input, and (5) the mature forested stage with 2.5 fold quantity of litter compared to the young forested stage. To assess the relative effect of the leaf quality as the direct substrate on the associated microbial communities compared to the effect of background litter standing stock, a small quantity of grass and tree leaves were exposed at each simulated successional stage. In detail, we expected higher activity and diversity of microbial communities associated to leaves (i) when background litter is present (comparing treatment 1 with 2, 3, 4, and 5), (ii) when tree litter enters the stream and composes at least part of the background litter (comparing treatment 1,2 with 3,4,5), (iii) when the

litter of better quality but same quantity forms the background litter standing stock (comparing treatment 2 with 4) and (iv) when the quantity of background litter is augmented (comparing treatment 4 with 5) and (v) when a mix of litter composes the background litter (comparing treatment 3 with 2 and 4).

5.3 Material and methods

5.3.1 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 Hühnerwasser catchment and set up in the catchment without cover to establish natural light and temperature conditions. Due to the initial situation in the catchment, level of nutrients in the groundwater, which was used to supply the channels, was low (Table 4.1, chapter 4). Concentrations of dissolved organic carbon were high but it 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. 20 % of the litter was buried in the sediment to simulate the natural distribution of POM in sand-bed streams [Fuss and Smock, 1996]. Grass litter from Wood small-grass (*Calamagrostis epigejos* (L.) Roth) was used as well as leaves from 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).

To obtain a general inoculum, natural stream water suspensions containing a mix of microorganism from open-land and forested streams were poured in each channel to allow colonization of microbial communities. 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 forested streams) in the Brandenburg region (Germany). Half of the amount of

leaves was placed in an ultrasonic bath containing 8 L of water from Chicken Creek and was sonified three minutes with an energy output of 35 W to detach bacterial cells from the leaf litter, whereas the other half was exposed 48 h in 8 L of water from the Chicken Creek under aerated conditions to increase 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 for 24 h to facilitate establishment of microorganisms [Dang *et al.*, 2005]. Subsequently, the channels received oxygen saturated water (see section 3.3.1) to replace 5 % of the water volume during each cycle. The current velocity was $1.7 \pm 0.3 \text{ cm s}^{-1}$ and the water depth of 1 cm. This resulted in an average water renewal time of 6 h.

Table 5.1.: Nutrient contents of the two exposed litter types and stoichiometric ratios.

Nitrogen (N) concentrations of the litter were measured on ground material using a Thermo--Finningan NC EA 1112 elemental analyzer (Strada Rivoltana, Milan, Italy), whereas phosphorus (P) was measured as ortho--phosphate after digestion with peroxodisulfate [Ebina *et al.*, 1983], carbon (C) content was assessed by combusting the leaves (550°C, 4h) and assuming a carbon content of 50% of the ash-free dry mass (AFDM) [Webster and Meyer, 1997].

Species	P ($\mu\text{g g}^{-1}$ DM)	N ($\mu\text{g g}^{-1}$ DM)	C (mg g^{-1} DM)	C/P	C/N	N/P
<i>Calamagrostis epigejos</i>	158 ± 28	3418 ± 159	368 ± 12	6020	126	48
<i>Betula pendula</i>	1176 ± 64	6900 ± 2365	469 ± 5	1029	79	13

5.3.2 Sampling and basic analyses

Pieces of grass and leaf 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 (DN = dissolved nitrogen, NO_2^- , NO_3^- , NH_4^+ , DP = dissolved phosphorus), pH and conductivity (Table 4.1, chapter4).

5.3.3 Extracellular Enzyme Activities (EEA)

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 5.2. 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 buffer (Tri(hydroxymethyl)aminomethane 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 was subtracted by measuring sample and substrate controls. Degradation of fluorescence was also taken into account by including a quench standard in each series of analyses. Potential enzyme activities were expressed in µmol substrate per g AFDM of sediment per h.

Table 5.2. Extracellular enzymes analysed, substrate analogues used to determine their potential activities, and biogeochemical function of the enzymes according to *Sinsabaugh et al.* [2008]. AP = aminopeptidase, MUB = 4-methylumbelliferone, AMC = 7-amino-4-methylcoumarin

Enzyme	Substrate analogue	Function
Carbon-acquiring enzymes		
β -Glucosidase	4-MUB- β -D-glucopyranoside	Cellulose degradation
β -Xylosidase	4-MUB- β -D-xylopyranoide	Hemicellulose degradation
Nitrogen-acquiring enzymes		
Chitinase	4-MUB-N-acetyl- β -D-glucosaminide	Degradation of β -1,4 glucosamines
Leucine-AP	L-Leucine-AMC	Degradation of hydrophobic amino acids
Phosphorus-acquiring enzyme		
Phosphatase	4-MUB-phosphate	Phosphomono- and -diester degradation
Lignin-degrading enzymes		
Phenol oxidase	3,4-dihydroxyphenylalanine (L-DOPA)	Polyphenol oxidation
Peroxidase	3,4-dihydroxyphenylalanine (L-DOPA) with H ₂ O ₂	Polyphenol oxidation

5.3.4 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).

5.3.5 Bacterial and fungal biomass and fungal sporulation rates

Bacterial abundance was determined by flow cytometry after detachment of bacterial cells from leaves with an ultrasonic probe [Buesing and Gessner, 2002]. For details of the method see section 3.3.3. The conversion factor used to calculate bacterial biomass was 58 fg cell^{-1} and was determined as followed; the mean cell-biovolume of 12 different stream sediment samples measured by epifluorescence microscopy [Frossard, 2011, chapter 2] was used to convert cell abundance to biovolume. Biovolume was converted into biomass using the relationship established by Loferer_Krössbacher *et al.* [1998] for pelagic freshwater bacteria: $\text{biomass} = 435 \text{ biovolume}^{0.86}$. Fungal biomass was determined by extracting and quantifying ergosterol as described in section 4.3.5.

5.3.6 Fungal and bacterial community fingerprints

Fungal and bacterial communities were also assessed by automated ribosomal intergenic spacer analysis (ARISA). Desoxyribonucleid acid (DNA) from 3 frozen leaf discs or pieces of grass ($-80 \text{ }^{\circ}\text{C}$) was extracted and purified in 3 steps which 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_2PO_4 and 947 ml l^{-1} of 120 mM Na_2HPO_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 $15230 \times g$. 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_2EDTA , 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 $15230 \times g$. 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 $15230 \times g$ 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 ($17135 \times g$), 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 .

The full 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 Switzzelrand; [Torzilli, 2006]. The PCR reaction mix (25 μL) contained (final concentrations): 1x GoTaq[®] Flexi reaction buffer (Promega, Switzerland), 2.5 mM MgCl_2 , 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, Switzerland), 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 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, Switzerland; Yanarell *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 (Promega, Switzerland), 3 mM MgCl_2 , 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 (Promega, Switzerland), 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 fungal and bacterial PCR products was checked on a 2% agarose gel containing a 100 bp ladder (Promega, Switzerland). A 1- μL volume of PCR product was mixed with 9 μL of HiDi formamide and 0.5 μL of standard LIZ1200 (Applied

Biosystems, 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, Switzerland) using a 50 cm capillary and a standard genemapper protocol (Applied Biosystems, Switzerland). The peak pattern was analysed with the software Peak Scanner V1.0 (Applied Biosystems, Switzerland). Relative peak area of the fragments with lengths between 200 and 1200 bp were extracted and the profiles of the different samples were compared with the interactive binning R script *interactive_binner.r* [Ramette, 2009].

5.3.7 Data Analysis

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 (Table 5.3), comparing treatment 1 vs. 2/3/4/5, 1/2 vs. 3/4/5, 2 vs. 4, 3 vs. 2/4 and 4 vs. 5.

Non-metric multidimensional scaling (NMDS) were performed on a matrix regrouping relative abundance of each peak from the ARISA with the function *meta.mds* of the package *vegan* [Oksanen *et al.*, 2011] of R; the distance matrix was calculated using the Bray-Curtis distance and 1000 permutations were calculated. Permutational multivariate analysis of variance using distance matrices were performed with the function *adonis* in the same package. 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.

Table 5.3. Specific contrasts between the treatments according to the hypotheses. Treatments simulate different successional stages achieved by input of either grass litter, tree litter or a mix of both litter types and increasing quantity of tree litter between treatments 4 and 5.

Contrasts	I	II	III	IV	V
Hypotheses	No litter vs. litter	No and grass litter vs. tree litter present	Grass litter vs. tree litter	Low vs. high quantity of tree litter	Mixed litter vs. one species litter
Treatments	1 vs. 2,3,4,5	1,2 vs. 3,4,5	2 vs. 4	4 vs.5	3 vs.(2,4)/2

5.4 Results

5.4.1 Physicochemical parameters

All physicochemical parameters were similar among the treatments of different background litter input. During the experiment pH values ranged between 7.1 and 7.6 (7.4) and conductivity between 406 and 948 (752) $\mu\text{S cm}^{-1}$. Water dissolved organic carbon (DOC), NO_3 , NH_4 , and PO_4 were temporarily increased after the start of the experiment due to the addition of the inoculum and leaf leaching. During the sampling period (between 6 and 10 weeks), DOC concentration ranged between 5.7 and 14.1 (9.5) mg C L^{-1} , NO_2 ranged between values below detection limit and 13.1 (3.5) $\mu\text{g N L}^{-1}$. NO_3 ranged from 30 to 80 (47.4) $\mu\text{g N L}^{-1}$, NH_4 from 5.4 to 40.1 (12.0) $\mu\text{g N L}^{-1}$ and PO_4 ranged from 1.2 to 3.4 (2.0) $\mu\text{g P L}^{-1}$.

5.4.2 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 II: $P=0.43$).

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 (Figure 5.1). Phosphatase generally showed the highest potential activity among the enzymes with higher values

for grass litter ($F_{1,60}=14.7$, $P<0.001$; Figure 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 ($F_{1,60}=231.3$, $P<0.001$; Figure 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 (Figure 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 (Figure 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 (Figure 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 (Figure 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).

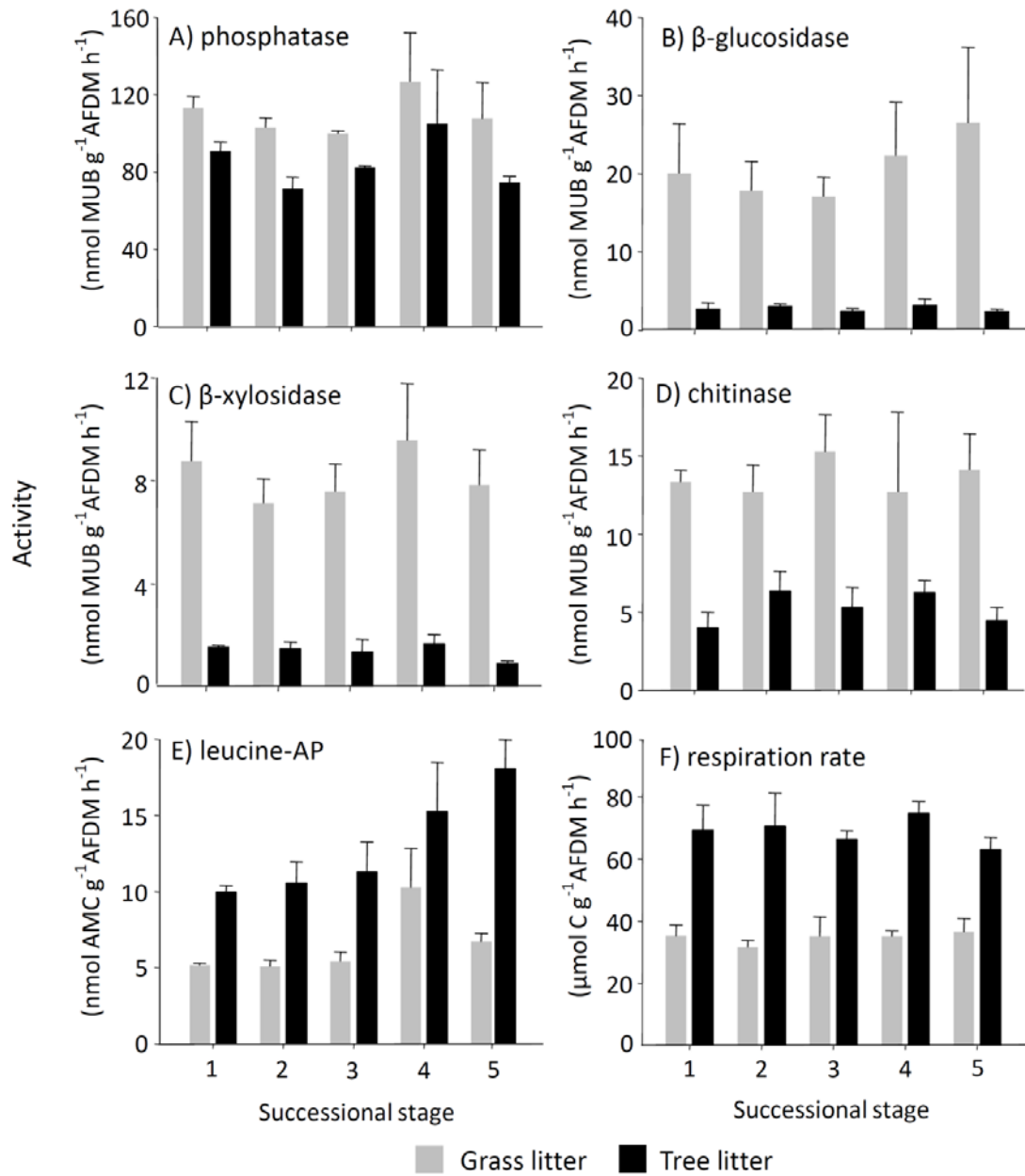


Figure 5.1: Potential activities of five enzymes and respiration of microbial communities associated with tree and grass litter exposed in the channels stocked with different types and amounts of leaf litter: N=No litter, G=Grass litter, M=Mix grass-tree litter, T=Tree litter, D= Doubled amount of tree litter. Histograms show means pooled of 3 sampling dates (after 6, 8 and 10 weeks), error bars denote standard errors (n = 3).

