

# Reciprocal influences of microbial community and hydrogeomorphology in sandy streambeds

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# Abstract

The main goal of this dissertation was to explore the interactions between the hydrogeomorphology of the streambed in sandy lowland low-order streams and the microbial community inhabiting it. In particular, (i) the influence of the vertical water exchange across the streambed and (ii) of the sediment transport on the function and structure of the streambed microbial community, (iii) and the potential of the microbial community to influence these physical factors were explored. The influences were studied with a model system approach (micro- and mesocosms).

Firstly, I examined the significance of vertical water exchange across the streambed for the microbial community. I determined the differences in the microbial community structure and function associated with sediments of differing grain sizes. The grain sizes differed in surface-to-volume ratio and hydraulic conductivity. The results revealed vertical water exchange as the major factor for the structure and function of the microbial community.

Secondly, I studied the ability of the microbial community to influence the vertical water exchange across two sandy streambeds: leveled and rippled. My results showed that the microbial community can reduce and even block the vertical water exchange by reducing pore space with gas bubbles formed due to high primary production.

Thirdly, I determined the effect of short-term sediment transport events on the function of the microbial community and on the influence of the microbial community on vertical water exchange. The results show that the mechanical stress associated with short-term sediment transport events does not influence the microbial community function. However, a single short-term sediment transport event increased vertical water exchange by (i) releasing the gas bubbles produced by the microbial community and (ii) creating irregularities in the flume bed.

Lastly, I ascertained the potential of benthic algal mats to transport sediment by means of buoyancy-mediated detachment from the bed. The results revealed the detachment of algal mats as a novel mechanism of sediment transport during low-flow periods.

Overall, the interactions studied show that in sandy streambeds (i) the pattern of vertical water exchange is the primary physical template for the microbial community, and (ii) the activity of the microbial community and sediment transport are stochastic sources of spatiotemporal heterogeneity in vertical water exchange. These results contribute to the understanding and prediction of stream ecosystem functions in sandy streams, which is of special significance in light of the increase in fine sediment load in streams worldwide.

# Zusammenfassung

Das Ziel dieser Dissertation war es, die Wechselwirkungen zwischen der Hydrogeomorphologie von sandigen Bachbetten (Bäche geringerer Ordnung - nach Strahler) und der darin lebenden mikrobiellen Gemeinschaft zu untersuchen. Im Speziellen galt es dabei, (i) den Einfluss des vertikalen Wasseraustausches zwischen Porenwasser und Bachwasser, als auch (ii) den Sedimenttransport bezogen auf die Struktur und die Funktion der mikrobiellen Gemeinschaft zu untersuchen. Zusätzlich wurde in diesem Zusammenhang (iii) das Potential der mikrobiellen Gemeinschaft genauer betrachtet, diese physikalischen Faktoren zu beeinflussen. Diese Interaktionen wurden in Modellökosystemen analysiert (Mikro- und Mesokosmos).

Als erstes habe ich geprüft, ob der vertikale Wasseraustausch zwischen Porenwasser und Bachwasser für die mikrobielle Gemeinschaft von Bedeutung ist. Die Struktur und Funktion der mikrobiellen Gemeinschaft wurde in Sedimenten unterschiedlicher Korngröße erfasst. Die Korngröße der Sedimente wurden so gewählt, dass sich das Verhältnis der besiedelbaren Oberfläche zum Nährstofftransport im Porenvolumen unterschieden hat. Die Ergebnisse zeigen, dass der vertikale Wasseraustausch der Haupteinflussfaktor für die Struktur und die Funktion der mikrobiellen Gemeinschaft ist.

Als zweites untersuchte ich die Fragestellung, in wie weit die mikrobielle Gemeinschaft den vertikalen Wasseraustausch sandiger Bachbette, die sich in ihrer Rauigkeit unterscheiden (eben und geriffelt), zu beeinflussen. Meine Ergebnisse zeigen, dass die mikrobielle Gemeinschaft den vertikalen Wasseraustausch einschränken und sogar blockieren kann. Der Porenraum wurde signifikant durch die Einlagerung von Gasblasen, die bei der Primärproduktion entstehen, reduziert.

Als drittes untersuchte ich den Effekt von kurzzeitigen Ereignissen des Sedimenttransports auf die Funktion der mikrobiellen Gemeinschaft und den damit verbundenen Einfluss auf den vertikalen Wasseraustausch. Der mechanisch wirkende Stress, verursacht durch den Sedimenttransport, hatte keinen Einfluss auf die Funktion der mikrobiellen Gemeinschaft. Dennoch erhöht ein einmaliger, kurzzeitiger Sedimenttransport den vertikalen Wasseraustausch durch (i) herauslösen der durch die mikrobiellen Gemeinschaft produzierten Gasblasen und (ii) Schaffung von Unregelmäßigkeiten im Rinnenbett.

Zuletzt ermittelte ich das Potential von auftreibenden, benthischen Algenmatten, Sediment vom Gewässerbett zu lösen und flussabwärts zu transportieren. In Fließrinnenexperimenten konnte ich zeigen, dass das Ablösen von Algenmatten ein wichtiger und bislang unberücksichtigter Mechanismus von Sedimenttransport während Perioden mit geringem Wasserabfluss darstellen kann.



Zusammenfassend zeigen die betrachteten Wechselwirkungen in sandigen Bachbetten, dass (i) das Modell des vertikalen Wasseraustausches der primäre physikalische Faktor ist, der die Struktur und die Funktion der mikrobiellen Gemeinschaft im Bachbett prägt. Des Weiteren sind (ii) die Aktivität der mikrobiellen Gemeinschaft sowie der Sedimenttransport stochastische Quellen raum-zeitlicher Heterogenität des vertikalen Wasseraustauschs. Vor allem im Hinblick auf die zunehmende Ablagerung von Feinsedimenten in Bächen weltweit tragen diese Ergebnisse wesentlich zum Verständnis und der Prognose von Ökosystemfunktionen in sandigen Bachläufen bei.

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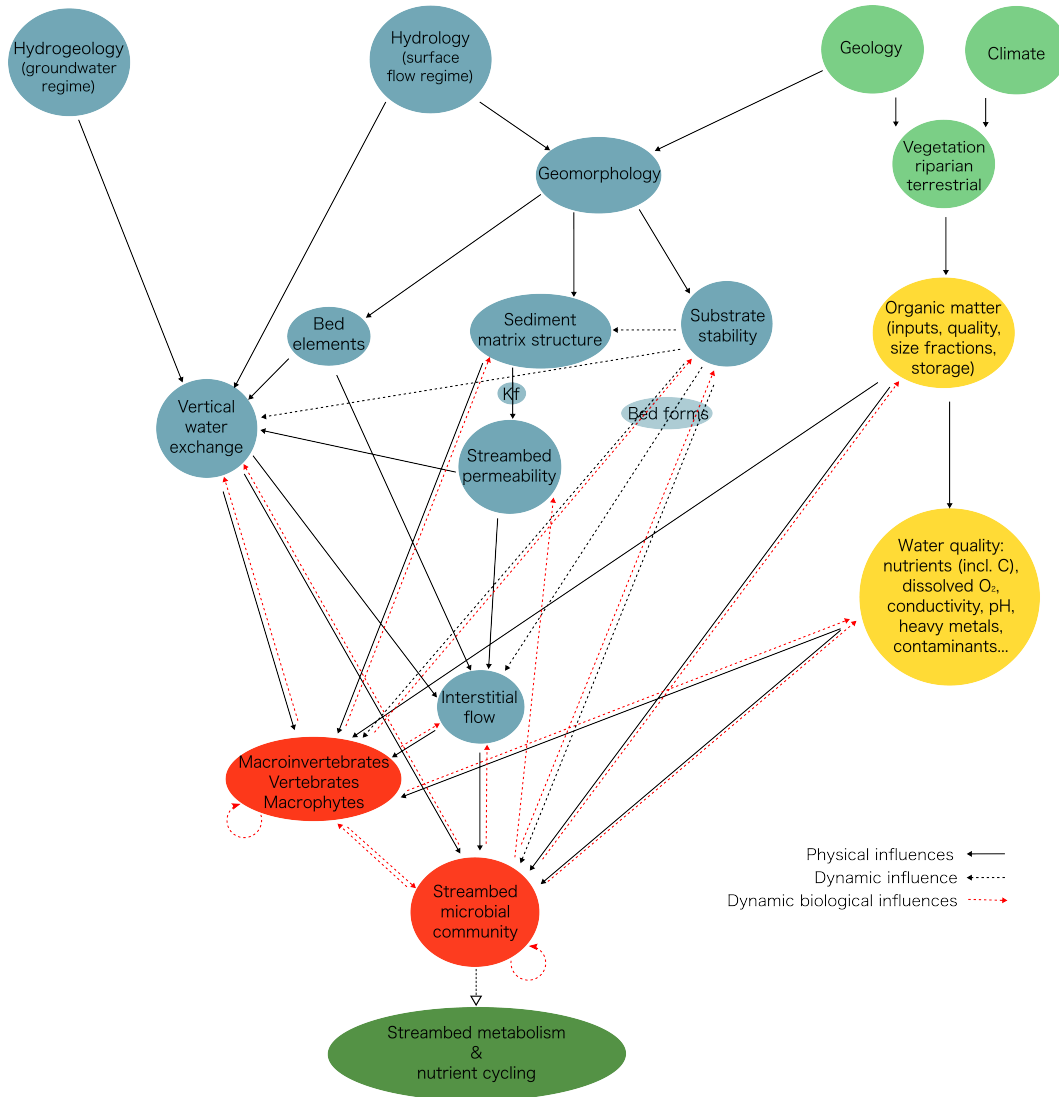
# 1. Introduction

## 1.1 The significance of microbial communities in lotic ecosystems

The streambed in lotic ecosystems, including the hyporheic zone, hosts a large and diverse microbial community colonising its surfaces and pore spaces (Brunke & Gonser, 1997; Battin et al., 2008). This streambed (benthic and hyporheic) microbial community carries out most of the metabolism and nutrient cycling of the ecosystem (i.e., ecosystem function; Findlay et al., 1993; Findlay & Sobczak, 1996; Mulholland et al., 1997; Lefebvre et al., 2004; Vervier et al., 2009; Argerich et al., 2011). In fact, it does it to such an extent that it is a strong determinant of the trophic and biogeochemical state of the ecosystem (Paerl & Pinckney, 1996). Additionally, because the streambed community may be consumed by other organisms, it constitutes an essential step in nutrient cycling (Allan & Castillo, 1995; Sabater et al., 2002; Singer et al., 2010).

The streambed microbial community consists of a community of microorganisms (algae, bacteria, fungi and protozoans) existing at the sediment surfaces. They are typically present within a layer of extracellular polysaccharide that is secreted by the community (Sigee, 2005). The structural and functional characteristics of the microbial community, which in turn, result in metabolism and nutrient cycling, are modulated by an array of (1) physical habitat factors, (2) biological interactions, and (3) interactions between the physical and the biological components (Figure 1.1; Grimm, 1995). Among these three factors, the influence of physical habitat on the inhabiting community and the biological interactions have been the main subject of ecological studies (Corenblit et al., 2011). Further research is needed to fully understand the mechanisms through which the physical habitat influences the microbial community and, consequently, ecosystem function (Marmonier et al., 2012).

The physical habitat where the organisms develop results from the interactions between physical factors (Figure 1.1, black continuous lines). Although not studied to such a great extent, the inhabiting organisms can also physically alter their physical habitat (Figure



**Figure 1.1:** Simplified ecosystem perspective of physical and biological influences that modulate the streambed microbial community and, in turn, the metabolism and nutrient cycling. Influences with a dynamic character are indicated by dotted lines. Blue bubbles correspond to hydrogeomorphological factors, red bubbles correspond to the streambed community, and the orange ones correspond to organic matter and water quality. Kf = hydraulic conductivity. Adapted from Boulton et al. (2010) and Brunke & Gonser (1997).

1.1, red dashed lines) (Turner, 2005; Corenblit et al., 2011). The biological influence on physical habitat is well acknowledged in geomorphological studies and it is increasingly becoming a relevant factor for ecologists (see Statzner, 2012, for a comprehensive review). Most studies focus on the biological influence of vertebrates such as beavers (Naiman, 1988) or fish (Montgomery et al., 1996), macrophytes (Fritz et al., 2004) or insects (Statzner,

2012), on the physical habitat. Although the influence of the microbial community is less known, recent research points out that the microbial community can significantly influence its physical habitat, e.g. the significance of the interaction of biofilm structure and flow for biofilm development in streams (Battin et al., 2007; Besemer et al., 2007), or the significance of biofilms for sediment stability (Vignaga et al., 2013; Gerbersdorf & Wieprecht, 2015) or sediment permeability (Baveye et al., 1998).

The physical-biological interactions that shape the physical habitat and control the microbial community and, subsequently, ecosystem function, vary temporally and spatially as a function of hydrology (Bridge, 2003a, b) and community dynamics (Figure 1.1, dotted lines) (Fisher & Grimm, 1991; Fisher, 1994; Grimm, 1995). The effect of bioturbation caused by fish or macroinvertebrates is linked to the life history of the species. Some fish species select their spawning sites according to the substrate and flow characteristics (Baxter & Hauer, 2000).