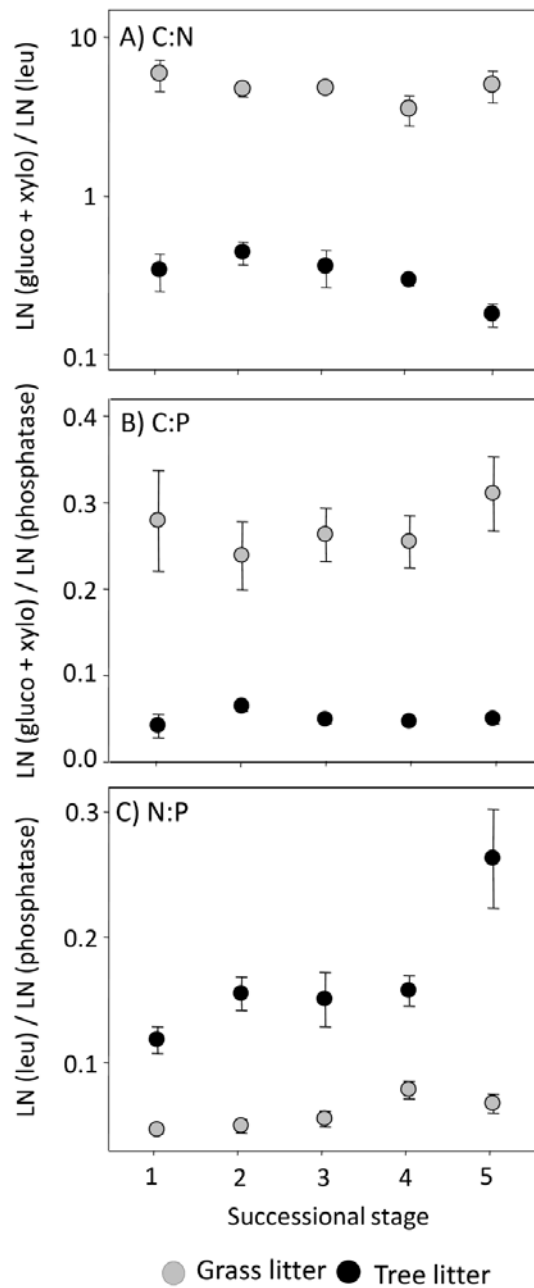


Figure 5.2: Ratios of enzymatic activities of microbial communities associated with grass and tree litter in the five different channels stocked with different types and amounts of leaf litter: N=No litter, G=Grass litter, M=Mix grass-tree litter, T=Tree litter, D=Doubled amount of tree litter. Means ± 1 SE of 3 sampling dates, n=3. Note the logarithm scale in panel A). Abbreviations: gluco= β -glucosidase, xylo= β -xylosidase, leucine=leucine-AP.

5.4.3 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) $\mu\text{g C g}^{-1}\text{AFDM h}^{-1}$ than for grass litter ranging between 18.6 and 74.0 (34.7) $\mu\text{g C g}^{-1}\text{AFDM h}^{-1}$ (Figure 5.1F).

5.4.4 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 % (Figure 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 ($F_{1,58}=0.56$, $P=0.46$).

5.4.5 Fungal sporulation rate

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

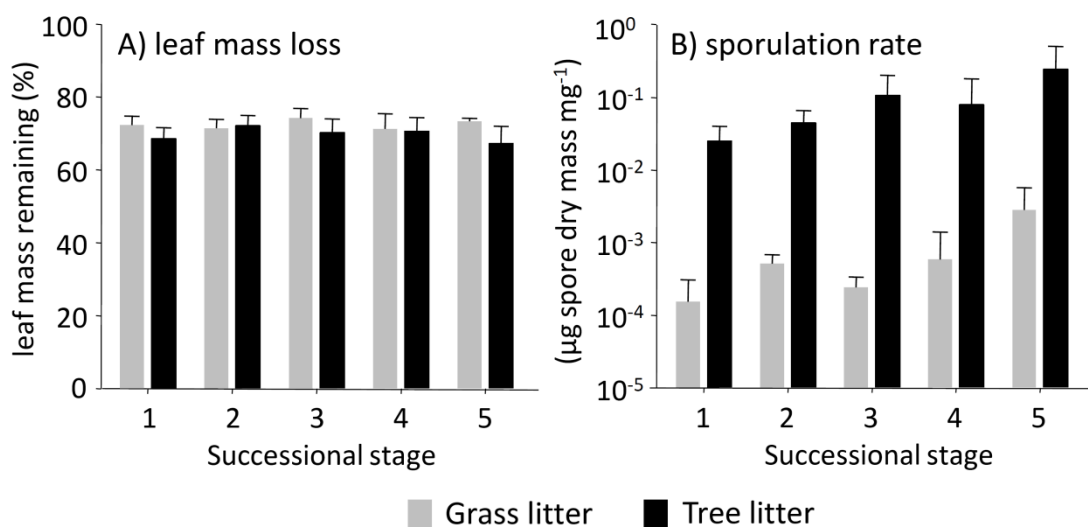


Figure 5.3: Leaf mass loss (A) and fungal sporulation rate (B) associated with grass and tree litter in experimental channels mimicking five stages of stream ecosystem succession. Histograms show means + 1 SE pooled across 3 (leaf mass loss) or 2 (sporulation rate, 8 and 10 weeks) sampling dates in each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

5.4.6 Fungal sporulation rates

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

5.4.7 Fungal and bacterial biomass

Biomass of the bacterial communities colonizing tree and grass litter showed no differences among treatments ($F_{4,10}=1.73$, $P=0.22$), (Figure 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) $\mu\text{g cells g}^{-1} \text{AFDM}$ on tree litter and between 245

and 834 (466) $\mu\text{g cells g}^{-1}$ 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 (Figure 5.4B).

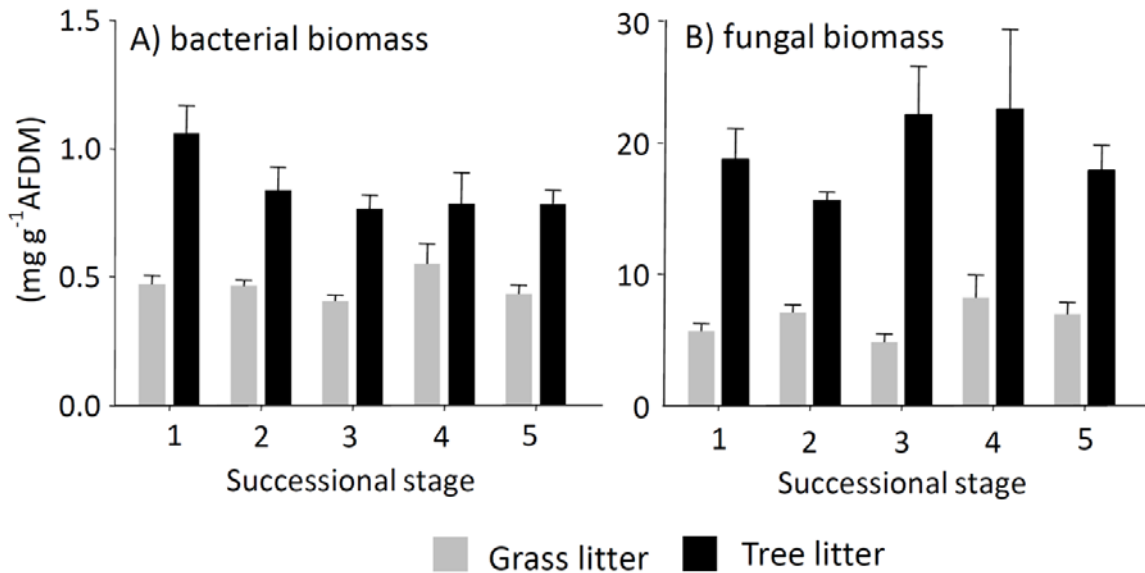


Figure 5.4: Biomass of bacteria (A) and fungi (B) associated with grass and tree litter in experimental channels mimicking five stages of stream ecosystem succession. Histograms show means + 1 SE pooled across 3 sampling dates in each of three channels, n=3.

5.4.8 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 , Figure 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 (Figure 5.6A,C) were more similar among litter type than after 10 weeks (Figure 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 I, $F_{1,44}=1.41$ and 1.47 , $P=0.002$ and 0.001) and on the presence of tree litter (contrast II, $F_{1,44}=1.95$ 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 III, $F_{1,44}=1.42$ and 1.27 , $P=0.001$), and depending on the quantity of tree litter in the channel (contrast V, $F_{1,44}=1.2$ 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 IV).

Fungal community structure differed between the two litter types ($F_{1,70}=2.06$, $P=0.002$), and changed with time (Figure 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 (Figure 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 II, $F_{1,37}$ 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 I, $F_{1,37}=1.62$, $P=0.034$) and on the litter type of background litter (contrast III, $F_{1,37}=1.77$, $P=0.022$). 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 (Figure 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.

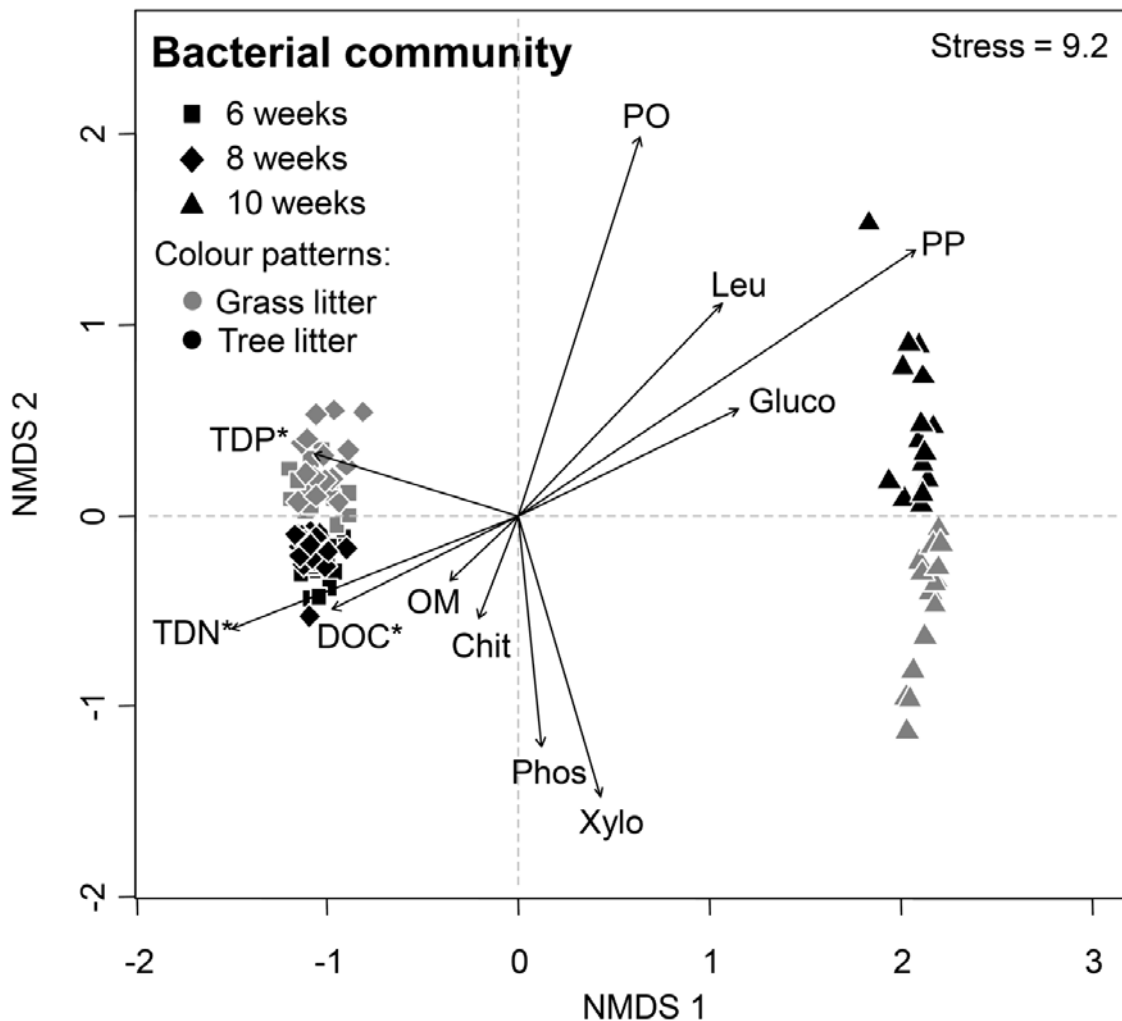


Figure 5.5A: NMDS ordination of bacterial community structure inferred from ARISA profiles in relation to a suite of potential enzyme activities associated with tree and grass litter in experimental channels mimicking five stages of stream ecosystem succession. Asterisks (*) indicate variables that are 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.

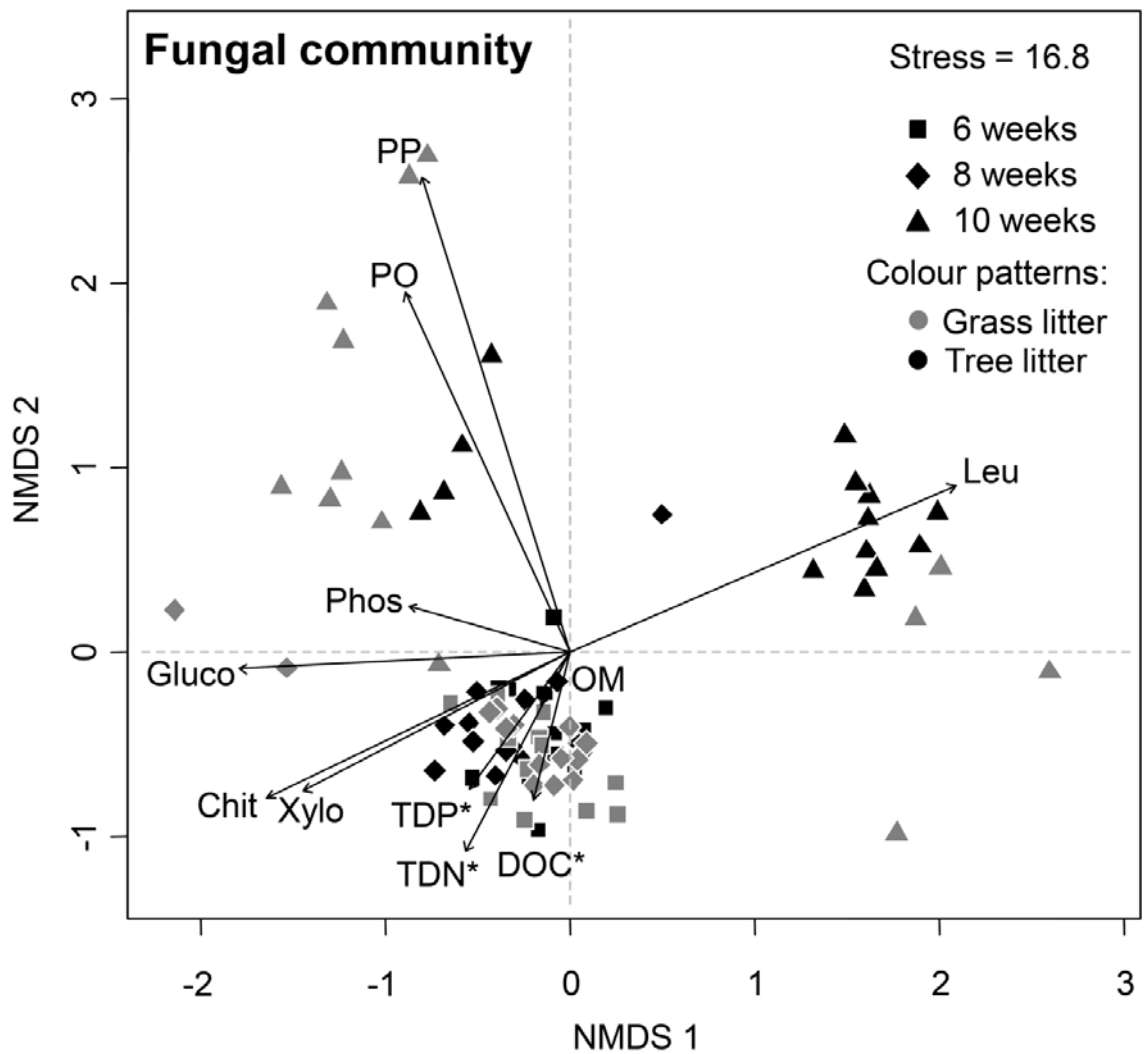


Figure 5.5B: NMDS ordination of fungal community structure inferred from ARISA profiles in relation to a suite of potential enzyme activities associated with tree and grass litter in experimental channels mimicking five stages of stream ecosystem succession. Asterisks (*) indicate variables that are 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.

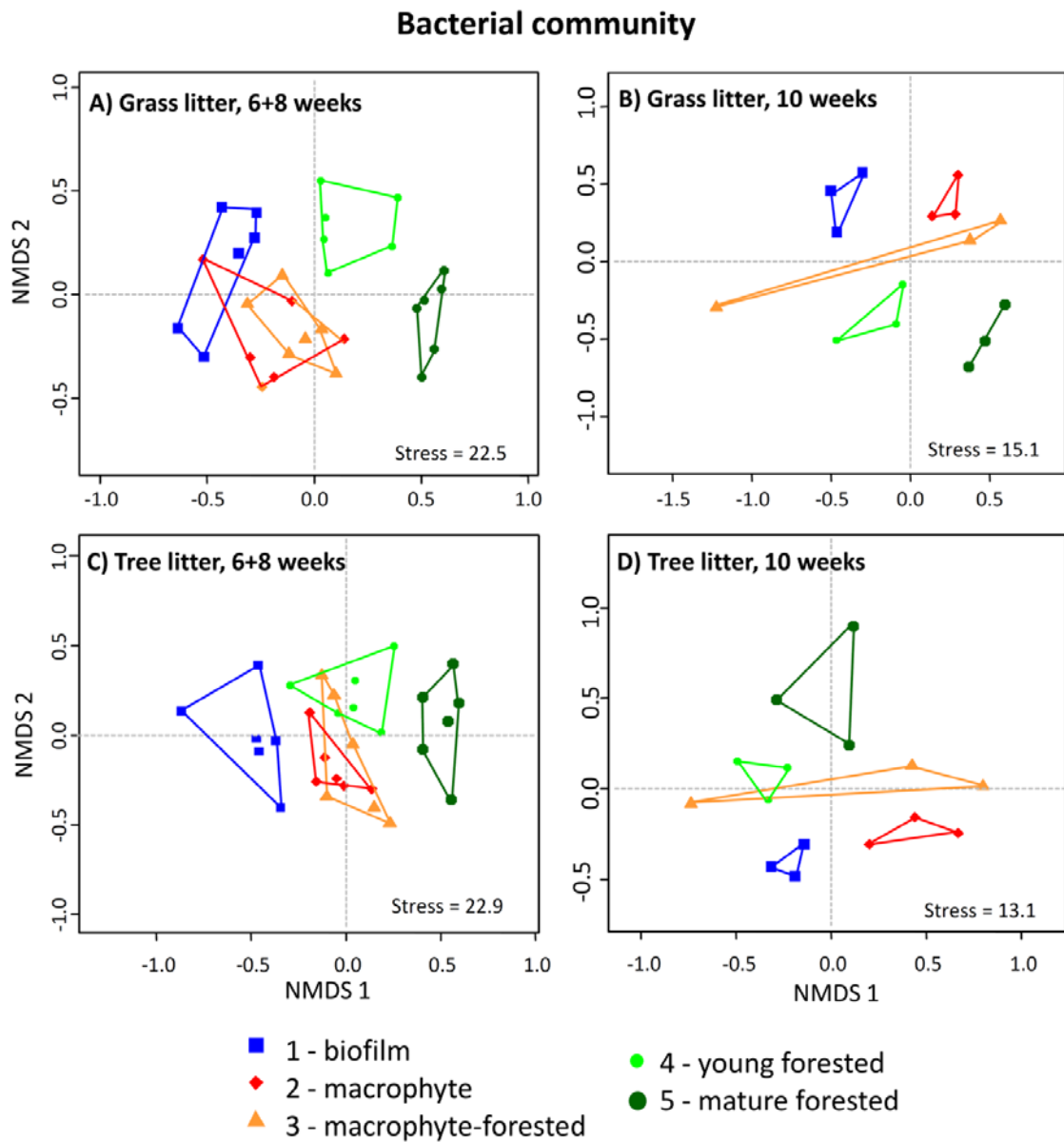


Figure 5.6: NMDS ordination of fungal communities inferred from ARISA profiles in A) grass litter, sampled after 6 + 8 weeks, B) grass litter sampled after 10 weeks, C) tree litter sampled after 6 + 8 weeks, and D) tree litter sampled after 10 weeks from experimental channels mimicking five stages of stream ecosystem succession.

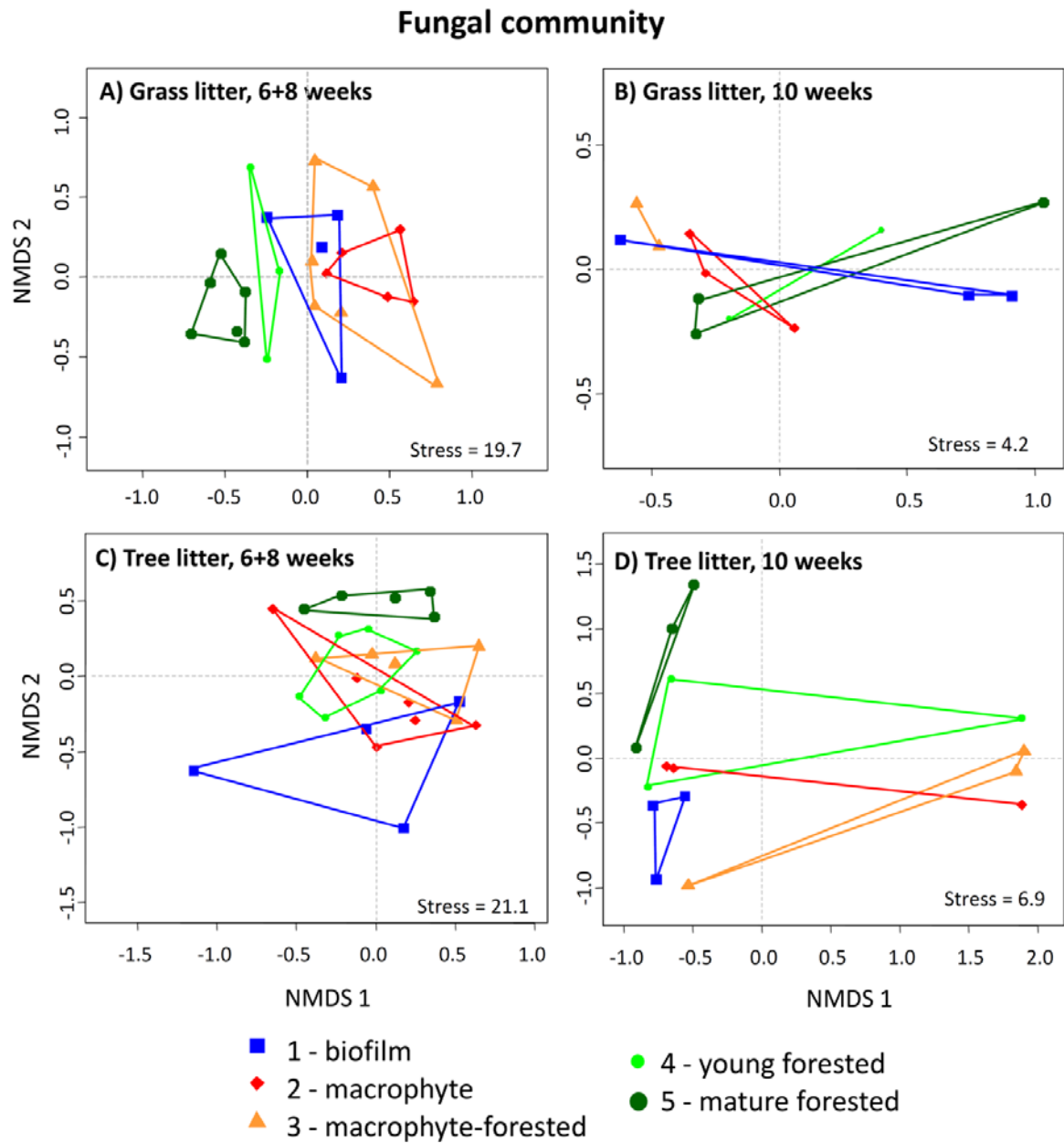


Figure 5.7: NMDS ordination of bacterial communities inferred from ARISA profiles in A) grass litter, sampled after 6 + 8 weeks, B) grass litter sampled after 10 weeks, C) tree litter sampled after 6 + 8 weeks, and D) tree litter sampled after 10 weeks from experimental channels mimicking five stages of stream ecosystem succession.

5.5 Discussion

5.5.1 Effects of the quality and quantity of the background litter

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 the treatment effects on microbial activities in sediment in a similar study [Chapter 4], there were no differences in biomass and respiration of leaf associated microbial communities between the treatments in this study. This is surprising regarding the dispersal of new colonizers from a higher amount of decomposing background litter, which should facilitate a larger microbial inoculum, increase colonization, and consequently accelerate microbial decomposition [*Treton et al.*, 2004]. Only a trend of higher sporulation rates in week 8 could be observed in the tree litter treatments before rates declined. By measuring after 8 weeks we may have missed this facilitating effect of background litter, as fungi are known to be rapid colonizers and reach high productivity within 2 to 8 weeks after colonization [*Gessner and Chauvet*, 1994]. However, the general null-effect of background litter 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 found before [*Tiegs et al.*, 2008] and studies which revealed 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 metabolic response to the treatments 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 II). 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, the nitrogen concentrations within the voids and on the leaves of the background tree litter layer colonized by active microbial communities might have been reduced [*Suberkropp and Chauvet, 1995*]. This could have resulted in the increased excretion of N-acquiring enzymes in the treatments with tree litter background.

The expected effect of mixed background litter on decomposition in the transitional treatment between open land and forested streams was not found. This was consistent with the findings in leaf pack decomposition experiments comparing mixed to single species litter [*Taylor et al., 2007; Swan and Palmer, 2004*]. However, other studies reported an effect of mixing litter species on decomposition rates [*Abelho, 2009; Kominoski et al., 2007; Lecerf et al., 2007*] and hence, the general effect of mixing litter on decomposition is still unclear. We can state from this experiment that the mix of background litter can not be the reason for increased decomposition rates and still other factors besides leaf quality and background litter seem to play an important role [*Gessner et al., 2010*].

5.5.2 Effects of litter quality on microbial communities

The distinct differences in microbial activity parameters and biomass of fungi and bacteria between the two litter types reflected the quality of the grass and tree litter which differed in P, N, C, C:N and C:P ratios. Decomposition rates have been shown to be correlated to condensed tannins, lignin, N and C:N content of litter material [*Gessner and Chauvet, 1994; Ostrofsky, 1997; Ardón et al., 2009; Hladyz, 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 with a high C:N has generally been found to decompose slowly [*Menninger and Palmer, 2007, Shaftel et al., 2011*]. The higher 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 and Chauvet, 1994*] and typically ranges between 13% and 39% in deciduous leaves [*Ostrofsky, 1997*] and between 3 and 6% in grasses

[Hoorens *et al.*, 2002; Rozema *et al.*, 1997; 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 polysaccharide decomposition [Snajdr *et al.*, 2011, Fioretto *et al.*, 2005]. Although differences in C were not substantial between tree and grass litter, the differences in the ratio of C to P and N have presumably caused the higher activity of C-acquiring enzymes associated with grass litter. Although respiration rates showed a higher metabolic activity associated with tree leaves, activity patterns of enzymes revealed the complexity of processes during decomposition and point at that part, that Ostrofsky [1997] (see above) found not to be explained just by nutrient and lignin contents of the leaves.

Microbial communities on both exposed leaf types were primarily P-limited as implied by the high activity of phosphatase compared to the other measured enzymes [Taylor *et al.*, 2003], most probably due to the low SRP concentration in the water supplying the flumes. Nutrients from the water column are significant nutrient source for leaf associated microbial communities in addition to the leaf itself [Suberkropp and 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 [Sturner and Elser, 2002]. Nevertheless, microbial communities were still rather limited by carbon as reflected by the C:N enzymatic ratio above 1 for grass 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 due 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 they are energetically and nutritionally expensive to produce and their activity may increase with increasing availability of labile C and N (e.g., Schimel and 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 (Figure 5.3A, B and 5.1F).