Despite these various pieces of evidence, we still lack a mechanistic understanding of how the factors represented in Figure 1.1 interact and, in turn, modulate ecosystem metabolism and nutrient cycling. The aim of the present dissertation is to elucidate several reciprocal influences of streambed physical habitat and microbial community, which are not yet fully understood or have not been tackled before.

## **1.2 Interacting factors that shape the physical habitat and microbial community**

The physical factors that can influence the microbial community can be grouped into three major interrelated factors, as shown in figure 1.1: (1) hydrogeomorphology, (2) organic matter and water quality, and (3) biological interactions. The latter two are being widely studied, even together with hydrogeomorphology, e.g. interactions between sediment grain size and macroinvertebrates (De Troch et al., 2006), protozoan grazing on biofilms (Wey et al., 2008), the role of different streambed microorganisms on nitrogen uptake (Pastor et al., 2013), the influence of organic matter on the microbial community (Freeman et al., 1990), the impact of interspecies interactions on organic matter processing (Ribblett et al., 2005; Risse-Buhl et al., submitted), the interactions between auto- and heterotrophic microorganisms (Romaní & Sabater, 1999), and the influence of meiofauna (Miall, 1977; Majdi et al., 2012) and macroinvertebrates (Nogaro et al., 2007) on microbial communities. The influence of factors 2 and 3 occurs within the constraints imposed by the hydrogeomorphology (factor 1). Therefore, the influence of hydrogeomorphology on the microbial community

has to be fully understood as a first step towards understanding streambed metabolism and nutrient cycling.

Hydrogeomorphology provides the physical substrate where the microbial community develops and defines the exchange of water between the surface and the streambed (hereafter called the vertical water exchange, VWE) and the interstitial flow pattern, which supply redox partners and nutrients to microorganisms (Battin, 2000). The VWE is facilitated by interactions between flow and morphological elements, such as riffle-pool sequences (Brunke & Gonser, 1997), bedforms, such as dunes, antidunes or ripples (e.g. Cardenas et al., 2004; Cardenas & Wilson, 2007; Cardenas et al., 2008), and bed elements such as wood (Mutz et al., 2007) or stones (Hutchinson & Webster, 1998), and is controlled by the hydraulic conductivity ( $K_f$ ) of the streambed sediments (Huggenberger et al., 1998; see Buffington & Tonina, 2009 and Tonina & Buffington, 2009, for a comprehensive review). The VWE and the heterogeneity in grain size distribution define the interstitial flow (Brunke & Gonser, 1997). Hydrogeomorphology is not a static factor but is rather dynamic as a result of the surface flow regime and its associated sediment transport (Sidle & Onda, 2004). The surface flow regime ranges from catastrophic flood events to drought. Within this range, the sediment transport dynamics alter the geomorphology and, thus, the VWE by means of migrating bedforms, altering streambed permeability and grain size distribution (Bridge, 2003a, b). From the microbial community point of view, sediment transport dynamics not only influence the VWE, but they are also a source of mechanical disturbance.

### **1.3 Hydrogeomorphological factors interacting with the microbial community**

As described above, hydrogeomorphology can influence the microbial community by means of VWE and sediment transport dynamics. Although the influence of hydrogeomorphology on the streambed microbial community has been studied, e.g. sediment stability (Uehlinger et al., 2002), flow velocity (Ribblett et al., 2005) or VWE heterogeneity (Argerich et al., 2011; Briggs et al., 2013), some aspects remain unclear, as well as the influence of the microbial community on streambed hydrogeomorphology. On this basis I have developed the following research questions.

#### *1.3.1 VWE – microbial community interactions*

Streambed hydraulic conductivity ( $K_f$ ), defined by the sediment grain size (Huggenberger et al., 1998), is the basic factor that modulates the VWE. Grain size influences the microbial

community in two ways. On the one hand, grain size defines the surface area for colonisation (physical habitat availability; hereafter called the colonisable area; Hargrave, 1972; Bott & Kaplan, 1985; Marxsen & Witzel, 1990). Finer grain sizes offer higher surface-to-volume ratios or colonisable area than coarser ones. On the other hand, grain size defines  $K_f$ , the ability of porous media to transmit water, and thus the advective supply of redox partners and nutrients (Battin, 2000; Higashino et al., 2009; Lowell et al., 2009). The transfer of redox partners and nutrients in low permeable to impermeable sediments ( $K_f < 0.01 \text{ cm s}^{-1}$ ), such as silt or clay, is limited to the slow process of molecular diffusion restricting microbial activity (Novitsky, 1983; Higashino, 2012). Ny contrast, in permeable bed sediments ( $K_f = 0.1\text{--}100 \text{ cm s}^{-1}$ ), from fine sand to cobbles, redox partners and nutrients are supplied to the sediment community by advective-dispersive mass transfer between the bulk flow and the sediment community (de Beer et al., 1996; Rasmussen & Lewandowski, 1998; Higashino, 2012). The effect of grain size, thus, depends on these two interrelated factors, whereas area has an additive effect (Hargrave, 1972; Santmire & Leff, 2007; Harvey et al., 2011), the effect of the advective supply of redox partners and nutrients remains unclear and, in turn, the influence of grain size on the microbial community. This derives in the following research questions:

**Research question 1.1: How does sediment grain size influence the streambed microbial community? What is the role of advective supply in this influence? (Chapter 2)**

The influence of the microbial community on the VWE is limited to the results of experiments in unidirectional percolated sediment columns (Vandevivere & Baveye 1992b, Nogaro et al. 2006). Sediment columns simulate only a small homogenous area of a streambed. Hence, they do not accurately simulate a natural bed with much more diverse and complex water fluxes. Based on the idea of upscaling effects observed from microcosms studies (Drake & Kramer, 2012), the following research questions were tested in a mesocosm experiment:

**Research question 1.2: Can the microbial community influence the VWE? Does the potential influence vary depending on the presence of bed elements such as sand ripples? (Chapter 3)**

### *1.3.2 Sediment transport dynamics – microbial community interactions*

Despite the disturbance-dependet nature of streams (Boulton, 2007), and especially of low-land sandy streams (see section 1.4), the significance of sediment disturbance for streambed

heterotrophic metabolism remains unclear (Boynton et al., 1981). There is no conclusive evidence for modifications in the streambed heterotrophic metabolism due to episodic sediment disturbances. While some authors report a decrease in ecosystem metabolism after sediment transport events (Uehlinger & Naegeli, 1998), other reported an increase (O'Connor et al., 2012) or even no change (Gerull et al., 2012). Changes after sediment transport events are attributed to several interacting mechanisms, e.g. loss of the community by abrasion or export to downstream sections (Uehlinger & Naegeli, 1998), or flushing/burying of organic matter (Matthaei et al., 1996; Olsen & Townsend, 2005). Even then, disparate findings have been reported. Sediment movements did not alter the ciliate community structure according to morphotypes (Risse-Buhl et al., 2014) and the content of organic matter (Naegeli, 1997; Romani et al., 1998). These disparate findings can be attributed to confounding factors in combination with changes in the level of activity between seasons (Uehlinger, 2000, 2006; Roberts et al., 2007) and riparian vegetation (Gerull, 2011). In order to mechanically understand the effect of sediment transport events, I propose the usage of microcosms to isolate confounding factors (Benton et al., 2007). One of the basic effects of sediment transport dynamics is the mechanical stress associated with particle collision (Bridge, 2003b). Using a microcosm approach, I isolated the mechanical stress from other confounding factors to test the following research question:

**Research question 2.1: Is mechanical stress associated with short-term disturbances a factor for the heterotrophic metabolism of the microbial community? (Chapter 4)**

The streambed microbial community can influence sediment transport dynamics by biostabilization of the sediments (Paterson & Daborn, 1991; Yallop et al., 1994; Graba et al., 2010; Vignaga, 2012; Vignaga et al., 2013). In the absence of disturbances, the development of algal mats in the sediment-water interface is known to end up in the detachment of the mats from the bed as a result of accumulation of oxygen gas bubbles resulting from primary production (Hillebrand, 1983; Cambra & Aboal, 1992; Fechner-Levy & Hemond, 1996). The detachment of large seaweeds (kelp) in coastal areas was reported to transport associated sediments. Although this transport is small, it is a significant source of sediment transported to beaches and should be considered in the sediment budgets for coastal areas (Garden & Smith, 2011). In streams, it is not known whether detaching algal mats can transport entangled streambed sediments. The following research questions arise based on these observation:

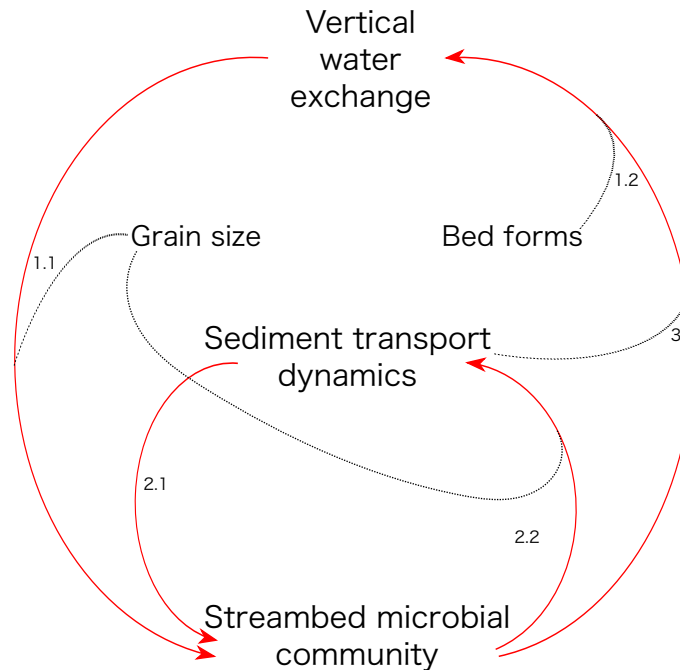
**Research question 2.2: Does the microbial community have the potential to transport sediment? Does the sediment transport potential vary with varying grain sizes? (Chapter 5)**

### 1.3.3 *Interactions between the VWE, microbial community and sediment transport dynamics*

Sediment transport events are very common in sandy streams (research question 2.1). Thus, if microbial community can influence the VWE by reducing the pore space in a model ecosystem with complex and diverse water fluxes (research question 1.2), sediment transport dynamics will, in turn, be a source of heterogeneity for the interaction between the sediment community and the VWE. Based on this idea, the following question was derived:

**Research question 3: Can sediment transport dynamics influence the interaction between the microbial community in the sediment and the VWE? (Chapter 2)**

The scope of the preceding research questions and of the present thesis is represented in Figure 1.2, which, furthermore focuses on a portion of the broad context depicted in Figure 1.1.



**Figure 1.2:** Representation of the influences studied. Numbers correspond to the research questions. Red arrows represent the influences regarded in the research questions. Black dotted lines represent the factors included in the influences studied.

## 1.4 Lowland sand-gravel streams

Lowland low-order, sand-gravel streams have some characteristics that make them especially suitable for the study of the reciprocal influences of hydrogeomorphology and the microbial community.

**Global importance.** First of all, first and second-order streams account for a large percentage of total stream length (Leopold et al., 1964; Wallace & Eggert, 2009) and drain 60–80% of the terrestrial landscape (Benda et al., 2005). The high surface-to-volume ratios of low-order streams allow for relatively greater rates of metabolism and nutrient cycling than high order rivers (Mulholland et al., 1997; Alexander et al., 2000; Peterson et al., 2001; Wondzell, 2011).

**Streambed mobility.** Sediment is transported from headwater mountain streams to large lowland rivers. In sandy lowland streams, however, sediment dynamics are of special significance. The small particle sizes and low cohesion make sand a highly mobile substratum (Morisawa, 1968), to the extent that small changes in shear stress, resulting from small changes in discharges, mobilize certain areas of the streambed (Powell et al., 2005) whereas others are in continuous motion, e.g. as migrating ripples (Bridge, 2003a; Segura et al., 2011; O'Connor et al., 2012). Additionally, heterogeneity in shear stress and sediment transport results in high spatial heterogeneity in grain size distribution (Gomi et al., 2002; Gooderham et al., 2007). Hence, sandy lowland streams are also a good model to understand the significance of sediment transport for the streambed microbial community.

**Anthropogenic impacts.** Different human activities influence lotic ecosystems. Changes in land use can alter the streambed hydrogeomorphology of small streams (Cummins, 1974; Sweeney, 1992; Minshall et al., 2000). Many human activities, e.g. forestry, mining, agriculture and urbanization (Hancock, 2002; Owens et al., 2005), increase fine sediment fluxes to rivers (Wood & Armitage, 1997; Owens et al., 2005; Datry et al., 2014). The deposition and infiltration of these fine sediments into the streambed reduces its permeability. This anthropogenic impact on many lotic ecosystems is therefore another argument to focus on sandy streams.