5.5.3 Effects of litter type and background litter on microbial community composition

The large difference of microbial community metabolism between the two litter types was also reflected in the fungal and bacterial community structure (Figure 5.5). Thus, the quality of the litter types was more relevant for microbial metabolism and community structure than the effect of the background litter standing stock (Figure 5.5). Substrate quality has been found to superimpose effects of environmental variation in other studies [*Mille-Lindblom et al.*, 2006; *Gulis*, 2001], but, these studies focused on differences in water chemistry which was constant among our treatments. Other studies found that stream water nutrient content was more relevant than variability in litter quality [*Harrop et al.*, 2009, *Marks et al.*, 2009].

In our experiment, differences among channels stocked with different amounts and types of background 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 *Frossard et al.* [2011] that microbial communities are often functionally redundant [*Kominoski et al.*, 2009]. Nevertheless, the observed differences between communities on grass and tree litter suggests 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 preferred grass litter over tree litter in spite of higher resource quality of the latter.

5.5.4 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). Hence, 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 and 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 background litter 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 grass litter to forested streams with higher quality tree litter.

6 Conclusions and outlook

6.1 Conclusions

To conclude this thesis, I attempt to answer the questions raised in the introduction.

(I) What are the factors regulating microbial activity in stream corridors of an early successional watershed?

The investigations performed in the Chicken Creek watershed revealed that permanent water availability was not a main factor determining potential microbial activity. Microbes associated with rewetted soils and sediments, formerly dry, were able to immediately raise their metabolism to full capacity, resembling adaptations of communities present in arid environments. However, the carbon turnover rates in perennial stream sections were still higher due to the permanent activity of microbes and low organic matter content in stream-bed sediments. Nevertheless, the high turnover of carbon at these permanently wet hot spots was insufficient to have a strong influence on the carbon flux of the entire watershed on an annual basis. Precipitation in that mesic climate caused frequent wetting of ephemeral stream sections and soils with hot moments of high microbial respiration which dominated the total carbon flux of the watershed.

Another factor potentially regulating microbial activity was the availability of nutrients. Due to the initial state of the watershed, with its scarce vegetation cover, the level of nutrients was low. This generally curbed microbial activity as compared to mature stream ecosystems. Similar to other mature open-land streams, benthic algal productivity was of major importance for the heterotrophic activity. There was a positive linkage between algal biomass and microbial activity in benthic sediments presumably due to the exudation of labile organic matter by algae fuelling heterotrophic bacteria. Compared to mature systems, this source might have been of eminent importance, as the availability of carbon sources in this early successional environment was low. The same could be observed for POM derived from the first established vascular plants. The fragments, seeds and other amorphous plant material, often buried in the sediments by sediment disturbance events, were shown to be of prime importance for microbial metabolic activity in an early successional landscape. But the establishment of in-stream vascular plants as well as benthic algae, both drivers for

microbial activity, is dependant on the stability of the bed sediments. The hydrologic regime typical for an open-land watershed with scarce vegetation is characterized by high surface-runoff and frequent flush flows even with minor precipitation events. The size of the sub-watersheds and the amount of run-off discharging into the network of rills and channels varies, which can lead to high variance in scour and fill in the streambeds. Thus, the size of the sub-watersheds and the evolved network of rills and channels were found to be an elemental initial structure for further development. The frequency of increased discharge resulting in bed-instability indirectly determined the speed of vegetation succession and algal colonization, both main factors driving microbial activity in initial streams.

(II) Do have moderate and severe sediment disturbances a similar effect on whole-stream metabolism? And do these effects differ depending on what stage of succession the disturbance occurs?

Effects of sediment disturbance differed for NCP and CR. NCP did not show any effect after sediment disturbance in an early successional stage but a strong decline in the advanced stage after moderate as well as severe sediment disturbance. The similarity of NCP after moderate and severe sediment disturbance suggests that algae were less affected by the disconnection from the light supply than by the disruption of the fine structure of the sediment. CR showed no effect and no differences between moderate and severe sediment disturbance in the early successional stage. However, in the late stage, severe sediment disturbance stimulated CR more than moderate sediment disturbance. A key finding was that sediment scour and fill can reduce NCP for several days even when a disturbance event is limited to 1 cm depth, which is only a moderate disturbance. This delayed recovery of NCP and the concomitant stimulation of CR have implications particularly for sand-bed streams experiencing frequent sediment movements. If such moderate sediment movements are frequent, metabolism could shift from autotrophy to heterotrophy, even in the absence of a riparian canopy. This reveals that even moderate sediment disturbances have a great effect on ecosystem metabolism in sand-bed streams in general and serves to corroborate the findings from chapter 2. Frequent sediment movements which are typical for early successional streams can thoroughly impact algal communities and primary production and as a consequence also heterotrophic microbial activity benefitting from algal exudates.

(III) Does the input of increasing quality and quantity of litter into streams lead to higher rates of whole-stream metabolism and higher activity of leaf associated microbial communities?

The results of the experiment conducted to answer this question showed no difference of whole-stream metabolism related to quality or quantity of litter input. The same was shown for leaf associated microbial activity. Although microbial communities associated with leaves showed substantial potential for activity and growth when measured in mesocosms in the laboratory, their ability to contribute to whole-stream metabolism in the experimental flumes was curbed. Obviously microbial communities in the densely packed leaf layer on the benthos were limited by nutrient and oxygen availability. Input of litter seemed to fuel microbial activity in the sediments but intensive respiration in sediments with insufficient surface-subsurface water exchange can lead to hypoxia, again limiting the contribution of hyporheic sediments to whole-stream metabolism. Results indicated a compensatory effect between absent and low quality litter input in the open-land treatments by higher algal biomass fuelling microbial activity with labile organic matter and better oxygen availability in the upper sediment layer.

Although leaves proved to be a good source and surface for colonization of microbes, including fungi as a new group of microbes entering the streams when leaves are present, the importance of litter input for stream succession and whole-stream metabolism in sand-bed streams was not evident in the experiment. As long as streams are not shaded and too frequently disturbed (chapter 3), benthic algae can be highly productive, also driving heterotrophic activity in the sediments similar to streams with allochthonous input of POM. The key factor controlling whole-stream metabolism seems to be the size and oxygenation of the hyporheic zone. Oxygenation of the sediments is dependant on the surface-subsurface water exchange which is greatly defined by the grain-size of the sediments. In the investigated sand-bed streams with low vertical water flux, input of coarse allochthonous organic matter did not show the proposed effect. In streams with coarser sediments, litter input might be able to substantially fuel hyporheic respiration and function as a driver of stream succession. If the hyporheic zone is large, well connected to the surface flow and fueled by allochthonous POM buried in the sediments (chapter 2), the difference compared to

streams without allochthonous POM sources will be large. Algal production seems to be able to compensate for the lack of allochthonous POM to some extent, but as algae are dependent on light, they are bound to the surface layers of the hyporheic zone only. Thus, there presumably is a shift from mainly autotrophic to heterotrophic fueled systems during stream succession which likely is dependent on the availability of oxygen and nutrients in the hyporheic zone. So to answer the initial question, I can state that litter input of increasing quality and quantity can have the potential to increase whole-stream metabolism and to be a driver for stream succession, but only if not constrained by oxygen and nutrient availability.

(IV) Is there a higher microbial activity and a more diverse microbial community on tree litter compared to grass litter? And is the leaf quality or the background litter standing stock more relevant for leaf associated microbial activity and community composition?

Results of the experiment showed that microbial activity associated with tree litter of *Betula pendula* was higher than with grass litter of *Calamagrostis epigejos* shown by higher microbial respiration, bacterial and fungal biomass and fungal sporulation rates. The tree litter proved to be a better quality resource for colonizing microbes with higher content of P, N and lower C:N ratio. The higher activity of C-acquiring enzymes on the grass litter showed the carbon limitation of associated microbial communities. The N-acquiring enzyme leucine-AP showed a higher potential activity on tree litter, indicating that if carbon was not a limiting factor and nitrogen available, microbial communities could invest in the acquisition of nitrogen for better growth. Phosphorus concentration in the stream water was very low and for microbes on both litter types limiting. Background grass or tree litter input had no effect on the activity associated with exposed leaves of either species, implying that for leaf associated microbial activity, the quality of the single leaf is more important than the background litter standing stock typically occurring during stream succession. Structure of the microbial communities differed between the two litter types and also between the treatments simulating different successional stages. As there were no treatment differences in metabolic parameters but in the community structure associated with litter, we can assume functional redundancy among the communities in the treatments of different quality and quantity of background litter.

6.2 Outlook

The research performed for this thesis aimed to gain insight into the processes of carbon transformation and accumulation due to microbial activity in early successional streams. The used approach to study these questions directly in an early successional watershed and simultaneously in an experimental flume system, which offered the possibility for manipulation, proved to be excellent. The Chicken Creek watershed is suitable for long-term investigations with the possibility to repeat the study assessing the spatio-temporal variability of metabolic activity described in this thesis at later successional stages. By continuing the investigation of stream communities during succession, other important organisms of the stream food web should be included into the research frame, e.g. protozoans and hyporheic meiofauna, which are important grazers on benthic communities. Further studies in that long-term perspective would also help to corroborate the succession concept of the supposed 3 main stages described in the general introduction (chapter 1) and could improve our understanding of stream succession. Nevertheless, it would be also useful to prove several aspects for generalization with comparative studies in other early successional landscapes and stream types around the world e.g. gravel bed streams in the glacier fore field. This might elucidate climate and site specific differences in ecosystem succession and help to derive general concepts on ecological succession, which can be central to the field of ecology. In a world with increasing anthropogenic disturbances and issues such as biodiversity loss, climate change and ecological restoration, to name only a few, the concept of succession can be suitable to address these concerns and help predict changes, relevant for future generations.

Future research within this field could preferably aim at verifying the role of the ecological interactions between autotrophic and heterotrophic microbial communities in benthic sediments under early successional environmental conditions and to what depth of sediment is this interaction important. In addition, the autotrophic contribution to respiratory activity associated with sediments would require assessment. Results in this thesis suggest the primary importance of sub-watershed size as an initial structure of the watershed. Frequency of sediment disturbance, able to set-back the stage of succession, seems to be related to the size of the sub-watershed. Hence I recommend to further elucidate this aspect. Questions remain regarding the method of measuring single

stream compartments separately in lab mesocosms and the transferability of these measured values to nature. As much of our knowledge today of in-stream processes originates from laboratory measurements, attention has to be paid to simulate natural in-stream conditions in the laboratory. Although studies in the field are often challenging and due to the complex setting difficult to interpret, the value for understanding processes in nature cannot be overestimated.

Acknowledgements

This thesis was performed at the Department of Freshwater Conservation at the BTU Cottbus. I would like to thank all colleagues of the Department. The friendly atmosphere and cooperativeness of all have contributed to the success of this work.

I would like to thank Michael Mutz for being an excellent supervisor. I am very grateful for his scientific advice and for his patience in answering all my questions as well as encouraging me throughout the last years. I also extend sincere thanks to Mark Gessner for additional supervision, for his time and support, interest, and fruitful discussions. They both initiated the project and gave me the opportunity to work on this interesting topic.

The last four years would not have been that much fun without the cooperation, help and company of Aline Frossard. With her good humour and encouraging manner, she made all the smaller and bigger catastrophes, usually occurring during field work, bearable and mostly turned them into a laugh.

I am very grateful for the support, both technical and otherwise of Remo Ender, the master of the flumes, who always helped me in the *Hühnerwasser*, regardless of weather conditions or holidays and Thomas Wolburg. Both of them always encouraged me when I had my frustrating moments.

Many students contributed to making these investigations possible. I want to thank Marika Stange, Margot Weber, Constanze Keßler, Lisa Odpalick, Christiane Peters, Julia and Katja Westphal, Jürgen Amonat, Matthias Schuster, Bianca Räßle and Michael Seidel for field and lab assistance.

I thank Gudrun Lippert and Ute Abel for the patience they had with all the chaos I produced in the lab and for water nutrient analyses. Thanks to Regina Müller and Gabi Franke for DOC analyses in Cottbus and the AUA lab at the Eawag for water nutrient analyses. Many thanks to Daniel Steiner, who introduced me to the method of bacterial and fungal production and also finished the measurements of fungal production in Switzerland. I am grateful to Doris Hohmann for counting and identifying fungal spores. Thanks to Massimo Merlini and Manuel Koller at the ETH and Andrew Dolman for statistical support.

I very much enjoyed being part of the team at the Department of Freshwater Conservation. At all times, I thoroughly enjoyed the friendly, cooperative and inspiring atmosphere and the great festivities around the year - thank you to all of you. Special thanks to Ute Risse-Buhl for proofreading and useful comments.

I thank my family and Holger for their support in every respect. I am very grateful to PAN for support regarding formatting and layout and for giving me insight into the advantages of LaTeX.

Special thanks are due to Vattenfall Europe Mining AG for providing the research site. My PhD work was part of the Transregional Collaborative Research Centre 38 (SFB/TRR 38), which was financially supported by the German Research Council (DFG, Bonn) and the Brandenburg Ministry of Science, Research and Culture (MWFK, Potsdam).

List of abbreviations

AFDM	ash free dry mass
ANOVA	analysis of variance
AP	aminopeptidase
ARISA	automated ribosomal intergenic spacer analysis
C	carbon
chl <i>a</i>	chlorophyll <i>a</i>
C:N	molar ratio of carbon to nitrogen
C:P	molar ratio of carbon to phosphorus
CPOM	coarse particulate organic matter
DM	dry mass
DNA	desoxyribonucleid acid
DOC	dissolved organic carbon
DON	dissolved organic nitrogen
EDTA	Ethylenediaminetetraacetic acid
EEA	extracellular enzyme activity
EPS	extracellular polymeric substances
ETS	electron transport system
GPP	gross primary production
HPLC	high-performance liquid chromatograph
k	carbon turnover rate
LAPOM	loosely associated particulate organic matter
n	sample size
N	nitrogen
NCP	net community production
NMDS	non-metric multidimensional scaling
N:P	molar ration of nitrogen to phosphorus
OM	organic matter
P	phosphorus
PAR	photosynthetically active radiation
pers. comm.	personal communication

POM	particulate organic matter
P/R	ratio of production and respiration <i>in sensu</i> GPP/CR
q	quantile
qq-plots	quantile-quantile-plots
r	correlation coefficient (r^2 = coefficient of determination)
R	respiration rate
rmANOVA	repeated measures analysis of variance
RQ	respiratory quotient
SAPOM	strongly associated particulate organic matter
SDS	sodium dodecyl sulfate
SE	standard error
SRP	soluble reactive phosphorus
TDN	total dissolved nitrogen
TDP	total dissolved phosphorus
TCA	trichloroacetic acid

Bibliography

- Abelho, M., 2009. Leaf-litter mixtures affect breakdown and macroinvertebrate colonization rates in a stream ecosystem, *International Review of Hydrobiology* 94: 436-451.
- Acuna, V., C. Vilches & A. Giorgi, 2011. As productive and slow as a stream can be-the metabolism of a Pampean stream, *Journal of the North American Benthological Society* 30: 71-83.
- Amalfitano, S., S. Fazi, A. Zoppini, A. B. Caracciolo, P. Grenni & A. Puddu, 2008. Responses of benthic bacteria to experimental drying in sediments from Mediterranean temporary rivers, *Microbial Ecology* 55: 270-279.
- Ardon, M., C. M. Pringle & S. L. Eggert, 2009. Does leaf chemistry differentially affect breakdown in tropical vs temperate streams? Importance of standardized analytical techniques to measure leaf chemistry, *Journal of the North American Benthological Society* 28: 440-453.
- Arevalo, C. B. M., J. S. Bhatti, S. X. Chang, R. S. Jassal & D. Sidders, 2010. Soil respiration in four different land use systems in north central Alberta, Canada, *Journal of Geophysical Research-Biogeosciences* 115- G01003.
- Arnon, S., L. P. Marx, K. E. Searcy & A. I. Packman, 2010. Effects of overlying velocity, particle size, and biofilm growth on stream-subsurface exchange of particles, *Hydrological Processes* 24: 108-114.
- Atkinson, B. L., M. R. Grace, B. T. Hart & K. E. N. Vanderkruk, 2008. Sediment instability affects the rate and location of primary production and respiration in a sand-bed stream, *Journal of the North American Benthological Society* 27: 581-592.
- Austin, A. T., L. Yahdjian, J. M. Stark, J. Belnap, A. Porporato, U. Norton, D. A. Ravetta & S. M. Schaeffer, 2004. Water pulses and biogeochemical cycles in arid and semiarid ecosystems, *Oecologia* 141: 221-235.
- Baas, J. H., 1999. An empirical model for the development and equilibrium morphology of current ripples in fine sand, *Sedimentology* 46: 123-138.
- Baker, M. A., H. M. Valett & C. N. Dahm, 2000. Organic carbon supply and metabolism in a shallow groundwater ecosystem, *Ecology* 81: 3133-3148.
- Baldwin, D. S. & A. M. Mitchell, 2000. The effects of drying and re-flooding on the sediment and soil nutrient dynamics of lowland river-floodplain systems: A synthesis, *Regulated Rivers-Research and Management* 16: 457-467.
- Baldy, V., M. O. Gessner & E. Chauvet, 1995. Bacteria, fungi and the breakdown of leaf-litter in a large river, *Oikos* 74: 93-102.

- Baldy, V. & M. O. Gessner, 1997. Towards a budget of leaf litter decomposition in a first-order woodland stream, *Comptes Rendus de l'Académie des Sciences Serie Iii-Sciences de la Vie-Life Sciences* 320: 747-758.
- Baldy, V., E. Chauvet, J. Y. Charcosset & M. O. Gessner, 2002. Microbial dynamics associated with leaves decomposing in the mainstem and floodplain pond of a large river, *Aquatic Microbial Ecology* 28: 25-36.
- Baldy, V., V. Gobert, F. Guerold, E. Chauvet, D. Lambrigot & J. Y. Charcosset, 2007. Leaf litter breakdown budgets in streams of various trophic status: effects of dissolved inorganic nutrients on microorganisms and invertebrates, *Freshwater Biology* 52: 1322-1335.
- Bastviken, D., M. Olsson & L. Tranvik, 2003. Simultaneous measurements of organic carbon mineralization and bacterial production in oxic and anoxic lake sediments, *Microbial Ecology* 46: 73-82.
- Battin, T. J. & D. Sengschmitt, 1999. Linking sediment biofilms, hydrodynamics, and river bed clogging: Evidence from a large river, *Microbial Ecology* 185-196.
- Battin, T. J., L. A. Kaplan, S. Findlay, C. S. Hopkinson, E. Marti, A. I. Packman, J. D. Newbold & F. Sabater, 2008. Biophysical controls on organic carbon fluxes in fluvial networks, *Nature Geoscience* 1: 95-100.
- Baveye, P., P. Vandevivere, B. L. Hoyle, P. C. Deleo & D. S. de Lozada, 1998. Environmental impact and mechanisms of the biological clogging of saturated soils and aquifer materials, *Critical Reviews in Environmental Science and Technology* 28: 123-191.
- Bärlocher, F. & M. Schweizer, 1983. Effects of leaf size and decay-rate on colonization by aquatic hyphomycetes, *Oikos* 41: 205-210.
- Bärlocher, F., 2000. Water-borne conidia of aquatic hyphomycetes: seasonal and yearly patterns in Catamaran Brook, New Brunswick, Canada, *Canadian Journal of Botany-Revue Canadienne de Botanique* 78: 157-167.
- Behrendt, H. & B. Nixdorf, 1993. The carbon balance of phytoplankton production and loss processes based on in-situ measurements in a shallow lake, *Internationale Revue der Gesamten Hydrobiologie* 78: 439-458.
- Bell, R. T. & I. Ahlgren, 1987. Thymidine incorporation and microbial respiration in the surface sediment of a hypereutrophic lake, *Limnology and Oceanography* 32: 476-482.
- Belnap, J., J. R. Welter, N. B. Grimm, N. Barger & J. A. Ludwig, 2005. Linkages between microbial and hydrologic processes in arid and semiarid watersheds, *Ecology* 86: 298-307.

- Berggren, M., H. Laudon & M. Jansson, 2007. Landscape regulation of bacterial growth efficiency in boreal freshwaters, *Global Biogeochemical Cycles* 21.
- Biggs, B. J. F. & M. E. Close, 1989. Periphyton biomass dynamics in gravel bed rivers - the relative effects of flows and nutrients, *Freshwater Biology* 22: 209-231.
- Biggs, B. J. F., 1996. Patterns in benthic algae. In Stevenson, R. J., M. L. Bothwell & R. L. Lowe (eds), *Algal Ecology*. Academic Press, San Diego: 31-56.
- Blenkinsopp, S. A. & M. A. Lock, 1994. The impact of storm flow on river biofilm architecture, *Journal of Phycology* 30: 807-818.
- Bond-Lamberty, B. & A. Thomson, 2010. Temperature-associated increases in the global soil respiration record, *Nature* 464: 579-U132.
- Bothwell, M. L., 1989. Phosphorus limited growth dynamics of lotic periphytic diatom communities - Areal biomass and cellular growth-rate responses, *Canadian Journal of Fisheries and Aquatic Sciences* 46: 1293-1301.
- Bott, T. L., 1996. Primary productivity and community respiration. In Hauer, R. & G. A. Lamberti (eds), *Methods in Stream Ecology*. Academic Press, San Diego, USA: 533-556.
- Bott, T. L., J. D. Newbold & D. B. Arscott, 2006. Ecosystem metabolism in piedmont streams: Reach geomorphology modulates the influence of riparian vegetation, *Ecosystems* 9: 398-421.
- Boulton, A. J., T. Datry, T. Kasahara, M. Mutz & J. A. Stanford, 2010. Ecology and management of the hyporheic zone: stream-groundwater interactions of running waters and their floodplains, *Journal of the North American Benthological Society* 29: 26-40.
- Brzezinski, M. A., 1985. The Si-C-N ratio of marine diatoms - Interspecific variability and the effect of some environmental variables, *Journal of Phycology* 21: 347-357.
- Buesing, N. & M. O. Gessner, 2002. Comparison of detachment procedures for direct counts of bacteria associated with sediment particles, plant litter and epiphytic biofilms, *Aquatic Microbial Ecology* 27: 29-36.
- Buesing, N. & M. O. Gessner, 2003. Incorporation of radiolabeled leucine into protein to estimate bacterial production in plant litter, sediment, epiphytic biofilms, and water samples, *Microbial Ecology* 45: 291-301.
- Buesing, N. & M. O. Gessner, 2004. Secondary production and growth of litter-associated bacteria. In Graca, M. A. S., F. Bärlocher & M. O. Gessner (eds), *Methods to Study Litter Decomposition: A Practical Guide*. Kluwer Academic Publishers, Netherlands: 189-194.

- Buesing, N. & M. O. Gessner, 2006. Benthic bacterial and fungal productivity and carbon turnover in a freshwater marsh, *Applied and environmental microbiology* 72: 596-605.
- Cable, J. M. & T. E. Huxman, 2004. Precipitation pulse size effects on Sonoran Desert soil microbial crusts, *Oecologia* 141: 317-324.
- Cahill, R. A., A. D. Autrey, R. V. Anderson & J. W. Grubaugh, 1987. Improved measurement of the organic-carbon content of various river components, *Journal of Freshwater Ecology* 4: 219-223.
- Caracciolo, A. B., P. Grenni, C. Cupo & S. Rossetti, 2005. In situ analysis of native microbial communities in complex samples with high particulate loads, *Fems Microbiology Letters* 253: 55-58.
- Carr, G. M., A. Morin & P. A. Chambers, 2005. Bacteria and algae in stream periphyton along a nutrient gradient, *Freshwater Biology* 50: 1337-1350.
- Chapin, F. S., L. R. Walker, C. L. Fastie & L. C. Sharman, 1994. Mechanisms of primary succession following deglaciation at Glacier Bay, Alaska, *Ecological Monographs* 64: 149-175.
- Chapman, S. K. & G. S. Newman, 2010. Biodiversity at the plant-soil interface: microbial abundance and community structure respond to litter mixing, *Oecologia* 162: 763-769.
- Chauvet, E. & K. Suberkropp, 1998. Temperature and sporulation of aquatic hyphomycetes, *Applied and environmental microbiology* 64: 1522-1525.
- Claret, C. & A. J. Boulton, 2003. Diel variation in surface and subsurface microbial activity along a gradient of drying in an Australian sand-bed stream, *Freshwater Biology* 48: 1739-1755.
- Clements, F. E., 1916. *Plant succession: an analysis of the development of vegetation*. Carnegie Institute of Washington Publication 242.
- Clements, F. E., 1936. Nature and structure of the climax, *Journal of Ecology* 24: 252-284.
- Cohn, S. A. & R. E. Weitzell, 1996. Ecological considerations of diatom cell motility - Characterization of motility and adhesion in four diatom species, *Journal of Phycology* 32: 928-939.
- Cole, J. J., 1982. Interactions between bacteria and algae in aquatic ecosystems, *Annual Review of Ecology and Systematics* 13: 291-314.
- Collins, S. L., R. L. Sinsabaugh, C. Crenshaw, L. Green, A. Porras-Alfaro, M. Stursova & L. H. Zeglin, 2008. Pulse dynamics and microbial processes in aridland ecosystems, *Journal of Ecology* 96: 413-420.

- Cook, F. J. & V. A. Orchard, 2008. Relationships between soil respiration and soil moisture, *Soil Biology & Biochemistry* 40: 1018.
- Crenshaw, C. L., H. M. Valett & J. L. Tank, 2002. Effects of coarse particulate organic matter on fungal biomass and invertebrate density in the subsurface of a headwater stream, *Journal of the North American Benthological Society* 21: 28-42.
- Cronin, G., J. H. McCutchan, J. Pitlick & W. M. Lewis, 2007. Use of Shields stress to reconstruct and forecast changes in river metabolism, *Freshwater Biology* 52: 1587-1601.
- Cushing, C. E. & E. G. Wolf, 1984. Primary production in Rattlesnake Springs, a cold desert spring-stream, *Hydrobiologia* 114: 229-236.
- Dahm, C. N., D. L. Carr & R. L. Coleman, 1991. Anaerobic carbon cycling in stream ecosystems. Schweizerbartsche Verlagsbuchhandlung, Stuttgart.
- Dalzell, B. J., T. R. Filley & J. M. Harbor, 2005. Flood pulse influences on terrestrial organic matter export from an agricultural watershed, *Journal of Geophysical Research-Biogeosciences* 110.
- Dang, C. K., E. Chauvet & M. O. Gessner, 2005. Magnitude and variability of process rates in fungal diversity-litter decomposition relationships, *Ecology Letters* 8: 1129-1137.
- Das, M., T. V. Royer & L. G. Leff, 2007. Diversity of fungi, bacteria, and actinomycetes on leaves decomposing in a stream, *Applied and environmental microbiology* 73: 756-767.
- Davidson, E. A. & I. A. Janssens, 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change, *Nature* 440: 165-173.
- Decho, A. W., 2000. Microbial biofilms in intertidal systems: an overview, *Continental Shelf Research* 20: 1257-1273.
- del Giorgio, P. A. & J. J. Cole, 1998. Bacterial growth efficiency in natural aquatic systems, *Annual Review of Ecology and Systematics* 503-541.
- Dodds, W. K., R. E. Hutson, A. C. Eichen, M. A. Evans, D. A. Gudder, K. M. Fritz & L. Gray, 1996. The relationship of floods, drying, flow and light to primary production and producer biomass in a prairie stream, *Hydrobiologia* 333: 151-159.
- Du, G. Y., J. H. Oak, H. Li & I. K. Chung, 2010. Effect of light and sediment grain size on the vertical migration of benthic diatoms, *Algae* 25: 133-140.
- Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers & F. Smith, 1956. Colorimetric method for determination of sugars and related substances, *Analytical Chemistry* 28: 350-356.