## 1.5 Methodological considerations – on working with meso- and microcosms

The temporal and spatial scales of ecosystems are sufficiently extensive that they are difficult to reproduce, especially through the traditional route of experimental manipulation



(Benton et al., 2007; McCann, 2007). Conducting replicated experiments in model systems (i.e., micro- and mesocosms) is an alternative that has been widely acknowledged and criticized. Critics argue that model systems are simple and insufficiently generalizable and, therefore, inapplicable to understanding other, less simple parts of nature (Drake & Kramer, 2012). However, natural ecosystems must be understood mechanistically in order to make predictions and generalizations, and micro- and mesocosms provide a tractable way to test mechanistic models (Morin, 1998; Huston, 1999). Therefore, the perceived weakness of model systems actually provide the very advantages that make them useful for isolating mechanisms and testing mechanisms that are difficult in natural ecosystems because it is impossible to control confounding variables (Lawler, 1998; Cadotte et al., 2005; Benton et al., 2007; Drake & Kramer, 2012).

Definitions of micro- and mesocosms are imprecise (Kampichler et al., 2001): some authors refer to the size of the model system (Srivastava et al., 2004) others to the number of environmental variations (Odum, 1984; Drake & Kramer, 2012), and, depending on the context, both are synonyms. Here, in the context of the present dissertation, I use the term micro- and mesocosms combining the two first approaches:

**Microcosm:** Contains a small (10–50 mL; Figure 1.3) volume of disturbed or undisturbed streambed sediment in which factors such as the VWE, light regime and temperature are controlled. Microcosms, thus, enable the demonstration of hydrogeomorphological effects on the streambed microbial community.

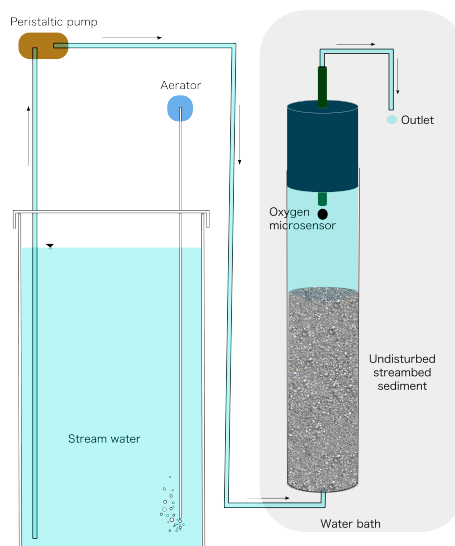


Figure 1.3: Schematic representation of the microcosms.

**Mesocosm** (i.e., flume): Contains a subset of a stream ( $4 \times 0.12 \times 0.10$  m, LWH; Figure 1.4) where the VWE, light, temperature, other meteorological variables and water quality

are defined by the surrounding environment. The VWE is induced by the interaction of the surface flow (defined by a recirculating pump) with the topology of the flume bed and the hydraulic conductivity of the sediments, thereby simulating the natural complexity of the VWE in the form of a mosaic of down- and upwelling spots connected by semicircular flow paths (Thibodeaux & Boyle, 1987; Salehin et al., 2004; Rehg et al., 2005; Bardini et al., 2013). Mesocosms, therefore, enable the observation of interactions between hydrogeomorphology and the microbial community, integrating more factors and their interactions than in a microcosm.

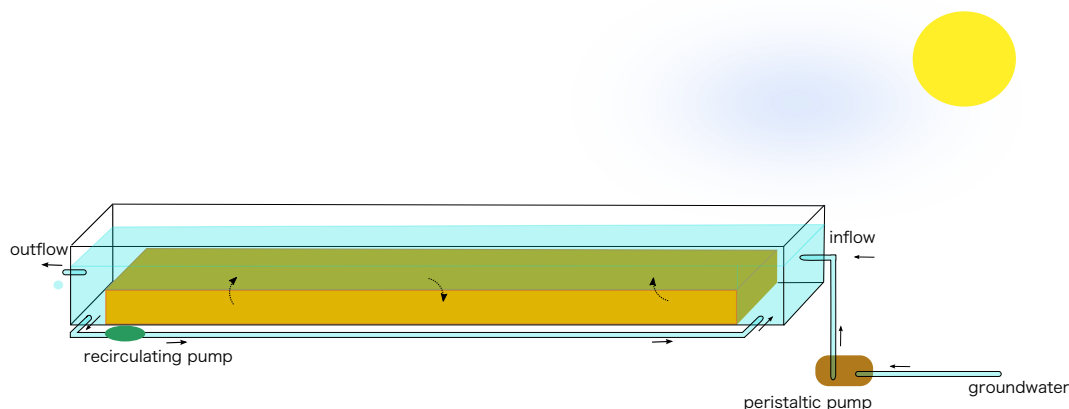


Figure 1.4: Schematic representation of the mesocosms. Arrows across the sediment represent VWE.

In the flumes, both the longitudinal and lateral dimension of lotic ecosystems are partially annulled (Ward, 1989). Flumes represent a stream section in which riparian inputs of organic matter are limited to aeolian processes. The longitudinal dimension, such as the upstream input of nutrients and organic matter, was limited to the water used to supply the flumes. The flumes used were supplied with oligotrophic groundwater from the experimental catchment Chicken Creek (Gerwin et al., 2009), which was enriched depending on the experimental design. The VWE was limited to the water exchange between the interstitial and the surface water, excluding groundwater inputs. Additionally, I used commercial sediment in order to exclude confounding factors such as sediment organic content (Santmire & Leff, 2007) in the experiments presented, in contrast to other studies in the same mesocosms using streambed sediments (Gerull et al., 2012; Frossard et al., 2013).

Small volumes of sediment were placed in the microcosms and percolated (bottom to top) with water from the same stream as the sediment. The streambed microbial community was isolated from the longitudinal and lateral dimension, in order to detect its response to the factor studied in the absence of confounding factors.

## 1.6 List of scientific papers and author publications

The following section gives an overview of the scientific papers (that have been published, submitted or are in preparation for submission) which were derived from the research for this thesis and explains the respective contributions of the authors involved.

### *List of scientific papers*

- Chapter 2: Mendoza-Lera C, Frossard A, Knie M, Federlein L L, Gessner M O & Mutz M. Advective supply drives the effect of grain size on streambed microbial communities. In preparation
- Chapter 3: Mendoza-Lera C & Mutz M. 2013. Microbial activity and sediment disturbance modulate the vertical water flux in sandy sediments. *Freshwater Science* 32:26-35.
- Chapter 4: Mendoza-Lera C. No effect of mechanical stress produced by short-term disturbances on sediment respiration. Submitted to *Hydrobiologia*
- Chapter 5: Mendoza-Lera C, Federlein L L, Knie M & Mutz M. The Algal Lift – Buoyancy-Mediated Sediment Dynamics in Sandy Streams. Submitted to *Water Resources Research*

### *Author contributions*

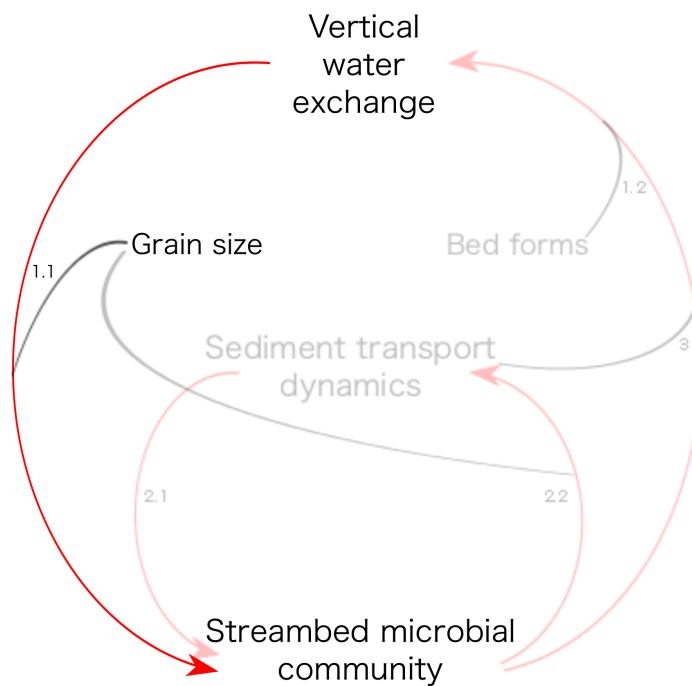
C Mendoza-Lera (CM-L), M Mutz (MM), L L Federlein (LLF), M Knie (MK), A Frossard (AF), M O Gessner (MOG).

- Chapter 2: CM-L and MM designed the experiment with contributions by MK, AF, MOG; CM-L performed the research with contributions by MK, AF, LLF; CM-L analyzed the data with contributions by MK and AF; CM-L wrote the paper with contributions by MM, AF, MK, LLF and MOG.
- Chapter 3: CM-L and MM designed the study; CM-L performed the research, analyzed the data and wrote the paper with contributions by MM.
- Chapter 4: CM-L designed the experiments; CM-L performed the research, analyzed the data and wrote the paper with contributions by MM.

- Chapter 5: CM-L designed the experiment with contributions by MK; CM-L and LLF performed the research and analyzed the data; CM-L wrote the paper with contributions by LLF, MM and MK.



## 2. Advective supply drives the effect of grain size on streambed microbial communities



## 2.1 Abstract

In lowland sand-gravel streams, the heterogenous grain size distribution is related to discharge and bed morphology. Sediment grain size can affect the inhabiting microbial community by determining (i) the available surface for microbial colonisation and (ii) the advective transport of water providing redox partners and nutrients. Due to the interaction between these two factors the influence of grain size on sediment microbial communities is not clear. We ran 12 flumes in a greenhouse with sand (0.2–0.8 mm), gravel (2–8 mm) or a sand-gravel mixture (25/75% mass) for 6 weeks before transferring the flume sediments to percolated microcosms under increased and uniform advective supply for 2 additional weeks. In the flumes, advective supply was repeatedly measured and was constant over time and 2 times higher in gravel than in sand or the sand-gravel mixture. After 6 weeks in the flumes and 2 weeks in the microcosms, we determined sediment algae and bacteria abundance, respiration and potential enzyme activities of the microbial community. Due to the higher advective supply, gravel-bed flumes had higher respiration rates and abundance of algae and bacteria per colonisable surface, and showed a trend towards higher potential enzyme activities. Also, higher advective supply in the microcosms increased bacterial abundance. Our results suggest that grain size influences the sediment microbial community by means of advective supply. This should be considered for the understanding of the heterogeneity in sediment communities among sediments with different grain sizes.

## 2.2 Introduction

Streambed granulometric features such as size, shape and composition of sediments derive from catchment scale geological processes and stream hydrodynamics (Boulton et al., 1998; Fischer et al., 2003), and from anthropogenic changes in sediment composition (Moring, 1982; Wallbrink, 2004; Hecky et al., 2010). These features shape the structure and function of the sediment associated microorganisms and meiofauna (hereafter named as sediment community) (Lock, 1993; Hakenkamp & Palmer, 2000; Santmire & Leff, 2007a).

In sand-gravel streambeds, sediment grain size distribution is spatially heterogeneous both in the horizontal and vertical directions. This is a consequence of heterogeneities in bed shear stress, sediment transport and sedimentation (Bridge, 2003a). This heterogeneity contributes to the high variation of microbial communities and metabolic activities in the streambed (Boulton et al., 1998). Sediment grain size can influence sediment community in two ways. On the one hand, grain size defines the surface area available for colonisation (i.e., physical habitat availability; hereafter named as colonisable area; Hargrave, 1972; Bott & Kaplan, 1985; Marxsen & Witzel, 1990); finer grain sizes offer higher surface-to-volume ratios or colonisable area than coarser ones. On the other hand, grain size defines hydraulic conductivity ( $K_f$ ), the ability of porous media to transmit water, and thus the advective supply of redox partners and nutrients (hereafter named as advective supply) (Battin, 2000; Higashino et al., 2009; Lowell et al., 2009). In low permeable to impermeable sediments ( $K_f < 0.01 \text{ cm}^{-1}$ ) such as silt or clay, the transfer of redox partners and nutrients is limited to the slow process of molecular diffusion restricting microbial activity (Novitsky, 1983; Higashino, 2012). In contrast, in permeable sediments from fine sand to cobbles ( $K_f = 0.1\text{--}100 \text{ cm}^{-1}$ ), redox partners and nutrients are supplied to the sediment community by advective-dispersive mass transfer between bulk flow and sediment community (de Beer et al., 1996; Rasmussen & Lewandowski, 1998; Higashino, 2012).

In absence of other confounding and interacting influences such as organic matter content (Hargrave, 1972; Santmire & Leff, 2007a; Harvey et al., 2011), the effect of grain size on sediment community will generally be a function of the colonisable area and the advective supply. Within permeable sediments the effect of colonisable area is commonly considered additive, and as consequence, abundances and metabolic activities of the sediment community are higher in finer permeable sediment than in coarser one, as reported by several studies (e.g. Bott & Kaplan, 1985; Marxsen & Witzel, 1990; Lefebvre et al., 2005; Santmire & Leff, 2007b). However, in field studies the effect of advective supply remains unclear due



to its high spatiotemporal heterogeneity. Advective supply in the streambed is not simply controlled by  $K_f$  (Huggenberger et al. 1998), but also by the hydraulic pressure in the streambed, which is modulated by factors such as surface waves (Huettel & Webster, 2001), bed elements (Mutz et al., 2007; Cardenas et al., 2008), and near-bed turbulence (Higashino et al., 2009). Still, previous research has reported that at similar sediment organic matter content, carbon uptake rates of gravel and cobble associated microbial communities decrease when surface flow is lowered. This seems to be the result of reduced advective mass transfer into the pore space (Gantzer et al., 1988). Similarly, a numerical model revealed a positive correlation between sediment oxygen demand and advective supply (Higashino, 2012).