- Ebina, J., T. Tsutsui & T. Shirai, 1983. Simultaneous determination of total nitrogen and total phosphorus in water using peroxodisulfate oxidation, *Water Research* 17: 1721-1726.
- Eggert, S. L. & J. B. Wallace, 2003. Litter breakdown and invertebrate detritivores in a resource-depleted Appalachian stream, *Archiv fur Hydrobiologie* 156: 315-338.
- Fazi, S., S. Amalfitano, J. Pernthaler & A. Puddu, 2005. Bacterial communities associated with benthic organic matter in headwater stream microhabitats, *Environmental Microbiology* 7: 1633-1640.
- Fierer, N., J. P. Schimel & P. A. Holden, 2003. Influence of drying-rewetting frequency on soil bacterial community structure, *Microbial Ecology* 45: 63-71.
- Findlay, S., 1995. Importance of surface-subsurface exchange in stream ecosystems: The hyporheic zone, *Limnology & Oceanography* 40 : 159-164.
- Findlay, S. & W. V. Sobczak, 2000. Microbial communities in hyporheic sediments. In Jones, J. B. Jr. & P. J. Mulholland (eds), *Streams and Ground Waters*. Academic Press, San Diego, California: 287-306.
- Fioretto, A., C. Di Nardo, S. Papa & A. Fuggi, 2005. Lignin and cellulose degradation and nitrogen dynamics during decomposition of three leaf litter species in a Mediterranean ecosystem, *Soil Biology & Biochemistry* 37: 1083-1091.
- Fischer, H., M. Pusch & J. Schwoerbel, 1996. Spatial distribution and respiration of bacteria in stream-bed sediments, *Archiv fur Hydrobiologie* 137: 281-300.
- Fischer, H. & M. Pusch, 2001. Comparison of bacterial production in sediments, epiphyton and the pelagic zone of a lowland river, *Freshwater Biology* 46: 1335-1348.
- Fischer, H., S. C. Wanner & M. Pusch, 2002. Bacterial abundance and production in river sediments as related to the biochemical composition of particulate organic matter (POM), *Biogeochemistry* 61: 37-55.
- Fisher, S. G. & G. E. Likens, 1973. Energy flow in Bear Brook, New Hampshire - integrative approach to stream ecosystem metabolism, *Ecological Monographs* 43: 421-439.
- Fisher, S. G., 1977. Organic-matter processing by a stream-segment ecosystem - Fort River, Massachusetts, USA, *Internationale Revue der Gesamten Hydrobiologie* 62: 701-727.
- Fisher, S. G., L. J. Gray, N. B. Grimm & D. E. BUSCH, 1982. Temporal succession in a desert stream ecosystem following flash flooding, *Ecological Monographs* 52: 93-110.
- Francoeur, S. N. & B. J. F. Biggs, 2006. Short-term effects of elevated velocity and sediment abrasion on benthic algal communities, *Hydrobiologia* 561: 59-69.

- Frossard, A., 2011. Microbial dynamics and function during stream ecosystem succession: community structure and enzymatic activities. ETH Zurich.
- Fuss, C. L. & L. A. Smock, 1996. Spatial and temporal variation of microbial respiration rates in a blackwater stream, *Freshwater Biology* 36: 339-349.
- Gerbersdorf, S. U., B. Westrich & D. M. Paterson, 2009. Microbial extracellular polymeric substances (EPS) in fresh water sediments, *Microbial Ecology* 58: 334-349.
- Gerull, L., A. Frossard, M. O. Gessner & M. Mutz, 2011. Variability of heterotrophic metabolism in small stream corridors of an early successional watershed, *Journal of Geophysical Research - Biogeosciences* 16- G02012.
- Gerwin, W., W. Schaaf, D. Biemelt, A. Fischer, S. Winter & R. F. Hüttl, 2009. The artificial catchment "Chicken Creek" (Lusatia, Germany)-A landscape laboratory for interdisciplinary studies of initial ecosystem development, *Ecological Engineering* 35: 1786-1796.
- Gessner, M. O. & E. Chauvet, 1993. Ergosterol-to-biomass conversion factors for aquatic hyphomycetes, *Applied and environmental microbiology* 59: 502-507.
- Gessner, M. O. & E. Chauvet, 1994. Importance of stream microfungi in controlling breakdown rates of leaf-litter, *Ecology* 75: 1807-1817.
- Gessner, M. O. & A. L. Schmitt, 1996. Use of solid-phase extraction to determine ergosterol concentrations in plant tissue colonized by fungi, *Applied and environmental microbiology* 62: 415-419.
- Gessner, M. O. & S. Y. Newell, 2002. Biomass, growth rate, and production of filamentous fungi in plant litter. In Hurst, C. J., R. L. Crawford, G. R. Knudsen, M. J. McInerney & L. D. Stelzenbach (eds), *Manual of Environmental Microbiology*. ASM Press, Washington D.C., USA: 390-408.
- Gessner, M. O., F. Bärlocher & E. Chauvet, 2003. Qualitative and quantitative analyses of aquatic hyphomycetes in streams. In Tsui, C. K. M. & K. D. Hyde (eds), *Freshwater Mycology*. Koeltz Scientific Books, Koenigstein, Germany: 127-157.
- Gessner, M. O., V. Gulis, K. A. Kuehn & K. Suberkropp, 2007. Fungal decomposers of plant litter in aquatic ecosystems. In Kubicek, C. P. & I. S. Druzhinina (eds), *EnThe Mycota, Environmental and Microbial Relationships*. Springer, Berlin, Heidelberg: 301-324.
- Gessner, M. O., C. M. Swan, C. K. Dang, B. G. Mckie, R. D. Bardgett, D. H. Wall & S. Hattenschwiler, 2010. Diversity meets decomposition, *Trends in Ecology & Evolution* 25: 372-380.
- Gleason, H. A., 1927. Further views on the succession-concept, *Ecology* 8: 299-326.

- Goedkoop, W., K. R. Gullberg, R. K. Johnson & I. Ahlgren, 1997. Microbial response of a freshwater benthic community to a simulated diatom sedimentation event: Interactive effects of benthic fauna, *Microbial Ecology* 34: 131-143.
- Golladay, S. W., 1997. Suspended particulate organic matter concentration and export in streams, *Journal of the North American Benthological Society* 16: 122-131.
- Graf, W. L., 1988. *Fluvial processes in dryland rivers*. Springer-Verlag, Berlin.
- Griffin, D. H., 1994. *Fungal physiology*. Wiley-Liss, New York.
- Griffiths, N. A., J. L. Tank, T. V. Royer, E. J. Rosi-Marshall, M. R. Whiles, C. P. Chambers, T. C. Frauendorf & M. A. Evans-White, 2009. Rapid decomposition of maize detritus in agricultural headwater streams, *Ecological Applications* 19: 133-142.
- Grimm, N. B. & S. G. Fisher, 1984. Exchange between interstitial and surface-water - Implications for stream metabolism and nutrient cycling, *Hydrobiologia* 111: 219-228.
- Grimm, N. B. & S. G. Fisher, 1989. Stability of periphyton and macroinvertebrates to disturbance by flash floods in a desert stream, *Journal of the North American Benthological Society* 8: 293-307.
- Grimm, N. B., 1994. Disturbance, succession, and ecosystem processes in streams: a case study from the desert. In Giller, P. S., A. G. Hildrew & D. G. Raffaelli (eds), *Aquatic ecology: scale pattern and processes*. Blackwell Scientific Publications, Oxford, England, 93-112.
- Gulis, V., 2001. Are there any substrate preferences in aquatic hyphomycetes?, *Mycological Research* 105: 1088-1093.
- Gulis, V. & K. Suberkropp, 2003. Effect of inorganic nutrients on relative contributions of fungi and bacteria to carbon flow from submerged decomposing leaf litter, *Microbial Ecology* 45: 11-19.
- Gupta, S. & S. C. Agrawal, 2007. Survival and motility of diatoms *Navicula grimmei* and *Nitzschia palea* affected by some physical and chemical factors, *Folia Microbiologica* 52: 127-134.
- Haack, T. K. & G. A. Mcfeters, 1982. Nutritional relationships among microorganisms in an epilithic biofilm community, *Microbial Ecology* 8: 115-126.
- Hammes, F. A. & T. Egli, 2005. New method for assimilable organic carbon determination using flow-cytometric enumeration and a natural microbial consortium as inoculum, *Environmental Science and Technology* 39: 3289-3294.
- Hargrave, B. T., 1973. Coupling carbon flow through some pelagic and benthic communities, *Journal of the Fisheries Research Board of Canada* 30: 1317-1326.

- Harms, T. K. & N. B. Grimm, 2008. Hot spots and hot moments of carbon and nitrogen dynamics in a semiarid riparian zone, *Journal of Geophysical Research-Biogeosciences* 113: G01020.
- Harrop, B. L., J. C. Marks & M. E. Watwood, 2009. Early bacterial and fungal colonization of leaf litter in Fossil Creek, Arizona, *Journal of the North American Benthological Society* 28: 383-396.
- Hedin, L. O., 1990. Factors controlling sediment community respiration in woodland stream ecosystems, *Oikos* 57: 94-105.
- Heffernan, J. B., 2008. Wetlands as an alternative stable state in desert streams, *Ecology* 89: 1261-1271.
- Hieber, M. & M. O. Gessner, 2002. Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates, *Ecology* 83: 1026-1038.
- Hill, B. H., A. T. Herlihy & P. R. Kaufmann, 2002. Benthic microbial respiration in Appalachian Mountain, Piedmont, and Coastal Plains streams of the eastern USA, *Freshwater Biology* 47: 185-194.
- Hillebrand, H. & U. Sommer, 1999. The nutrient stoichiometry of benthic microalgal growth: Redfield proportions are optimal, *Limnology and Oceanography* 44: 440-446.
- Hladyz, S., M. O. Gessner, P. S. Giller, J. Pozo & G. Woodward, 2009. Resource quality and stoichiometric constraints on stream ecosystem functioning, *Freshwater Biology* 54: 957-970.
- Holmes, R. M., S. G. Fisher, N. B. Grimm & B. J. Harper, 1998. The impact of flash floods on microbial distribution and biogeochemistry in the parafluvial zone of a desert stream, *Freshwater Biology* 40: 641-654.
- Hoorens, B., R. Aerts & M. Stroetenga, 2002. Litter quality and interactive effects in litter mixtures: more negative interactions under elevated CO₂?, *Journal of Ecology* 90: 1009-1016.
- Houser, J. N., P. J. Mulholland & K. O. Maloney, 2005. Catchment disturbance and stream metabolism: patterns in ecosystem respiration and gross primary production along a gradient of upland soil and vegetation disturbance, *Journal of the North American Benthological Society* 24: 538-552.
- Hurny, A. D., R. H. Riley, R. G. Young, C. J. Arbuckle, K. Peacock & G. Lyon, 2001. Temporal shift in contribution of terrestrial organic matter to consumer production in a grassland river, *Freshwater Biology* 46: 213-226.
- Jones, J. B., S. G. Fisher & N. B. Grimm, 1995. Vertical hydrologic exchange and ecosystem metabolism in a Sonoran desert stream, *Ecology* 76: 942-952.

- Jones, J. B., 1995. Factors controlling hyporheic respiration in a desert stream, *Freshwater Biology* 34: 91-99.
- Jones, J. B., 1997. Benthic organic matter storage in streams: Influence of detrital import and export, retention mechanisms, and climate, *Journal of the North American Benthological Society* 16: 109-119.
- Judd, K. E., B. C. Crump & G. W. Kling, 2006. Variation in dissolved organic matter controls bacterial production and community composition, *Ecology* 87: 2068-2079.
- Kaplan, L. A. & T. L. Bott, 1989. Diel fluctuations in bacterial-activity on streambed substrata during vernal algal blooms - Effects of temperature, water, chemistry and habitat, *Limnology and Oceanography* 34: 718-733.
- Kirchman, D., E. Knees & R. Hodson, 1985. Leucine incorporation and its potential as a measure of protein-synthesis by bacteria in natural aquatic systems, *APPLIED AND ENVIRONMENTAL MICROBIOLOGY* 49: 599-607.
- Klausmeier, C. A., E. Litchman, T. Daufresne & S. A. Levin, 2004. Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton, *Nature* 429: 171-174.
- Kominkova, D., K. A. Kuehn, N. Busing, D. Steiner & M. O. Gessner, 2000. Microbial biomass, growth, and respiration associated with submerged litter of *Phragmites australis* decomposing in a littoral reed stand of a large lake, *Aquatic Microbial Ecology* 22: 271-282.
- Kominoski, J. S., C. M. PRINGLE, B. A. Ball, M. A. Bradford, D. C. Coleman, D. B. Hall & M. D. Hunter, 2007. Nonadditive effects of leaf litter species diversity on breakdown dynamics in a detritus-based stream, *Ecology* 88: 1167-1176.
- Kominoski, J. S., T. J. Hoellein, J. J. Kelly & C. M. PRINGLE, 2009. Does mixing litter of different qualities alter stream microbial diversity and functioning on individual litter species?, *Oikos* 118: 457-463.
- Koutny, J. & M. Rulik, 2007. Hyporheic biofilm particulate organic carbon in a small lowland stream (Sitka, Czech republic): Structure and distribution, *International Review of Hydrobiology* 92: 402-412.
- Kritzberg, E. S., J. J. Cole, M. M. Pace & W. Graneli, 2005. Does autochthonous primary production drive variability in bacterial metabolism and growth efficiency in lakes dominated by terrestrial C inputs?, *Aquatic Microbial Ecology* 38: 103-111.
- Kuehn, K. A., D. Steiner & M. O. Gessner, 2004. Diel mineralization patterns of standing-dead plant litter: Implications for CO₂ flux from wetlands, *Ecology* 85: 2504-2518.