To understand the effect of grain size on the sediment community, assuming an additive effect of colonisable area and given similar organic matter content, our two main research questions to be answered are: firstly, is advective supply a factor for sediment community?, and secondly, how does the interaction between the advective supply and the colonisable area of a given grain size influence the inhabiting community?

To simulate the complexity of advective supply in lowland streams and to overcome the limitations imposed in field studies, we used an experimental approach combining mesocosms (i.e., flumes) and percolated microcosms. To our knowledge such an approach to determine the significance of grain size for sediment community has not been used before. Using this approach, we studied the sediment communities under two different advective supply scenarios. We first imitated advective supply in the streambed by generating grain-size-defined advective supply (hereafter named as  $K_f$ -defined supply) in flumes with three different sediment types and two hydraulic conductivities (gravel > [sand/gravel mixture = sand]). Secondly, we transferred a subset of the sediments from the above-mentioned flumes to percolated microcosms with controlled and uniform advective supply (hereafter named as controlled supply). Specifically, we hypothesized that: (1) higher advective supply in gravel will result in higher functional descriptors and abundances of the sediment community compared to sand and sand/gravel mixture; (2) the interaction between the inherent advective supply and colonisable area will result in similar sediment community descriptors, due to a compensation of higher colonisable area in finer sediments and higher advective supply in coarser sediments.

## 2.3 Methods

### 2.3.1 *Experimental set-up*

We used three sediment types based on grain size: (1) gravel, (2) sand, and (3) sand/gravel mixture (hereafter named as mixture; Table 2.1). Sediment mixture was composed of 25/75% sand/gravel proportions by mass, according to field measurements (Song et al., 2007; Salant et al., 2008). The sediment types differed in colonisable area and had differing Kf (Table 2.1). We ran the experiment for 54 days divided in two successive phases: (1) Kf-defined supply (days 1–40) and (2) controlled supply (days 40–54). The Kf-defined supply phase was carried out in 12 flumes. We measured vertical water flux and water physicochemical characteristics throughout the 40 days and at the end of the phase, we collected samples from the flumes to determine organic matter and Chlorophyll *a* content, algal and bacterial abundance, sediment respiration and extracellular enzymatic activity. The controlled supply phase started after day 40, when after sampling the sediment in the flumes, a subset of sediment was transferred to 9 percolated microcosms. After 14 days in the percolated microcosms, we determined sediment respiration and collected sediment samples to determine organic matter content, algal and bacterial abundance, and extracellular enzymatic activity.

### 2.3.2 *Flumes – Kf defined*

We achieved Kf-defined conditions by filling 12 flumes in a greenhouse at the Technical University of Brandenburg (Cottbus, Germany; LWH 4×0.12×0.12 m, for more details see Mendoza-Lera & Mutz, 2013, Chapter 3) to a height of 4 cm with either one of the three types of sediment grain size mentioned above. We filled 4 replicated flumes with each sediment type. The Kf of the sand and the mixture were comparable to each other but lower than that of the gravel (Table 2.1). Pre-experimental tests revealed that under the same flow conditions, as a result of differences in Kf, Vertical Water Flux (VWF) in flumes with gravel sediment was higher than in flumes with mixture and sand, which were similar (Table 2.1).

For 40 days, a peristaltic pump (Watson Marlow, Falmouth, UK) constantly supplied well-aerated groundwater (Table 2.2) collected from the experimental site Chicken Creek (Germany; lat 51°36'N, long 14°16'E, 171 m asl; Gerwin et al., 2009). We added phosphorous to the supplied water to reach oligotrophic conditions (Table 2.2). In addition, a

pump in each flume simulated surface flow conditions of low-energy sand-gravel-bed streams ( $0.029 \pm 0.017 \text{ m s}^{-1}$ ; DDC pump, Laing, Remseck, Germany), well below the bedload threshold for sand and gravel (Bridge, 2003b).

We increased the rate of microbial colonisation by adding inocula to each flume 48 h after the start (day 2). The inocula constituted of randomly collected  $12 \times 500\text{-mL}$  sediment samples from the bed of the stream at Chicken Creek, which were mixed and shaken into 12 L of the water supplied to the flumes for 3 minutes to produce a suspension of the sediment community. We filtered the resulting suspension through a  $125\text{-}\mu\text{m}$  mesh to avoid macroinvertebrates and coarse particulate organic matter and subsequently added the suspension carefully to the surface water of each flume (1 L per flume), avoiding any disturbance of the flume bed.

### 2.3.3 *Percolated microcosms – controlled supply*

At the end of the Kf-defined phase, on day 40, we transferred 50 mL of sediment from 3 randomly selected flumes of each sediment type into the percolated microcosms. We collected 3 cores of the whole sediment depth at three randomly selected points per flume. We pooled together the cores of each flume and mixed well before transferring them into the percolating microcosms (50 ml glass syringes, Poulten & Graf, Werheim, Germany; diameter 2.65 cm and length 12.7 cm). We placed the microcosms vertically in a water bath kept at constant temperature ( $20^\circ\text{C}$ ; Julabo, Seelbach, Germany) and in the dark for 14 days. We connected each microcosm to a peristaltic pump (Ismatec, Glattbrugg, Switzerland) and percolated them (bottom to top direction) with the same well-aerated groundwater supplied to the flumes. Subsequently, we sealed the top of the microcosm with a rubber stopper to prevent gas exchange from the atmosphere into the microcosm. We inserted a metallic tube (4 mm  $\varnothing$ ) into the stopper to allow the percolated water to flow out. The resulting flow rate in the microcosms was  $0.1 \text{ mL min}^{-1}$ , which on average generated an interstitial velocity of  $5.84 \pm 0.35 \text{ cm h}^{-1}$ . This corresponds to the lower interstitial velocities measured *in situ* in sand-gravel sediments of lowland streams (Angermann et al., 2012). The interstitial velocity was one order of magnitude higher than the VWF in the flumes, in order to simultaneously test the response to uniform advective supply and to higher advective supply.

Table 2.1: Initial sediment and hydraulic characteristics of the flumes and percolated microcosms. Where applicable, values are mean  $\pm$  SD,  $n = 5$ ,  $n_{particle\ properties} = 15$ ,  $n_{VWF} = 4$ .

	Gravel	Mixture	Sand
Sediment grain size (mm)	2-8	0.2-8	0.2-0.8
Hydraulic conductivity (Kf; cm s <sup>-1</sup> )	0.77	0.004 $\pm$ 0.0019	0.0062 $\pm$ 0.0025
Porosity	0.18 $\pm$ 0.004	0.18 $\pm$ 0.005	0.20 $\pm$ 0.005
Num of particles g <sup>-1</sup> sed	5.14 $\pm$ 2.6	2,005.73 $\pm$ 706.1	2,969.56 $\pm$ 125.44
Particle roundness	0.71 $\pm$ 0.051; well rounded		0.75 $\pm$ 0.021; well rounded
Sediment specific area (cm <sup>2</sup> g <sup>-1</sup> sed)	6.48 $\pm$ 1.52	44.90 $\pm$ 0.38	57.70 $\pm$ 4.24
Initial OM (mg AFDM g <sup>-1</sup> sed)	1.35 $\pm$ 1.26	1.51 $\pm$ 0.37	1.57 $\pm$ 0.13
Flumes - Vertical water flux (VWF; cm h <sup>-1</sup> )	0.82 $\pm$ 0.30	0.35 $\pm$ 0.06	0.37 $\pm$ 0.05
Percolated microcosms – Pumping rate (mL min <sup>-1</sup> )	0.1	0.1	0.1
Percolated microcosms – Interstitial velocity (cm h <sup>-1</sup> )	6.04	6.04	5.04

### 2.3.4 VWF, sediment and physicochemical variables

We assessed sediment colonisable area as sediment specific area (SSA; i.e., particle area per unit dry mass of sediment) according to Pidgeon & Dodd (1954) using a stereomicroscope (10 $\times$  magnification; Zeiss, Jena, Germany) for gravel and a microscope (400 $\times$  magnification; Nikon, Düsseldorf, Germany) for sand. We took digital pictures from 15 replicates of 0.5 g of gravel and of 0.05 g of sand (Olympus, Tokyo, Japan) and we analyzed them using ImageJ (Rasband, 1997-2012; Abramoff et al., 2004; Schneider et al., 2012). We obtained particle roundness index as described by Russel & Taylor (1937).

We determined hydraulic conductivity (Kf, cm<sup>-1</sup>) of sand and mixture from parallel sediment samples before the start ( $n = 5$ ) and from each flume at day 36 by means of falling head tests (Eijkelkamp, Giesbeek, The Netherlands). We estimated Kf of gravel from the permeability ( $\kappa$ , m<sup>2</sup>) as a function of the sediment porosity ( $\phi$ ) and the sediment grain diameter ( $d_s$ ) (Bear, 1988; Boudreau et al., 2005);

$$\kappa = \frac{5.6 \times 10^{-3} \cdot \phi^3 \cdot d_s^2}{(1 - \phi)^2}$$

$$\kappa = \text{Kf} \frac{\mu}{\rho \cdot g}$$

where  $\mu$  is the dynamic viscosity of water (kg m<sup>-1</sup> s<sup>-1</sup> at 20°C),  $\rho$  is the density of water (kg m<sup>-3</sup> at 20°C) and  $g$  is acceleration due to gravity (m s<sup>-2</sup>).

We assessed surface flow velocity of flumes from the breakthrough curves of a pulse injection of conservative fluorescent tracer measured by with a field fluorometer (uranine; Gotschy Optotechnik, Adnet, Austria). We calculated the Froude number ( $Fr$ ) as

$$Fr = \frac{v}{(g \cdot d)^{0.5}}$$

where  $v$  is the mean surface-water velocity (m s<sup>-1</sup>), and  $d$  is the flow depth (m).

We calculated Reynolds number ( $Re$ ) as

$$Re = \frac{v \cdot L}{\nu}$$

$L$  is the mean flow depth (m), and  $\nu$  is the kinematic viscosity (m<sup>2</sup> s<sup>-1</sup> at 20°C).

We determined VWF according to the method of Mutz et al. (2007). To describe it briefly, we calculated VWF from the decrease in the concentrations of conservative tracer in the surface water caused by vertical hydraulic exchange of labeled surface water with unlabeled pore water (uranine; final concentration = 3  $\mu\text{g L}^{-1}$ ). During the measurement, we operated the flumes in recirculating mode without additional water inflow. Surface

Table 2.2: Inflowing water physicochemical characteristics. Where applicable, values are mean  $\pm$  SD,  $n = 8$ ,  $n_{\text{temperature}} = 40$ .

NO <sub>3</sub> -N ( $\mu\text{g N L}^{-1}$ )	$9.2 \pm 10.3$
NH <sub>4</sub> -N ( $\mu\text{g N L}^{-1}$ )	$11.1 \pm 3.7$
PO <sub>4</sub> -P ( $\mu\text{g L}^{-1}$ ) <sup>a</sup>	2
SRP ( $\mu\text{g L}^{-1}$ ) <sup>b</sup>	< 0.05
Conductivity ( $\mu\text{S cm}^{-1}$ )	$555.8 \pm 32.1$
pH	$8.2 \pm 0.1$
DOC ( $\text{mg C L}^{-1}$ )	$10.3 \pm 0.9$
Water temperature ( $^{\circ}\text{C}$ )	$18.7 \pm 0.3$

<sup>a</sup> Final concentration of PO<sub>4</sub>-P after enrichment.

<sup>b</sup> Values < 0.05 indicate that the concentration was below detection limit.

water was uniformly labeled with tracer after 60 min (based on previous tests). We assessed decrease in tracer concentration 12 h after the start of the experiment and at days 2, 9 and 15 by measuring the decrease of tracer in the surface water of each flume every 15 min for 5 h with a field fluorometer (Gotschy Optotechnik, Adnet, Austria). Due to failure of the field fluorometer at day 36, we assessed the decrease in tracer concentration from surface water samples (20 mL) collected every 60 minutes and measured within the next 8 hours with a lab spectrofluorometer (Carl Zeiss, Jena, Germany). Quenching of the tracer was excluded because pH was constant during the measurements. We measured VWF at night to avoid photochemical decay of uranine (Leibundgut et al., 2009).

We determined the penetration of photosynthetically active radiation (PAR) into the sediment bed in a laboratory replicate of the flumes (20×12×12 cm) filled with the same type of sediment as for the experiment. We estimated the depth of the limit of the photic zone (1% of incident light intensity; de Winder et al. 1999) by covering a spherical light meter (4 mm diameter; Walz, Effeltrich, Germany) with sediment (incident light PAR =  $300 \mu\text{E m}^{-2} \text{s}^{-1}$ ). We established the photic zone in the sediment streambed at 0–1 cm deep and the aphotic zone at 1–4 cm.

We measured flume surface water temperatures continuously (HOBO Data Logger; Onset, Bourne, Massachusetts), and conductivity and pH weekly (WTW, Weilheim, Germany). We collected and filtered (0.45  $\mu\text{m}$  cellulose acetate filters; Sartorius, Göttingen, Germany) water samples for analysis of dissolved organic carbon (DOC), NO<sub>x</sub>-N, NH<sub>4</sub>-N, and soluble reactive phosphorous (SRP) every five days. We measured NO<sub>x</sub>-N, NH<sub>4</sub>-N, and SRP spectrophotometrically with a segmented flow injection analyzer (PERSTORP Analytical, Rodgau, Germany) and UV/VIS spectrometer (Perkin Elmer, Rodgau, Germany), accord-

ing to standard methods (DEV, 1976-2009). The DOC was measured with a total organic carbon analyzer (Shimadzu, Tokyo, Japan).

### *2.3.5 Sediment community – structural and functional descriptors*

We collected sediment samples with PVC corers (1.5 cm  $\varnothing$ ) from each flume at the end of phase 1 (days 38, 39 and 40) at both light zones (i.e., photic 0–1cm and aphotic zone 1–4 cm) at 5 arbitrarily selected sampling points which were pooled together to constitute one representative sample per light zone and flume. At the end of phase 2 (Kf-defined supply), we collected the sediment from each percolated microcosms and mixed them well. We divided the samples from each phase into subsamples and preserved them at 4°C processed them within 24 h after sampling.

We measured sediment organic matter (OM) as loss on ignition and reported it as ash-free dry mass (AFDM). We dried 10-g subsamples to a constant mass (65°C) and then burned them (4 h, 500°C). We placed 8–10-g subsamples the flumes and 8 mL of 90% ethanol in brown glass bottles for Chlorophyll *a* (Chl *a*) analysis following Mendoza-Lera & Mutz (2013, Chapter 3).

### *Algal and bacterial abundance*

In order to structurally characterize the sediment community, we enumerated and identified bacteria and algae. For algal identification, we fixed 17–18-g subsamples with formaldehyde (2%). We diluted the samples (10 $\times$ ) with deionized water and to mechanically detach adhesive organisms from sediment particles, we shook the samples on a vortex (max. velocity). We quantified and measured algal cells from 1 mL of supernatant in a 10 mL Utermöhl settling chamber (Hydrobios, Germany) under an inverted microscope at 400 $\times$  magnification (Nikon, Düsseldorf, Germany). We scanned at least 6% of the chamber area. We categorized algae into four groups: Bacillariophytes, Cyanobacteria, Eucaryotes and unknown, according into seven size-classes: 0–10  $\mu\text{m}$ , 10–25  $\mu\text{m}$ , 25–50  $\mu\text{m}$ , 50–75  $\mu\text{m}$ , 75–100  $\mu\text{m}$ , 100–150  $\mu\text{m}$  and 200–250  $\mu\text{m}$ . We further transformed algal abundance into projected area according to Hillebrand et al. (1999), to determine the potential area colonised by the algae.

For bacterial abundance, we preserved about 5-g subsamples in 10 mL of formaldehyde (2%) containing 0.1% pyrophosphate and stored at 4°C. We detached bacterial cells from the sediment particles following Buesing & Gessner (2002). We determined bacterial abundance by epifluorescence microscopy using DAPI staining in 0.2  $\mu\text{m}$  polycarbonate filters (Nixdorf & Jander, 2003) We calculated potential area colonised by transforming bacterial abundance

into projected area assuming  $0.1 \mu\text{m}^3 \text{ cell}^{-1}$  (Romaní et al., 2009).

### ***Functional descriptors of the sediment community***

We characterized the function of the sediment community determining sediment respiration and the potential activity of 6 enzymes.

We measured sediment respiration from Kf-defined sediments in the same system as the controlled supply was simulated but using smaller microcosms. Within 6h from sampling, we determined sediment respiration from sediments under Kf-defined supply as  $\text{O}_2$  consumption in 10-mL subsamples in percolated microcosms at a constant temperature in a water bath ( $20^\circ\text{C}$ ; Julabo, Seelbach, Germany). We sealed the microcosms (10-mL glass syringes) at the top with a rubber stopper fitted with an  $\text{O}_2$  micro-optode (PreSens, Regensburg, Germany). We also measured control microcosms filled with axenic sediments. During the measurement, we supplied the microcosms constantly with the same well-aerated groundwater supplied to the flumes ( $0.1 \text{ mL min}^{-1}$ ; ISMATEC, Glattbrugg, Switzerland). We began measurements when the water in the microcosms had been completely replaced twice and  $\text{O}_2$  consumption was stable. We calculated sediment respiration from the difference in  $\text{O}_2$  concentration between the sample and control microcosms. We measured sediment respiration under controlled supply directly in the microcosms, following the same procedure as for the sediment from Kf-defined supply. During controlled supply, we determined  $\text{O}_2$  concentration at the outflowing water every three days to avoid anoxic conditions in the microcosms.

We determined the potential activity of 6 enzymes (hereafter referred to as enzymatic activity) involved in the degradation of organic compounds,  $\beta$ -glucosidase (glucosidase; indicates cellulose degradation),  $\beta$ -xylosidase (xylosidase; indicates hemicelluloses degradation),  $\beta$ -N-acetylglucosaminidase (glucosaminidase; indicates C and N acquisition), leucine-aminopeptidase (peptidase; indicates N acquisition), alkaline phosphatase (phosphatase; indicates P acquisition), and phenol oxidase (oxidase; indicates lignin degradation) with substrate analogues linked to fluorescent molecules (4-methylumbelliferone, MUB; or 7-amino-4-methyl-coumarin, AMC) or to 3,4-dihydroxyphenylalanine (L-DOPA), according to Frossard et al. (2013). We mixed 10-g subsamples thoroughly with 100 mL of Tris buffer and added 200  $\mu\text{L}$  of the resulting sediment slurry to 96-well microplates. We added substrate analogues to the microplates at saturation level (50  $\mu\text{L}$  of 200  $\mu\text{M}$ ; based on previously tested saturation curves). We shook the microplates for 15 seconds, incubated at  $1^\circ\text{C}$  for 1.5h, and added NaOH (0.5 N, 10  $\mu\text{L}$ ) before shaking the microplates again prior to the measurement. We measured fluorescence on a microplate reader (Biotek Synergy 2, Winoosky, USA) at an emission wavelength of 445 nm and 450 nm, for assays with MUB



and AMC substrate respectively, and an excitation wavelength of 365 nm for both types of substrate. We subtracted background fluorescence from the sediment and substrate analogues by measuring sample and substrate controls. We assessed quenching with a quench coefficient (0.8–1.0), calculated as the quotient of fluorescence of each individual sample spiked with a fluorescence standard (MUB or AMC) and the same standard added only to the buffer. For oxydase assays, we measured absorbance at 460 nm. We also measured absorbance in sediment sample controls (containing sediment and buffer only) and substrate control (containing substrate only) in order to calculate a background absorbance, and deducting it from the sample absorbance values during the calculation of enzymatic rates.

### 2.3.6 *Data analysis*

We report all values as means and standard deviation (SD). In order to determine the effect of advective supply, we excluded the effect of colonisable area from the observed patterns by standardizing all the structural and functional descriptors of the microbial community to the colonisable area of each sediment type. To determine the interaction between advective supply and colonisable area, we expressed the microbial community descriptors per volume of sediment. We transform mass of sediment into volume assuming a density of  $1.52 \text{ kg dm}^{-3}$  for gravel,  $1.58 \text{ kg dm}^{-3}$  for mixture and  $1.6 \text{ kg dm}^{-3}$  for sand.

We tested differences in VWF throughout the flume experiment by repeated measures analysis of variance (rmANOVA), with time as within-subjects factor and sediment type as between-subjects factor. We determined the response of bacterial and algal abundance, potential area colonised, OM, Chl *a* and sediment respiration by two-way permutation ANOVA with sediment type and light zone as factors (permANOVA; R-package *lmPerm* 1.1-2). We tested the general response of enzymatic activity by means of multivariate 2-way mixed-model ANOVA (MANOVA; factors: sediment type, light zone; based on Pillai-Bartlett trace statistic) from which we extracted the response for each enzyme by ANOVAs. We used MANOVAs in order to prevent alpha error inflation due to the use of multiple univariate tests. We tested the significance of the deviation of slopes of potential activity ratios (i.e., C-acquiring and lignin-degrading to N- or P-acquiring enzymes) from the 1:1 line by calculating the deviations between the observed values and those on the 1:1 line for each independent variable and using regression analysis to test whether the slope of the resulting relationship between the independent variable and the deviation was significantly greater than zero. When necessary, we transformed data ( $\log(x + 1)$ ). Post hoc tests were done with Tukey's HSD (R-package *agricolae* 1.2-0). When variances were not homogeneous, we performed permutational ANOVA (permANOVA). We ran 5000 permutations for all

permutation analyses (Anderson, 2001). We considered the results to be significant at  $p < 0.05$ . We ran all statistical analyses in R (version 2.11.1; R Development Core Team, Vienna, Austria).

## 2.4 Results

### 2.4.1 *Physicochemical characteristics*

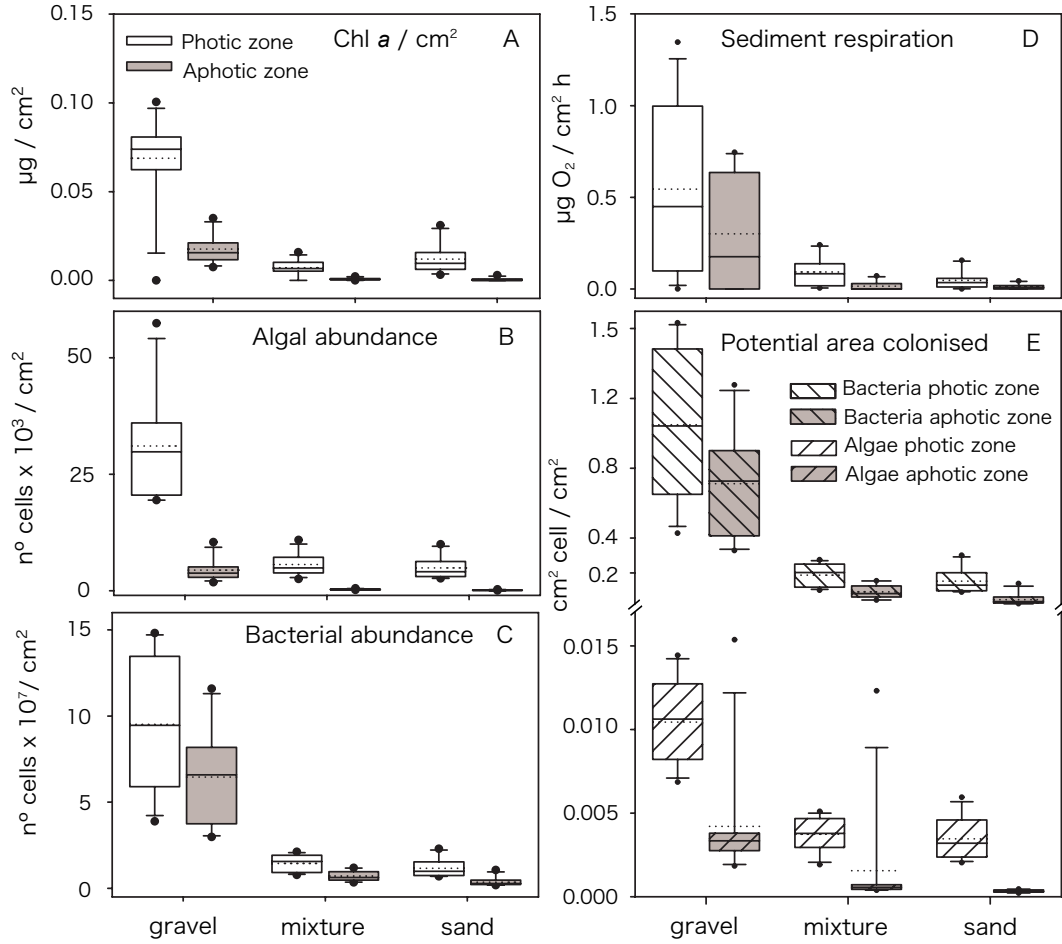
Throughout the experiment physicochemical characteristics of the water supplied to flumes and microcosms did not vary (Table 2.2). The flow in the surface water was tranquil and laminar ( $Fr = 0.09 \pm 0.05$ ,  $Re = 296 \pm 172$ ), and the shear stress was insufficient to scour the bed surface and detach the benthic community (Bridge, 2003b). VWF throughout the experiment was twice as high in gravel-bed than in mixture and sand (rmANOVA:  $F_{2,34} = 6.767$ ,  $p = 0.0034$ ). After 40 days, Kf of the mixture- and sand-beds was reduced by one order of magnitude from the initial values, but the differences with respect to gravel remained the same ( $0.015 \pm 0.040 \text{ cm s}^{-1}$  and  $0.028 \pm 0.066 \text{ cm s}^{-1}$  respectively; ANOVA:  $F_{1,13} = 36.22$ ,  $p < 0.0001$ ).

### 2.4.2 *The effect of advective supply on sediment's community – under Kf-defined supply (flumes)*

After 40 days under Kf-defined supply, structural and functional descriptors of the sediment community showed higher values in gravel than in sandy sediments (mixture and sand; Figures 1 and 2). OM increased by 25% in the photic zone and by 30% in the aphotic zone (ANOVA:  $F_{1,76} = 11.46$ ,  $p = 0.0011$ ). Chl *a* content was more than 8× higher in gravel-bed (Figure 2.1; ANOVA:  $F_{2,66} = 77.68$ ,  $p < 0.0001$ ) than in sandy sediments.