- Laitung, B., J. L. Pretty, E. Chauvet & M. Dobson, 2002. Response of aquatic hyphomycete communities to enhanced stream retention in areas impacted by commercial forestry, *Freshwater Biology* 47: 313-323.
- Lake, P. S., 2000. Disturbance, patchiness, and diversity in streams, *Journal of the North American Benthological Society* 19: 573-592.
- Larned, S. T., T. Datry & C. T. Robinson, 2007. Invertebrate and microbial responses to inundation in an ephemeral river reach in New Zealand: effects of preceding dry periods, *Aquatic Sciences* 69: 554-567.
- Larned, S. T., T. Datry, D. B. Arscott & K. Tockner, 2010. Emerging concepts in temporary-river ecology, *Freshwater Biology* 55: 717-738.
- Leichtfried, M., 1985. Organic matter in gravel streams (Project RITRODAT-LUNZ), *Verhandlungen des Internationalen Verein Limnologie* 22: 2058-2062.
- Loch, R. J., 2000. Effects of vegetation cover on runoff and erosion under simulated rain and overland flow on a rehabilitated site on the Meandu Mine, Tarong, Queensland, *Australian Journal of Soil Research* 38: 299-312.
- Lock, M. A., R. R. Wallace, J. W. Costerton, R. M. Ventullo & S. E. Charlton, 1984. River epilithon - Toward a structural-functional model, *Oikos* 42: 10-22.
- Loferer-Krössbacher, M., L. Klima & R. Psenner, 1998. Determination of bacterial cell dry mass by transmission electron microscopy and densitometric image analysis, *Applied and environmental microbiology* 64: 688-694.
- Luce, J. J., A. Cattaneo & M. F. Lapointe, 2010. Spatial patterns in periphyton biomass after low-magnitude flow spates: geomorphic factors affecting patchiness across gravel-cobble riffles, *Journal of the North American Benthological Society* 29: 614-626.
- Ludwig, J. A., B. P. Wilcox, D. D. Breshears, D. J. Tongway & A. C. Imeson, 2005. Vegetation patches and runoff-erosion as interacting ecohydrological processes in semiarid landscapes, *Ecology* 86: 288-297.
- Mackay, W. P., J. Zak & W. G. Whitford, 1992. Litter decomposition in a Chihuahuan desert playa, *American Midland Naturalist* 128: 89-94.
- Malard, F., K. Tockner & J. V. Ward, 2002. A landscape perspective of surface-subsurface hydrological exchanges in river corridors, *Freshwater Biology* 47: 621-640.
- Malcolm, I. A., C. A. Middlemas, C. Soulsby, S. J. Middlemas & A. F. Youngson, 2010. Hyporheic zone processes in a canalised agricultural stream: implications for salmonid embryo survival, *Fundamental and Applied Limnology* 176: 319-336.

- Maltchik, L. & F. Pedro, 2001. Responses of aquatic macrophytes to disturbance by flash floods in a Brazilian semiarid intermittent stream, *Biotropica* 33: 566-572.
- Marks, J. C., G. Haden, B. L. Harrop, E. G. Reese, J. L. Keams, M. E. Watwood & T. G. Whitham, 2009. Genetic and environmental controls of microbial communities on leaf litter in streams, *Freshwater Biology* 54: 2616-2627.
- Martin, J. G. & P. V. Bolstad, 2009. Variation of soil respiration at three spatial scales: Components within measurements, intra-site variation and patterns on the landscape, *Soil Biology & Biochemistry* 41: 530-543.
- Marxsen, J., 2006. Bacterial production in the carbon flow of a central European stream, the Breitenbach, *Freshwater Biology* 51: 1838-1861.
- Marxsen, J., A. Zoppini & S. Wilczek, 2010. Microbial communities in streambed sediments recovering from desiccation, *Fems Microbiology Ecology* 71: 374-386.
- McArthur, J. V., G. R. Marzolf & J. E. Urban, 1985. Response of bacteria isolated from a pristine prairie stream to concentration and source of soluble organic-carbon, *Applied and environmental microbiology* 49: 238-241.
- McArthur, M. D. & J. S. Richardson, 2002. Microbial utilization of dissolved organic carbon leached from riparian litterfall, *Canadian Journal of Fisheries and Aquatic Sciences* 59: 1668-1676.
- McCallister, S. L. & P. A. del Giorgio, 2008. Direct measurement of the delta C-13 signature of carbon respired by bacteria in lakes: Linkages to potential carbon sources, ecosystem baseline metabolism, and CO₂ fluxes, *Limnology and Oceanography* 53: 1204-1216.
- McClain, M. E., E. W. Boyer, C. L. Dent, S. E. Gergel, N. B. Grimm, P. M. Groffman, S. C. Hart, J. W. Harvey, C. A. Johnston, E. Mayorga, W. H. McDowell & G. Pinay, 2003. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems, *Ecosystems* 6: 301-312.
- McCormick, P. V. & R. J. Stevenson, 1991. Mechanisms of benthic algae succession in lotic environments, *Ecology* 72: 1835-1848.
- Menninger, H. L. & M. A. Palmer, 2007. Herbs and grasses as an allochthonous resource in open-canopy headwater streams, *Freshwater Biology* 52: 1689-1699.
- Merz, R. & G. Blöschl, 2003. A process typology of regional floods, *Water Resources Research* 39.
- Metzler, G. M. & L. A. Smock, 1990. Storage and dynamics of subsurface detritus in a sand-bottomed stream, *Canadian Journal of Fisheries and Aquatic Sciences* 47: 588-594.
- Meyer, J. L., 1994. The Microbial Loop in Flowing Waters, *Microbial Ecology* 28: 195-199.

- Mille-Lindblom, C. & L. J. Tranvik, 2003. Antagonism between bacteria and fungi on decomposing aquatic plant litter, *Microbial Ecology* 45: 173-182.
- Mille-Lindblom, C., H. Fischer & L. J. Tranvik, 2006. Litter-associated bacteria and fungi - a comparison of biomass and communities across lakes and plant species, *Freshwater Biology* 51: 730-741.
- Milner, A. M., E. E. Knudsen, C. Soiseth, A. L. Robertson, D. Schell, I. T. Phillips & K. Magnusson, 2000. Colonization and development of stream communities across a 200-year gradient in Glacier Bay National Park, Alaska, USA, *Canadian Journal of Fisheries and Aquatic Sciences* 57: 2319-2335.
- Milner, A. M. & I. T. Gloyne-Phillips, 2005. The role of riparian vegetation and woody debris in the development of macroinvertebrate assemblages in streams, *River Research and Applications* 21: 403-420.
- Milner, A. M., C. L. Fastie, F. S. Chapin, D. R. Engstrom & L. C. Sharman, 2007. Interactions and linkages among ecosystems during landscape evolution, *Bioscience* 57: 237-247.
- Milner, A. M. & A. L. Robertson, 2010. Colonization and succession of stream communities in Glacier Bay, Alaska; What has it contributed to general successional theory?, *River Research and Applications* 26: 26-35.
- Minshall, G. W., R. C. Petersen, K. W. Cummins, T. L. Bott, J. A. Sedell, C. E. Cushing & R. L. Vannote, 1983. Interbiome Comparison of Stream Ecosystem Dynamics, *Ecological Monographs* 53: 2-25.
- Mitchell, C. P. J. & B. A. Branfireun, 2005. Hydrogeomorphic controls on reduction-oxidation conditions across boreal upland-peatland interfaces, *Ecosystems* 8: 731-747.
- Moore, S., T. Wallington, R. Hobbs, P. Ehrlich, C. Holling, S. Levin, D. Lindenmayer, C. Pahl-Wostl, H. Possingham, M. Turner & M. Westoby, 2009. Diversity in current ecological thinking: implications for environmental management, *Environmental Management* 43: 17-27.
- Morisawa, M., 1968. *Streams, their dynamics and morphology*. McGraw-Hill, New York.
- Mulholland, P. J., A. D. Steinman, A. V. Palumbo, D. L. Deangelis & T. E. Flum, 1991. Influence of nutrients and grazing on the response of stream periphyton communities to a scour disturbance, *Journal of the North American Benthological Society* 10: 127-142.
- Mulholland, P. J., E. R. Marzolf, J. R. Webster, D. R. Hart & S. P. Hendricks, 1997. Evidence that hyporheic zones increase heterotrophic metabolism and phosphorus uptake in forest streams, *Limnology & Oceanography* 42: 443-451.
- Mulholland, P. J., C. S. Fellows, J. L. Tank, N. B. Grimm, J. R. Webster, S. K. Hamilton, E. Marti, L. Ashkenas, W. B. Bowden, W. K. Dodds, W. H. McDowell, M. J. Paul & B. J.

- Peterson, 2001. Inter-biome comparison of factors controlling stream metabolism, *Freshwater Biology* 46: 1503-1517.
- Mutz, M., D. Leßmann, R. Ender & J. Schließ, 2002. Development of sustainable streams after opencast coal mining in Lusatia, Germany. Eigenverlag des Forums für Abfallwirtschaft und Altlasten e.V., Technische Universität Dresden, 125-129.
- Naegeli, M. W., U. Hartmann, E. I. Meyer & U. Uehlinger, 1995. POM-Dynamics and community respiration in the sediments of a floodprone prealpine river (Necker, Switzerland), *Archiv für Hydrobiologie* 133: 339-347.
- Naegeli, M. W. & U. Uehlinger, 1997. Contribution of the hyporheic zone to ecosystem metabolism in a prealpine gravel-bed river, *Journal of the North American Benthological Society* 16: 794-804.
- Neely, R. K., 1994. Evidence for positive interactions between epiphytic algae and heterotrophic decomposers during the decomposition of *Typha-latifolia*, *Archiv für Hydrobiologie* 129: 443-457.
- Neely, R. K. & R. G. Wetzel, 1997. Autumnal production by bacteria and autotrophs attached to *Typha latifolia* detritus, *Journal of Freshwater Ecology* 12: 253-267.
- Newell, S. Y., R. D. Fallon & J. D. Miller, 1989. Decomposition and microbial dynamics for standing, Naturally positioned leaves of the salt-marsh grass *Spartina-alterniflora*, *Marine Biology* 101: 471-481.
- Niyogi, D. K., K. S. Simon & C. R. Townsend, 2003. Breakdown of tussock grass in streams along a gradient of agricultural development in New Zealand, *Freshwater Biology* 48: 1698-1708.
- Norland, S., 1993. The relationship between biomass and volume of bacteria. In Kemp, P. F., B. F. Sherr, E. B. Sherr & J. J. Cole (eds), *Handbook of Methods in Aquatic Microbiology*. Lewis Publishers, 303-307.
- Noy-Meir, I., 1973. Desert ecosystems: environment and producers, *Annual Review of Ecology and Systematics* 4: 25-51.
- Odum, E. P., 1969. Strategy of ecosystem development, *Science* 164: 262-270.
- Odum, H. T., 1956. Primary production in flowing waters, *Limnology and Oceanography* 1: 102-117.
- Oksanen, J., R. Kindt, P. Legendre, B. O'Hara, G. L. Simpson & M. H. H. Stevens, 2011. *vegan: Community ecology package*, 2010.

- Ostrofsky, M. L., 1997. Relationship between chemical characteristics of autumn-shed leaves and aquatic processing rates, *Journal of the North American Benthological Society* 16: 750-759.
- Pascoal, C. & F. Cassio, 2004. Contribution of fungi and bacteria to leaf litter decomposition in a polluted river, *Applied and environmental microbiology* 70: 5266-5273.
- Peterson, C. G. & R. J. Stevenson, 1992. Resistance and resilience of lotic algal communities - Importance of disturbance timing and current, *Ecology* 73: 1445-1461.
- Peterson, C. G., 1996. Response of algae to natural physical disturbance. In Stevenson, R. J., M. L. Bothwell & R. L. Lowe (eds), *Algal Ecology - Freshwater Benthic Ecosystems*. Academic Press, San Diego: 375-402.
- Peterson, C. G., 1996. Mechanisms of lotic microalgal colonization following space-clearing disturbances acting at different spatial scales, *Oikos* 77: 417-435.
- Pickett, S. T. A., 1982. Population-patterns through 20 years of oldfield succession, *Vegetatio* 49: 45-59.
- Pickett, S. T. A. & P. S. White, 1985. The ecology of natural disturbance and patch dynamics, Pickett, S. T. A. and P. S. White (Ed.). *the Ecology of Natural Disturbance and Patch Dynamics*. Xv+472P. Academic Press, Inc. , Publishers: Orlando, Fla. , Usa; London, England. Illus. Maps.
- Pinheiro, P., S. DebRoy, D. Sarkar, D. Bates & R Development Team, 2011. nlme: linear and nonlinear mixed effects models.
- Powell, D. M., R. Brazier, J. Wainwright, A. Parsons & J. Kaduk, 2005. Streambed scour and fill in low-order dryland channels, *Water Resources Research* 41.
- Powell, D. M., R. Brazier, A. Parsons, J. Wainwright & M. Nichols, 2007. Sediment transfer and storage in dryland headwater streams, *Geomorphology* 88: 152-166.
- Power, M. E. & A. J. Stewart, 1987. Disturbance and recovery of an algal assemblage following flooding in an Oklahoma stream, *American Midland Naturalist* 117: 333-345.
- Power, M. E., 1990. Resource enhancement by indirect effects of grazers - Armored catfish, algae, and sediment, *Ecology* 71: 897-904.
- Pusch, M. & J. Schwoerbel, 1994. Community respiration in hyporheic sediments of a mountain stream (Steina, Black Forest), *Archiv fur Hydrobiologie* 130: 35-52.
- Pusch, M., 1996. The metabolism of organic matter in the hyporheic zone of a mountain stream, and its spatial distribution, *Hydrobiologia* 323: 107-118.

- R Development Core Team, 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria.
- Rabeni, C. F., K. E. Doisy & L. D. Zweig, 2005. Stream invertebrate community functional responses to deposited sediment, *Aquatic Sciences* 67: 395-402.
- Ramette, A., 2009. Quantitative community fingerprinting methods for estimating the abundance of operational taxonomic units in natural microbial communities, *Applied and environmental microbiology* 75: 2495-2505.
- Rees, G. N., P. M. Bowen & G. O. Watson, 2005. Variability in benthic respiration in three southeastern Australian lowland rivers, *River Research and Applications* 21: 1147-1156.
- Reice, S. R., 1991. Effects of detritus loading and fish predation on leafpack breakdown and benthic macroinvertebrates in a woodland stream, *Journal of the North American Benthological Society* 10: 42-56.
- Rennie, C. D. & R. G. Millar, 2000. Spatial variability of stream bed scour and fill: a comparison of scour depth in chum salmon (*Oncorhynchus keta*) redds and adjacent bed, *Canadian Journal of Fisheries and Aquatic Sciences* 57: 928-938.
- Resh, V. H., A. V. Brown, A. P. Covich, M. E. Gurtz, H. W. Li, G. W. Minshall, S. R. Reice, A. L. Sheldon, J. B. Wallace & R. C. Wissmar, 1988. The role of disturbance in stream ecology, *Journal of the North American Benthological Society* 7: 433-455.
- Rier, S. T., K. A. Kuehn & S. N. Francoeur, 2007. Algal regulation of extracellular enzyme activity in stream microbial communities associated with inert substrata and detritus, *Journal of the North American Benthological Society* 26: 439-449.
- Riis, T. & B. J. F. Biggs, 2003. Hydrologic and hydraulic control of macrophyte establishment and performance in streams, *Limnology and Oceanography* 48: 1488-1497.
- Riis, T., B. J. F. Biggs & M. Flanagan, 2004. Colonisation and temporal dynamics of macrophytes in artificial stream channels with contrasting flow regimes, *Archiv fur Hydrobiologie* 159: 77-95.
- Roberts, B., P. J. Mulholland & W. Hill, 2007. Multiple scales of temporal variability in ecosystem metabolism rates: Results from 2 years of continuous monitoring in a forested headwater stream, *Ecosystems* 10: 588-606.
- Romani, A. M. & S. Sabater, 1997. Metabolism recovery of a stromatolitic biofilm after drought in a Mediterranean stream, *Archiv fur Hydrobiologie* 140: 261-271.
- Romani, A. M., A. Butturini, F. Sabater & S. Sabater, 1998. Heterotrophic metabolism in a forest stream sediment: surface versus subsurface zones, *Aquatic Microbial Ecology* 16: 143-151.

- Romani, A. M. & S. Sabater, 1999. Effect of primary producers on the heterotrophic metabolism of a stream biofilm, *Freshwater Biology* 41: 729-736.
- Romani, A. M. & S. Sabater, 2000. Influence of algal biomass on extracellular enzyme activity in river biofilms, *Microbial Ecology* 40: 16-24.
- Romani, A. M., H. Fischer, C. Mille-Lindblom & L. J. Tranvik, 2006. Interactions of bacteria and fungi on decomposing litter: Differential extracellular enzyme activities, *Ecology* 87: 2559-2569.
- Rozema, J., M. Tosserams, H. J. M. Nelissen, L. van Heerwaarden, R. A. Broekman & N. Flierman, 1997. Stratospheric ozone reduction and ecosystem processes: Enhanced UV-B radiation affects chemical quality and decomposition of leaves of the dune grassland species *Calamagrostis epigeios*, *Plant Ecology* 128: 284-294.
- Sabater, F., A. Butturini, E. Marti, I. Munoz, A. Romani, J. Wray & S. Sabater, 2000. Effects of riparian vegetation removal on nutrient retention in a Mediterranean stream, *Journal of the North American Benthological Society* 19: 609-620.
- Sala, O. E. & W. K. Lauenroth, 1982. Small rainfall events - An ecological role in semi-arid regions, *Oecologia* 53: 301-304.
- Scarsbrook, M. R. & C. R. Townsend, 1994. The roles of grass leaf-litter in streams draining tussock grassland in New-Zealand - retention, food-supply and substrate stabilization, *Freshwater Biology* 32: 429-443.
- Schaaf, W., O. Bens, A. Fischer, H. H. Gerke, W. Gerwin, U. Gruenewald, H. M. Hollaender, I. Koegel-Knabner, M. Mutz, M. Schloter, R. Schulin, M. Veste, S. Winter & R. F. Huettl, 2011. Patterns and processes of initial terrestrial-ecosystem development, *Journal of Plant Nutrition and Soil Science* 174: 229-239.
- Schade, J. D. & S. G. Fisher, 1997. Leaf litter in a Sonoran Desert stream ecosystem, *Journal of the North American Benthological Society* 16: 612-626.
- Schanz, F. & H. Juon, 1983. 2 different methods of evaluating nutrient limitations of periphyton bioassays, using water from the river Rhine and 8 of its tributaries, *Hydrobiologia* 102: 187-195.
- Schelske, C. L. & E. F. Stoermer, 1971. Eutrophication, silica depletion and predicted changes in algal quality in Lake Michigan, *Science* 173: 423-424.
- Schimel, J. P. & M. N. Weintraub, 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model, *Soil Biology & Biochemistry* 35: 549-563.
- Schwendel, A. C., R. G. Death & I. C. Fuller, 2010. The assessment of shear stress and bed stability in stream ecology, *Freshwater Biology* 55: 261-281.

- Shaftel, R. S., R. S. King & J. A. Back, 2011. Breakdown rates, nutrient concentrations, and macroinvertebrate colonization of bluejoint grass litter in headwater streams of the Kenai Peninsula, Alaska, *Journal of the North American Benthological Society* 30: 386-398.
- Shields, F. D., 2009. Do we know enough about controlling sediment to mitigate damage to stream ecosystems?, *Ecological Engineering* 35: 1727-1733.
- Sinsabaugh, R. L., C. L. Lauber, M. N. Weintraub, B. Ahmed, S. D. Allison, C. Crenshaw, A. R. Contosta, D. Cusack, S. Frey, M. E. Gallo, T. B. Gartner, S. E. Hobbie, K. Holland, B. L. Keeler, J. S. Powers, M. Stursova, C. Takacs-Vesbach, M. P. Waldrop, M. D. Wallenstein, D. R. Zak & L. H. Zeglin, 2008. Stoichiometry of soil enzyme activity at global scale, *Ecology Letters* 11: 1252-1264.
- Snajdr, J., T. Cajthaml, V. Valaskova, V. Merhautova, M. Petrankova, P. Spetz, K. Leppanen & P. Baldrian, 2011. Transformation of *Quercus petraea* litter: successive changes in litter chemistry are reflected in differential enzyme activity and changes in the microbial community composition, *Fems Microbiology Ecology* 75: 291-303.
- Sobczak, W. V. & T. M. Burton, 1996. Epilithic bacterial and algal colonization in a stream run, riffle, and pool: A test of biomass covariation, *Hydrobiologia* 332: 159-166.
- Sobczak, W. V. & S. Findlay, 2002. Variation in bioavailability of dissolved organic carbon among stream hyporheic flowpaths, *Ecology* 83: 3194-3209.
- Sponseller, R. A. & S. G. Fisher, 2006. Drainage size, stream intermittency, and ecosystem function in a Sonoran Desert landscape, *Ecosystems* 9: 344-356.
- Sponseller, R. A., 2007. Precipitation pulses and soil CO₂ flux in a Sonoran desert ecosystem, *Global Change Biology* 13: 426-436.
- Stanley, E. H., S. M. Powers & N. R. Lottig, 2010. The evolving legacy of disturbance in stream ecology: concepts, contributions, and coming challenges, *Journal of the North American Benthological Society* 29: 67-83.
- Sterner, R. W. & J. J. Elser, 2002. *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton University Press, New Jersey, USA.
- Stevenson, R. J., 1996. An Introduction to algal ecology in freshwater benthic habitats. In Stevenson, R. J., M. L. Bothwell & R. L. Lowe (eds), *Algal Ecology - Freshwater Benthic Ecosystems*. Academic Press, San Diego: 3-30.
- Strickland, M. S., C. Lauber, N. Fierer & M. A. Bradford, 2009. Testing the functional significance of microbial community composition, *Ecology* 90: 441-451.
- Suberkropp, K. & E. Chauvet, 1995. Regulation of leaf breakdown by fungi in streams - influences of water chemistry, *Ecology* 76: 1433-1445.

- Suberkropp, K., 1995. The influence of nutrients on fungal growth, productivity, and sporulation during leaf breakdown in streams, *Canadian Journal of Botany-Revue Canadienne de Botanique* 73: S1361-S1369.
- Suberkropp, K. & M. O. Gessner, 2004. Aceate incorporation into ergosterol to determine fungal growth rates and production. In Graca, M. A. S., F. Bärlocher & M. O. Gessner (eds), *Methods to Study Litter Decomposition: A Practical Guide*. Kluwer Academic Publishers, Netherlands: 177-181.
- Swan, C. M. & M. A. Palmer, 2004. Leaf diversity alters litter breakdown in a Piedmont stream, *Journal of the North American Benthological Society* 23: 15-28.
- Tank, J. L., E. J. Rosi-Marshall, N. A. Griffiths, S. A. Entekin & M. L. Stephen, 2010. A review of allochthonous organic matter dynamics and metabolism in streams, *Journal of the North American Benthological Society* 29: 118-146.
- Taylor, B. R., C. Mallaley & J. F. Cairns, 2007. Limited evidence that mixing leaf litter accelerates decomposition or increases diversity of decomposers in streams of eastern Canada, *Hydrobiologia* 592: 405-422.
- Taylor, G. T., J. Way, Y. Yu & M. I. Scranton, 2003. Ectohydrolase activity in surface waters of the Hudson River and western Long Island Sound estuaries, *Marine Ecology-Progress Series* 263: 1-15.
- Thomas, A. D. & S. R. Hoon, 2010. Carbon dioxide fluxes from biologically-crusted Kalahari sands after simulated wetting, *Journal of Arid Environments* 74: 131-139.
- Tiegs, S. D., F. D. Peter, C. T. Robinson, U. Uehlinger & M. O. Gessner, 2008. Leaf decomposition and invertebrate colonization responses to manipulated litter quantity in streams, *Journal of the North American Benthological Society* 27: 321-331.
- Tornblom, E., 1996. Bacterial production and total community metabolism in sediments of a eutrophic lake, *Ergebnisse der Limnologie* 0: 207-216.
- Torzilli, A. P., M. Sikaroodi, D. Chalkley & P. M. Gillevet, 2006. A comparison of fungal communities from four salt marsh plants using automated ribosomal intergenic spacer analysis (ARISA), *Mycologia* 98: 690-698.
- Treton, C., E. Chauvet & J. Y. Charcosset, 2004. Competitive interaction between two aquatic hyphomycete species and increase in leaf litter breakdown, *Microbial Ecology* 48: 439-446.
- Uehlinger, U. & M. W. Naegeli, 1998. Ecosystem metabolism, disturbance, and stability in a prealpine gravel bed river, *Journal of the North American Benthological Society* 17: 165-178.

- Uehlinger, U., 2000. Resistance and resilience of ecosystem metabolism in a flood-prone river system, *Freshwater Biology* 45: 319-332.
- Uehlinger, U., M. Naegeli & S. G. Fisher, 2002. A heterotrophic desert stream? The role of sediment stability, *Western North American Naturalist* 62: 466-473.
- Uehlinger, U., B. Kawecka & C. T. Robinson, 2003. Effects of experimental floods on periphyton and stream metabolism below a high dam in the Swiss Alps (River Spol), *Aquatic Sciences* 65: 199-209.
- Valett, H. M., S. G. Fisher, N. B. Grimm & P. Camill, 1994. Vertical hydrologic exchange and ecological stability of a desert stream ecosystem, *Ecology* 75: 548-560.
- Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell & C. E. Cushing, 1980. River continuum concept, *Canadian Journal of Fisheries and Aquatic Sciences* 37: 130-137.
- Vendramini, F., S. Diaz, N. Perez-Harguindeguy, M. Cabido, J. M. Llano-Sotelo & A. Castellanos, 2000. Chemical composition and leaf traits of different plant functional types from central-western Argentina, *Kurtziana* 28: 181-193.
- Veraart, A. J., A. M. Romani, E. Tornes & S. Sabater, 2008. Algal response to nutrient enrichment in forested oligotrophic stream, *Journal of Phycology* 44: 564-572.
- Vitousek, P. M., 2004. Nutrient cycling and limitation: Hawai'i is a model system. *Princeton University Press, Princeton, NJ, USA*.
- Wallbrink, P. J., 2004. Quantifying the erosion processes and land-uses which dominate fine sediment supply to Moreton Bay, Southeast Queensland, Australia, *Journal of Environmental Radioactivity* 76: 67-80.
- Webster, J. R., J. B. Waide & B. C. Patten, 1975. Nutrient recycling and the stability of ecosystems. In Howell, F. G., Gentry J.B. & M. H. Smith (eds), *Mineral Cycling in Southeastern Ecosystems*. Illus. Maps. U.S. Energy Research, Augusta, Ga., U.S.A: 1-27.
- Webster, J. R. & E. F. Benfield, 1986. Vascular plant breakdown in fresh-water ecosystems, *Annual Review of Ecology and Systematics* 17: 567-594.
- Webster, J. R. & J. L. Meyer, 1997. Organic matter budgets for streams: A synthesis, *Journal of the North American Benthological Society* 16: 141-161.
- Wehr, J. D. & R. G. Sheath, 2003. Freshwater habitats of algae. In Wehr, J. D. & R. G. Sheath (eds), *Freshwater algae of North America*. Elsevier Academic Press, San Diego: 11-50.
- White, P. S. & S. T. A. Pickett, 1985. Natural disturbance and patch dynamics, an introduction, Pickett, S. T. A. and P. S. White (eds). *The ecology of natural disturbance and patch dynamics*. Xv+472P. Academic Press, Inc., Publishers: Orlando, Fla. , USA; London, England. Illus. Maps 3-14.

- Whittaker, R. H., 1953. A consideration of climax theory - the climax as a population and pattern, *Ecological Monographs* 23: 41-78.
- Wilcock, R. J. & G. F. Croker, 2004. Distribution of carbon between sediment and water in macrophyte dominated lowland streams, *Hydrobiologia* 520: 143-152.
- Winemiller, K. O., A. S. Flecker & D. J. Hoeinghaus, 2010. Patch dynamics and environmental heterogeneity in lotic ecosystems, *Journal of the North American Benthological Society* 29: 84-99.
- Wood, P. J. & P. D. Armitage, 1997. Biological effects of fine sediment in the lotic environment, *Environmental Management* 21: 203-217.
- Yannarell, A. C., A. D. Kent, G. H. Lauster, T. K. Kratz & E. W. Triplett, 2003. Temporal patterns in bacterial communities in three temperate lakes of different trophic status, *Microbial Ecology* 46: 391-405.
- Ylla, I., C. Borrego, A. M. Romani & S. Sabater, 2009. Availability of glucose and light modulates the structure and function of a microbial biofilm, *Fems Microbiology Ecology* 69: 27-42.
- Young, R. G. & A. D. Huryn, 1999. Effects of land use on stream metabolism and organic matter turnover, *Ecological Applications* 9: 1359-1376.
- Young, R. G., C. D. Matthaei & C. R. Townsend, 2008. Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health, *Journal of the North American Benthological Society* 27: 605-625.