The sediment community (bacteria and algae) showed similar patterns according to light zone and advective supply. In both light zones, algal abundance was higher in gravel than in sandy sediments (Figure 2.1; ANOVA:  $F_{2,66} = 71.62$ ,  $p < 0.0001$ ). At any depth and for every sediment type, the dominant groups were Bacillariophytes and Eucaryotes, which together accounted for more than 70% of the total abundance. The distribution of algae in size classes was independent of the sediment grain size.

Bacterial abundance was higher in gravel at any light zone and similar as for sandy sediments (Figure 2.1; ANOVA:  $F_{2,61} = 100.67$ ,  $p < 0.0001$ ). From the projected area of algae and bacteria, we calculated the potential area colonised. In the photic zone, the microbial community (algae and bacteria) in gravel colonised all the colonisable area (on



**Figure 2.1:** Boxplots of Chl *a* (A), algal abundance (B), bacterial abundance (C), sediment respiration (D) and potential area colonised by bacteria and algae (E) for each sediment treatment expressed per SSA under Kf-defined supply (flumes;  $n = 12$ ). Top and bottom edge of each box represents the 75<sup>th</sup> and 25<sup>th</sup> percentile, respectively, the lines bisecting the box represent the median (solid line) and the mean (dotted line) and the ends of the whiskers represent the 90<sup>th</sup> and 10<sup>th</sup> percentile. Note differences in y-axes.

average  $1.059 \pm 0.42 \text{ cm}^2 \text{ cell cm}^{-2}$ ; Figure 2.1) while in sandy sediments the microbial community colonised less the 20% of the colonisable area (Figure 2.1; ANOVA:  $F_{2,61} = 96.49$ ,  $p < 0.0001$ ). In the aphotic zone, the same pattern was observed, however, the potential area colonised was lower than for the photic zone due to the lower abundance of algae (Figure 2.1; ANOVA:  $F_{1,66} = 11.96$ ,  $p < 0.0001$ ).

Functional descriptors of the sediment community showed a similar pattern among advective supplies and depths. In the photic zone, respiration was higher in gravel than in mixture and sand (ANOVA:  $F_{2,61} = 21.69$ ,  $p < 0.0001$ ), and no difference was detected

between mixture and sand. The level of activity of all enzymes tended to be higher in gravel than in mixture and sand (Figure 2.2). However, in the photic zone, only the activity of peptidase was higher in gravel than in mixture and sand (ANOVA:  $F_{2,33} = 12.34$ ,  $p < 0.0001$ ). In the aphotic zone, the activities of glucosidase, glucosaminidase, phosphatase and oxydase were higher in gravel than mixture and sand (respectively: permANOVA:  $p = 0.0016$ , permANOVA:  $p = 0.045$ , ANOVA:  $F_{2,33} = 9.1$ ,  $p < 0.001$ , ANOVA:  $F_{2,33} = 3.9$ ,  $p = 0.029$ ). Ratios of natural log values of potential activities for C-acquiring hydrolytic enzymes (glucosidase and xylosidase) to N- (peptidase and glucosaminidase) to P-acquiring (phosphatase) enzymes were 1:3:5.1, 1:0.26:8.7 and 1:16:130 for gravel, mixture and sand respectively.

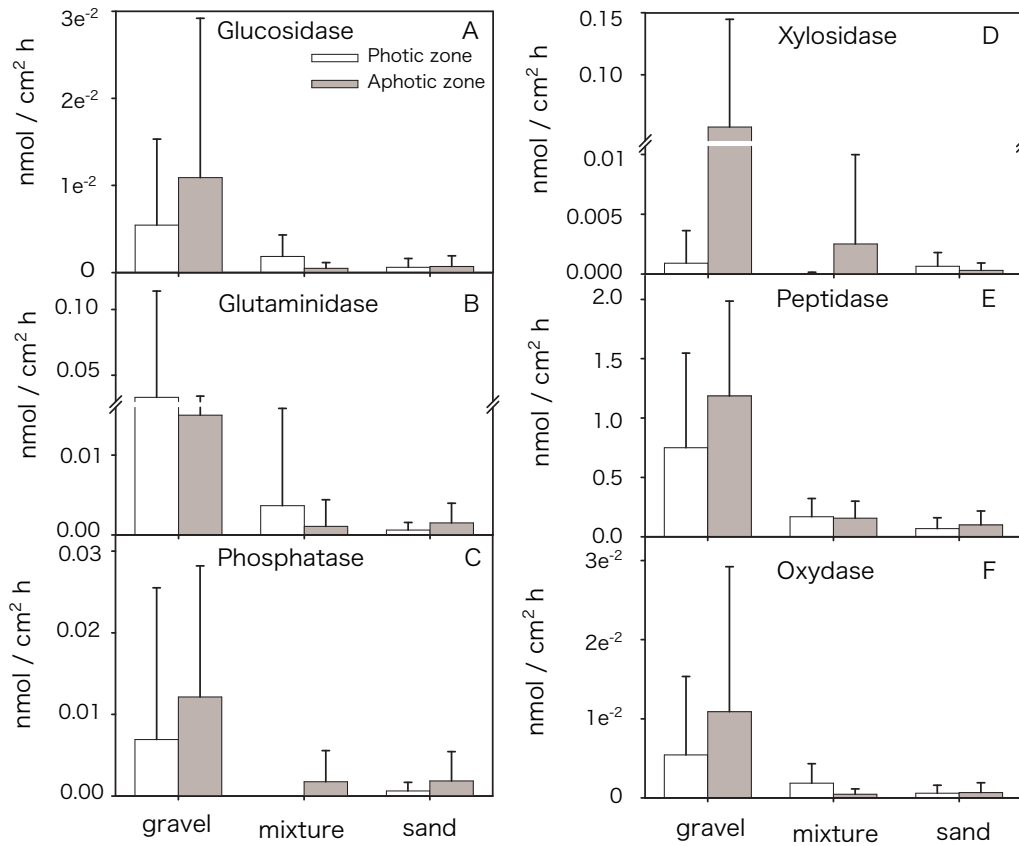


Figure 2.2: Mean ( $\pm$  SD,  $n = 12$ ) enzymatic activity under Kf-defined supply. Glucosidase (A), xylosidase (B), glucosaminidase (C), peptidase (D), phosphatase (E) and oxydase (F). Note differences in y-axes.

### 2.4.3 The effect of advective supply on sediment's community – under controlled supply (percolated microcosms)

After 14 days in darkness and under uniform and identical supply, the sediment community was modified, while OM remained constant. The dark conditions reduced algae abundance for all sediment types (on average  $62.91 \pm 19.9\%$ ; ANOVA:  $F_{1,12} = 6.55$ ,  $p = 0.025$ ). Bacterial abundance strongly increased in all treatments (ANOVA:  $F_{1,12} = 33.80$ ,  $p < 0.0001$ ) but this increase varied among the different treatments (gravel  $287.14 \pm 127.57\%$ , mixture  $166.14 \pm 53.4\%$  and sand  $349.64 \pm 42.24\%$ ). As a consequence, the potential area colonised by bacteria was higher under controlled than under Kf-defined supply (Figure 2.3; ANOVA:  $F_{1,12} = 371.59$ ,  $p = 0.0001$ ). Still bacterial abundance and potential area colonised were higher in gravel than in sandy sediments (Figures 2.2 and 2.3; ANOVA<sub>bacterial abundance</sub>:  $F_{2,6} = 37.93$ ,  $p = 0.0004$ ; ANOVA<sub>colonised area</sub>:  $F_{2,126} = 49.5$ ,  $p < 0.0001$ ).

The functional descriptors of the sediment community changed after 14 days in the per-

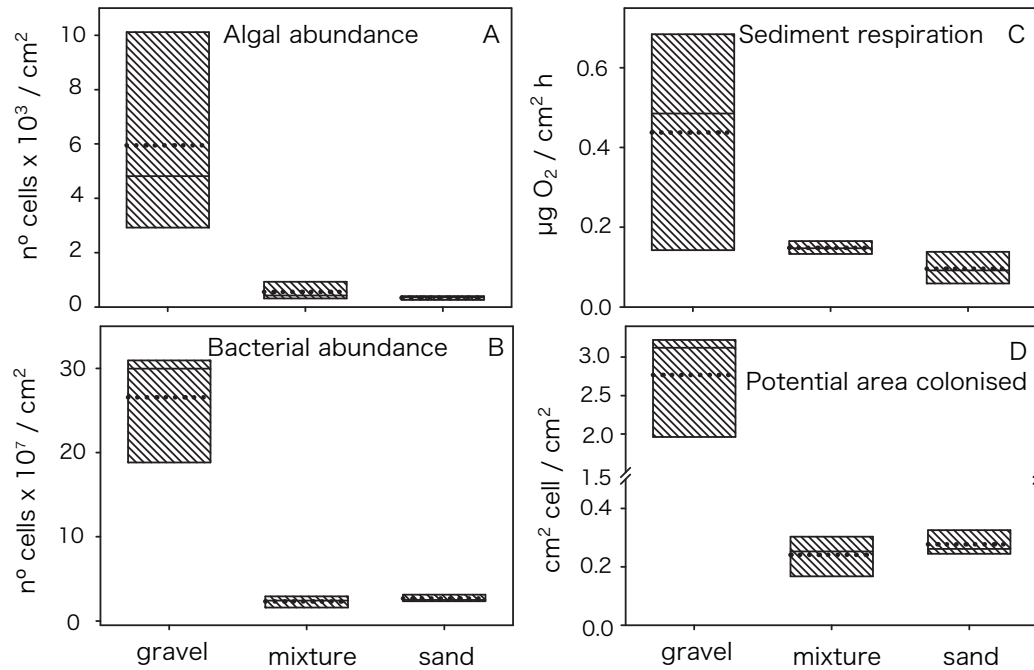


Figure 2.3: Boxplots of algae abundance (A), bacterial abundance (B), sediment respiration (C) and potential area colonised by bacteria (D) for each sediment treatment under controlled supply expressed per SSA ( $n = 3$ ). Top and bottom edge of each box represents the 75<sup>th</sup> and 25<sup>th</sup> percentile, respectively; the lines bisecting the box represent the median (solid line) and the mean (dotted line). Note differences in y-axes.

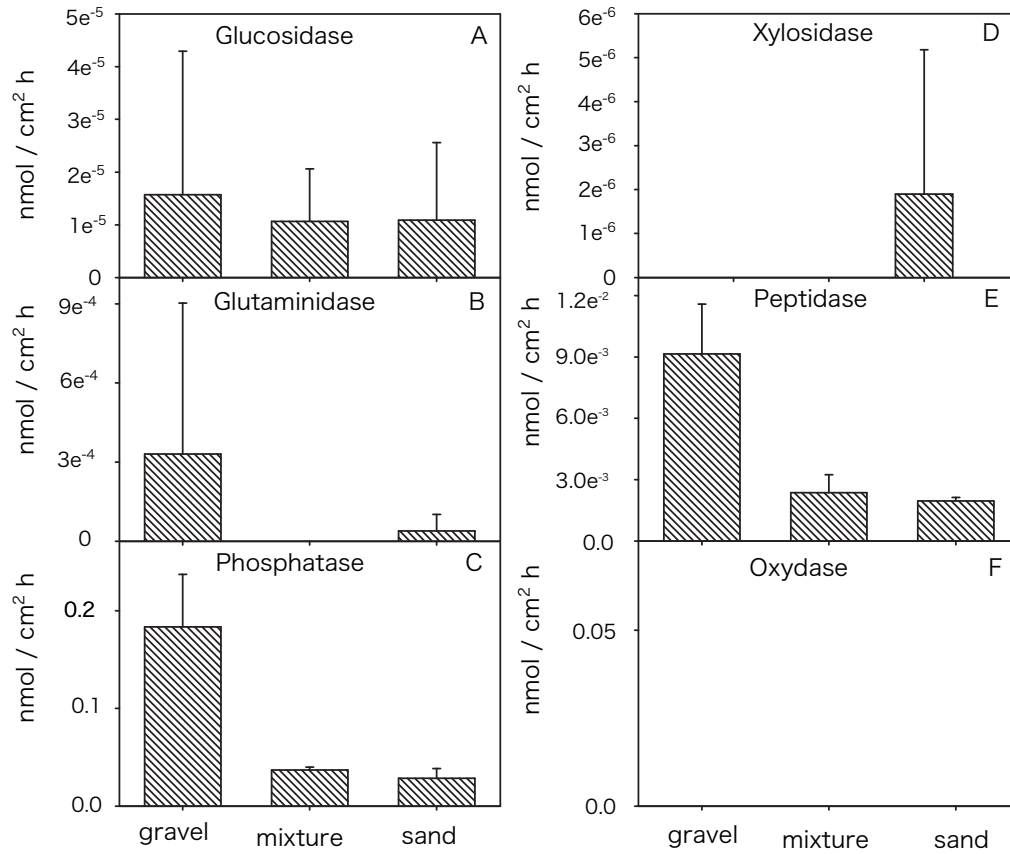


Figure 2.4: Mean ( $\pm$  SD,  $n=3$ ) enzymatic activity under controlled supply glucosidase (A), xylosidase (B), glucosaminidase (C), peptidase (D), phosphatase (E) and oxydase (F). Note differences in y-axes.

colated microcosms. Differences in sediment respiration among sediment types disappeared (Figure 2.3). The level of enzymatic activity was reduced considerably in the sediment transferred from the flumes to the percolated microcosms (Figure 2.4; MANOVA Pillai-Bartlett:  $F_{1,6} = 179.93$ ,  $p < 0.00001$ ). Under controlled supply, the activities of glucosidase, xylosidase, glucosaminidase were very low or even absent for certain sediment types. Moreover, the lignin-degrading enzyme (oxydase) showed no activity. In general, the activities of all enzymes were similar among sediment types except for peptidase and phosphatase, which remained higher in gravel (Figure 2.4; respectively ANOVA:  $F_{2,6} = 18.41$ ,  $p = 0.0027$ ;  $F_{2,6} = 20.66$ ,  $p = 0.0020$ ). Ratios of natural log values of potential activities in the microcosms for C-acquiring hydrolytic enzymes (glucosidase and xylosidase) to N- (peptidase and glucosaminidase) to P-acquiring (phosphatase) enzymes were highly imbalanced (1:601:10633, 1:221:3389 and 1:157:2198 for gravel, mixture and sand respectively).

#### 2.4.4 Interaction between colonisable area and advective supply

In the flumes, sandy sediments had  $7\times$  more colonisable area than gravel, and gravel had  $2\times$  higher advective supply (Table 2.1). Despite this heterogeneity among sediment types, there was not much variation in either the structural or the functional descriptors of the sediment community per sediment volume (Figure 2.5).

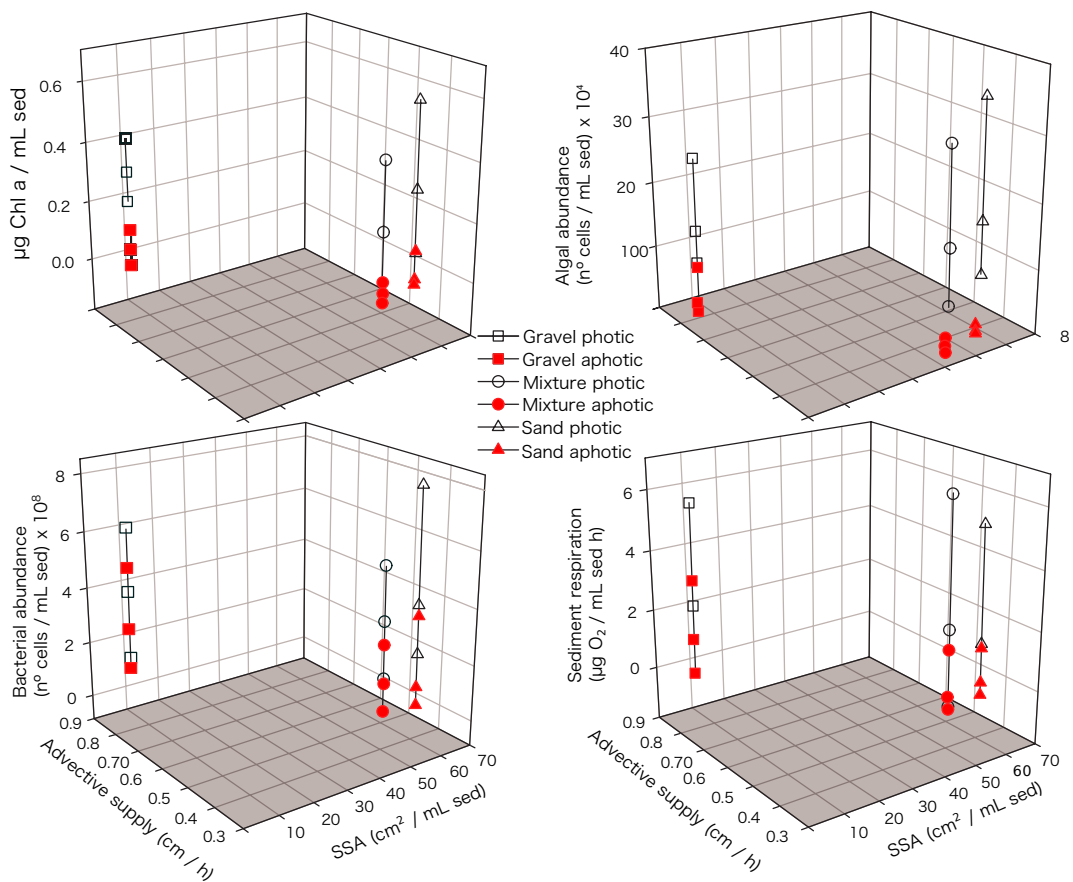


Figure 2.5: Three-dimensional representation of Chl *a* (A), algae abundance (B), bacterial abundance (C) and sediment respiration (D) (y-axes) per volume of sediment vs. advective supply (z-axes) and colonisable area (x-axes) under Kf-defined supply. Note differences in y-axes.

## 2.5 Discussion

### 2.5.1 *The effect of advective supply on sediment community*

Our first research question was: is advective supply a factor for the sediment community? Higher advective supply resulted in higher abundances and activities of the microbial community, both auto- and heterotrophic, in gravel bed flumes than in sandy ones. If advective supply were not a factor for the sediment community, community descriptors per unit of colonisable area would have been similar among sediment types. The influence of advective supply was observed even under the complex pattern of VWF created in the flumes. This, like in a natural stream, was a result of the differences in sediment hydraulic conductivity (Mendoza-Lera & Mutz, 2013, Chapter 3). VWF was comparable with values measured in situ in sand-gravel streams (Angermann et al., 2012). Also, the interstitial velocity (i.e., advective supply) in the percolated microcosms (controlled supply) was also comparable with interstitial velocities in sand-gravel streams (Angermann et al., 2012). The results from the percolated microcosms were influenced by the imposed darkness. Although, most community descriptors were reduced probably as a result of the reduction in algae, bacterial abundance increased after 14 days in the percolated microcosms. We attribute this to the lack of algal exudates that reduces the production of nutrient and carbon acquiring enzymes by the bacteria (Jones & Lock, 1993; Espeland et al., 2001). The lower abundance of bacteria in the aphotic zone compared to the photic sediments suggests that the increase in bacterial abundance in the percolated microcosms resulted from the increased advective supply and not from the reduced algal abundance.

Extracellular enzyme activities can be considered as an indicator of functional microbial responses to environmental conditions. Imbalances among sediment types under Kf-defined and controlled supply suggest that the sediment community was limited by the oligotrophic conditions. The higher rate of N- (peptidase and glucosaminidase) and P- (phosphatase) acquiring enzyme compared to carbon acquiring enzyme (glucosidase, xylosidase and oxidase) resulted in an imbalance of C:N:P ratio (Frossard et al., 2013), deviating from the 1:1:1 ratio of the natural log values of enzymatic activity involved in C, N and P acquisition (Sinsabaugh et al., 2009). This indicates that under natural advective supply, the demand for P and N was greater than for carbon. The same pattern was observed in a previous flume experiment with natural sand bed sediment, where it was found that the microbial community inhabiting the sediments was affected more by nutrient acquisition than by carbon demand (Frossard et al., 2013).

In contrast to responses of benthic algae to eutrophication (Smith, 2003; Townsend et



al., 2012), oligotrophic conditions were limiting in all the sediment types and differences in advective supply were not sufficient to cause a major shift in algal composition. Under the oligotrophic conditions of this experiment, nutrient uptake was most likely controlled by mass transfer (Larned et al., 2004) and nutrient concentrations below saturation, because nutrient uptake can decline with increasing concentration (Horner et al., 1990; Townsend et al., 2012). Therefore, under eutrophic conditions, low advection may support sufficient nutrient mass transfer to the sediment community. Consequently the significance of advective supply will depend on the nutrient load in the water. Under the oligotrophic conditions in our experiment, bacterial and algal abundance increased with supply from sandy to gravel bed flumes and to the percolated microcosms. Our results reveal that the sediment community colonises the available area depending on advective supply. Therefore mass transfer of redox partners and nutrients modulates the microbial community, similar to the positive correlation between benthic biofilm thickness and supply of solutes and oxygen (Wäsche et al., 2002; Townsend et al., 2012). Thicker biofilms developed under higher substrate load and under hydrodynamic conditions that favor mass transfer to biofilms such as turbulence. In our study, without altering community composition, higher mass transfer (i.e., advective supply) favored sediment community abundance and activity.

### *2.5.2 The effect of grain size on sediment community as a function of advective supply and colonisable area*

Our second research question was: How does the interaction between the advective supply and the colonisable area of a given grain size influence the inhabiting community? Our results show that due to the interrelation between advective supply and colonisable area in permeable sediments with  $K_f$  within a range of  $1.5 \text{ cm s}^{-1}$  to  $0.4 \text{ cm s}^{-1}$ , the influence of grain size is not evident. Previous studies in sandy sediments proposed the colonisable area as the dominant factor for the sediment community (Bott & Kaplan, 1985; Marxsen & Witzel, 1990; Lefebvre et al., 2005; Santmire & Leff, 2007b). If this were the case, we would expect an increase in the community descriptors per volume of sediment from gravel to sand, according to the increase in colonisable area. However this was not the case in our experiments. In our sediments, where mixture and sand had the same advective supply but mixture had a lower colonisable area, microbial community descriptors were similar for both sediments. The observed changes depending on the advective supply suggest that within permeable sediments ( $K_f = 0.1\text{--}100 \text{ cm s}^{-1}$ ) and oligotrophic conditions, the additive effect of colonisable area may in fact be a function of advective supply.

Upscaling our results to reach scale, we can infer that, in oligotrophic streams, the

contribution of permeable streambeds to stream metabolism will depend primary on the vertical water exchange (i.e., advective supply) driven mainly by hydraulic conductivity and flow obtruding bed elements (Huggenberger et al., 1998; Huettel & Webster, 2001; Mutz et al., 2007; Cardenas et al., 2008; Higashino et al., 2009; Datry et al., 2014). Grain size distribution results from heterogeneities in shear stress, and coarser sediments are found in areas with higher shear stress and subsequently higher advective supply (Bridge, 2003a; Higashino et al., 2009; Segura et al., 2011). Thus, advective supply in gravelly sediment will be even higher in the streambed than that measured in the flumes. Therefore our results emphasize the need to consider advective supply when stream metabolism experiments are designed and analyzed.

## 2.6 Conclusion

The present study highlights the significance of advective supply to understand the heterogeneity in sediment communities among sediments with different grain sizes. We show that the structure and function of the microbial communities are principally modulated by advective supply.

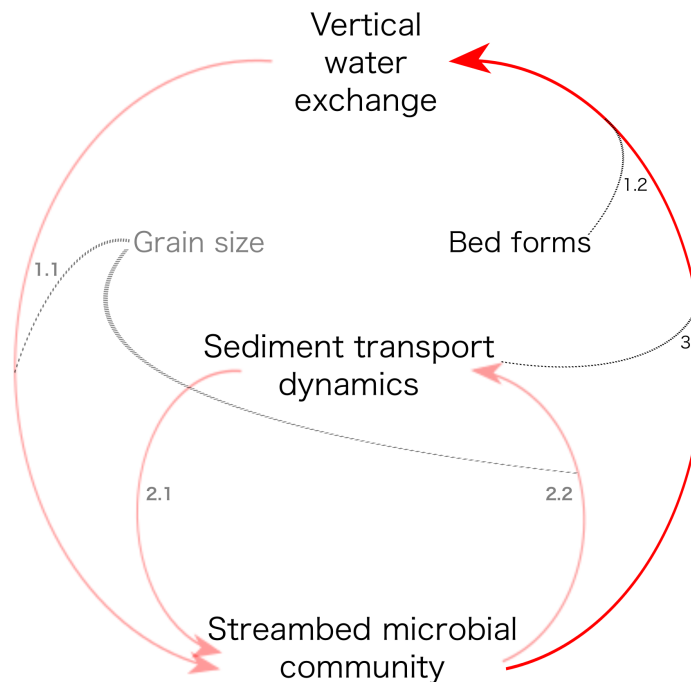
In light of the worldwide decrease in bed permeability and hyporheic water exchange due to increases in catchment erosion and proportion of fine sediments (Moring, 1982; Wallbrink, 2004; Hecky et al., 2010), our results indicate that ecosystem functions and services in oligotrophic conditions will be altered.

## 2.7 Acknowledgments

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### 3. Microbial activity and sediment disturbance modulate the vertical water flux in sandy sediments<sup>\*</sup>



<sup>\*</sup>Mendoza-Lera C & Mutz M. 2013. Microbial activity and sediment disturbance modulate the vertical water flux in sandy sediments. *Freshwater Science* 32:26-35.

### 3.1 Abstract

Little research has been conducted on the influence of microbial communities, sediment disturbances, and their interaction on the Vertical Water Flux (VWF) across a hydraulically complex streambed. Our study was aimed at the effects of microbial activity and shallow-sediment disturbance on VWF in sandbed flumes. We assessed the dynamics of VWF and the development of a microbial community during 30 days (June-July 2010) in 16 outdoor flumes with 2 types of bedform shape (level and ripple). We operated 8 flumes in constant darkness (no-light), and 8 in daylight to gain information on the relative significance of algae and heterotrophic microorganisms. We simulated a shallow-sediment disturbance after 21 days and compared microbial activity and VWF before and after the disturbance. We measured organic matter content, abundance of organisms, microbial activity, precipitation of  $\text{CaCO}_3$ , and  $\text{O}_2$  bubbles resulting from primary production before and after the disturbance. VWF differed among treatments after 13 days. Algal and bacterial cells embedded in an extracellular polymer network, algal and microbial precipitation of  $\text{CaCO}_3$ , and production of  $\text{O}_2$  bubbles in the uppermost sediment blocked the sediment pore space and disrupted VWF under daylight conditions, whereas bacterial cells and microbial precipitation of  $\text{CaCO}_3$  reduced VWF in no-light flumes. Microbial activity and organic matter were not affected by the shallow-sediment disturbance, but VWF was restored. VWF can be controlled by microbial activity and shallow-sediment disturbances and should be seen as spatially heterogeneous and fluctuating in time on small scales.

## 3.2 Introduction

Hydrological exchange between the water column and bed sediments influences the ecology and biogeochemical processes in streams (Boulton et al., 2010). Permeable hyporheic sediments provide a habitat for the microbial communities and can support a large percentage of stream respiration (Jones et al. 1995). The importance of hyporheic processes in the stream metabolism is largely a function of physical constraints on water exchange across the streambed (Findaly, 1995).

Vertical water Exchange or flux (VWF) across the streambed is facilitated by interactions between flow and morphological elements, such as bedforms like ripples and dunes (Cardenas et al., 2008) or wood (Mutz et al., 2007), and is controlled by the hydraulic conductivity of the sediments (Huggenberger et al., 1998). Researchers have demonstrated through laboratory-flume experiments an important transport pathway in which solutes enter the streambed at the upstream slopes of individual ripples or dunes (stoss face), follow semicircular flow paths, and exit into the water column at the downstream slopes (lee face; Thibodeaux & Boyle, 1987). Numerical simulations have been used to elucidate the porewater-flow processes in a bed with ripples (e.g. Salehin et al., 2004; Cardenas et al., 2008), but these simulations have not included biological processes or sediment dynamics. Hydraulic conductivity changes when the pore space is modified (Baveye et al., 1998). Sediment pores are affected by occupation of pore space by algal and bacterial cells and by their metabolic products, such as extracellular polymers, gas bubbles, and salt precipitation (Baveye et al., 1998). In addition, organisms, such as macroinvertebrates (Nogaro et al., 2006) or nematodes (Mauclaire et al., 2006), can affect VWF by creating tubes and galleries. Ward et al. (2011) reported the importance of interconnecting physical and biological processes, but knowledge of the significance of biological processes on VWF is limited to results of experiments in unidirectional percolated sediment columns (Vandevivere & Baveye, 1992b; Nogaro et al., 2006). Sediment columns simulate only a small homogenous area of a streambed. Hence, they do not accurately simulate a natural bed with much more diverse and complex water fluxes.

The other factor not included in the numerical simulations of VWF controlling features is the dynamic character of lotic sediments. Streams are disturbance-dependent ecosystems (Boulton, 2007). For instance, low sediment stability and frequent sediment disturbances turn desert streams towards heterotrophy when they are expected to have autotrophic metabolism (Fisher et al., 1982). A disturbance is a discrete event in time that disrupts the physical environment (White & Pickett, 1985). Different kinds of sediment disturbances have been reported. Drastic sediment transport and rearrangement of an entire streambed

occur during severe floods (Lake, 2000) causing changes in nutrient retention (Martí et al., 1997) and in ecosystem metabolism (Uehlinger et al., 2000). Moderate movements of sand occur frequently at lower flows, and they often are limited to patches of the bed that are exposed to high bed shear (Atkinson et al., 2008; Segura et al., 2011), e.g. ripple patches moving over a level bed (Bridge, 2003). Such moderate sand relocation is limited to the uppermost layer (0–2 cm depth; Atkinson et al., 2008). These physical disturbances, which have not been included in previous simulations models, affect the biological processes in the sediments (Atkinson et al., 2008) and, therefore, potentially influence VWF. The objectives of our research were to answer 2 major questions: 1) How do autotrophic and heterotrophic microbial communities affect VWF in realistic simulations of the hydraulic conditions and bedform of lotic ecosystems? 2) How does a shallow-sediment disturbance affect VWF and the microbial community?

We used a comparative approach and measured the dynamics of VWF in 16 outdoor flumes during the development of a microbial community in 2 different types of bedform (level and ripple bed). We operated 8 flumes in the dark and 8 in daylight to discern the relative significance of algae and heterotrophic microorganisms. VWF was induced by the interaction of the current with the topology of the sandbed in the flumes, thereby simulating the natural complexity of VWF, a mosaic of down- and upwelling spots connected by semicircular hyporheic flow paths (Thibodeaux & Boyle, 1987; Salehin et al., 2004; Rehget al., 2005). We simulated a shallow-sediment disturbance after 3 wk and compared the VWF and microbial activity before and after the disturbance.

### 3.3 Methods

#### 3.3.1 Study site and experimental set-up

We conducted the experiment from 15 June through 16 July 2010 at the experimental site Chicken Creek (Germany; lat 51°36'N, long 14°16'E, 171 m asl; Gerwin et al., 2009). We filled a series of 16 outdoor flumes with silica sand of medium grain size (mean particle size = 0.55 mm) and a permeability of  $7.2 \times 10^{-7} \text{ cm}^2$  (initial organic matter content = 0.001 mg ash-free dry mass [AFDM]  $\text{mg}^{-1}$  dry mass [DM]; Table 3.1). The flumes were continuously supplied with groundwater from the catchment (peristaltic pump, 520-SN, Watson Marlow, Falmouth, UK), well-aerated, and nutrient-enriched (Table 3.2) to ensure development of algal and microbial communities.

A pump recirculated water in each flume to simulate the flow conditions of low-energy sandbed streams, well below the threshold for scour of the bed (16 DDC pumps, 12V DDV-

Table 3.1: Flumes and sediment characteristics. Current velocity is given as mean  $\pm$  SD,  $n = 16$ . Sediment permeability was categorized according to Shepherd (1989) and Bear (1972).

Flume characteristic	Value
Lenght (m)	4
Width (m)	0.12
Height (m)	0.12
Level-bed sediment depth (m)	0.04
Water column depth (m)	0.01
Surface water (L)	9.1
Pore water (%)	22
Pore water (L)	4.9
Current velocity ( $\text{m s}^{-1}$ )	$0.017 \pm 0.003$
Surface water residence time (h)	6
Sediment grain size (mm)	0.2–0.8
Permeability ( $\text{cm}^2$ )	$7.2 \times 10^{-7}$ ; semipervious sand
Ripple characteristics	
Number of ripples per flume	14
Wavelength (m)	0.3
Crest height (m)	0.03
Valley sediment depth (m)	0.01

1RT; Laing, Remseck, Germany; Table 3.1). We divided the series of flumes into 2 sets of bedform shape (Gordon et al., 2004): 1) 8 flumes had a leveled sandbed (level-bed flumes), and 2) 8 flumes had consecutive sand ripples (ripple-bed flumes; Table 3.1). We created ripples and simulated their natural development by moving the sand with a soft-haired paintbrush in the flow direction. We exposed half of each set to daylight and protected them with a 5-mm-mesh white screen that transmitted photosynthetically active radiation (PAR). We permanently covered the other half with an opaque, gas-permeable tarpaulin to prevent algal growth and to separate algae and bacteria effects. We operated the flumes for 12 h before making initial measurements.

We increased the rate of algal colonization by adding inocula to the daylight flumes. We cultivated algae from a benthic algal biofilm from a 2<sup>nd</sup>-order quasinatural sandbed stream in a nutrient solution ( $31 \text{ mg NO}_3^- \text{ L}^{-1}$ ;  $9 \text{ mg NH}_4^+ \text{ L}^{-1}$ ;  $1.55 \text{ mg PO}_4^{3-} \text{ L}^{-1}$ ) at constant light and temperature (PAR:  $50 \mu\text{E m}^{-2} \text{ s}^{-1}$ ;  $20^\circ\text{C}$ ) for 7 days. We inoculated



Table 3.2: Physicochemical characteristics of the flume water during the experiment. Nutrient concentration corresponds to one measurement on day 21. Where applicable, values are mean  $\pm$  SD ( $n = 16$ ), except temperature is daily mean  $\pm$  SD ( $n = 30$ ). DOC = dissolved organic C.

Characteristics	Inflowing water	No-light flumes	Daylight flumes
NH <sub>4</sub> -N (mg N L <sup>-1</sup> ) <sup>a</sup>	0.12	< 0.05	< 0.05
NO <sub>3</sub> -N (mg N L <sup>-1</sup> )	18.3	14.9 $\pm$ 2.70	13.6 $\pm$ 2.90
PO <sub>4</sub> -P (mg P L <sup>-1</sup> ) <sup>a</sup>	< 0.05	< 0.05	< 0.05
Conductivity ( $\mu$ S cm <sup>-1</sup> )	975.68 $\pm$ 18.12	893.84 $\pm$ 79.76	878.4 $\pm$ 129.59
pH	6.3 $\pm$ 0.1	6.4 $\pm$ 0.2	6.5 $\pm$ 0.2
DOC (mg C L <sup>-1</sup> ) <sup>b</sup>	10.2 $\pm$ 0.3		
Alkalinity (meq L <sup>-1</sup> ) <sup>b</sup>	7.24		
Water temperature (°C)	21.7 $\pm$ 2.95	21.4 $\pm$ 3.13	20.8 $\pm$ 3.77

<sup>a</sup> Values < 0.05 indicate that the concentration was below detection limit.

<sup>b</sup> Data from 2009 ( $n = 12$ )

each flume with a 1-L suspension of benthic algae (day-1 algal abundance =  $2.13 \times 10^5$  cells mL<sup>-1</sup>) by adding it to the water column, avoiding any disturbance of the sediments.

We divided the experiment into 2 periods: 16 June – 6 July and 6 – 16 July that were separated by a shallow-sediment disturbance. During the 1<sup>st</sup> period, we focused on the effect of microbial colonization on VWF. At the end of this period (day 21), we carefully and manually redeposited the upper 2 cm of sediment in level-bed flumes (we used a metallic spoon to turn the upper 2 cm of sediments upside-down). During the 2<sup>nd</sup> period, we focused on the effect of the shallow-sediment disturbance on the VWF and microbial community (at day 30). The lapse of time between the disturbance of the uppermost sediments and the sampling of the sediments was a result of the time needed to process the sediment samples.

### 3.3.2 Light penetration and physicochemical variables

We tested light penetration into the sediments in a laboratory replicate of the flumes. We estimated the depth of the photic zone (1% of incident light intensity; de Winder et al. 1999) by covering a light meter (ULM-500; Walz, Effeltrich, Germany) with the same silica sand used for the experiment to increasing depth (incident light PAR = 300  $\mu$ E m<sup>-2</sup> s<sup>-1</sup>). We measured water temperatures in the flumes continuously (HOBO Pro v2 Water Temperature Data Logger-U22-001; Onset, Bourne, Massachusetts). We measured conductivity and pH of the water column in situ on each sampling date (WTW Cond315 and WTW pH320;

WTW, Weilheim, Germany).

We calculated the Froude number ( $Fr$ ) as

$$Fr = \frac{v}{(g \cdot d)^{0.5}}$$

where  $v$  is the mean surface-water velocity ( $\text{m s}^{-1}$ ),  $g$  is the acceleration caused by gravity ( $9.8 \text{ m s}^{-2}$ ), and  $d$  is the flow depth (m).

We calculated the Reynolds number ( $Re$ ) as

$$Re = \frac{v \cdot L}{\nu}$$

where  $v$  is the mean water-column velocity ( $\text{m s}^{-1}$ ),  $L$  is the mean flow depth (m), and  $\nu$  is the kinematic viscosity ( $1.007 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$  at  $20^\circ\text{C}$ ).

### 3.3.3 VWF

We measured VWF on days 1, 13, 21, and 30 of the experiment. We followed the methods of Mutz et al. (2007) to measure vertical exchange using uranine (sodium fluorescein;  $\text{C}_{20}\text{H}_{10}\text{O}_5\text{Na}_2$ ) as the conservative solute tracer. Uranine (excitation wavelength 470 nm, emission wavelength 510 nm) is highly water soluble and essentially nonadsorbing to sand and calcareous material (Käss & Behrens 1998, Flury & Wai 2003, Geyer et al. 2007). We operated the flumes in recirculating mode without additional water supply and added uranine to the water column of each flume (final concentration =  $3 \mu\text{g L}^{-1}$ ). Surface water was uniformly labeled with uranine after 60 min (based on previous tests). Hence, we used a field fluorometer (LLF-M Fiber-Optic fluorometer; Gotschy Optotechnik, Adnet, Austria) to take measurements of the time series for calculations of the VWFs directly in the water column at the upstream end of the flume every 15 min for 5 h. Quenching of the tracer was excluded because pH was constant during the measurements. We measured VWFs in the daylight-exposed flumes at night to avoid photochemical decay of uranine (Leibundgut et al., 2009). Decreases in the concentrations of uranine in the water column were caused by vertical hydraulic exchange of labeled surface water with unlabeled water from the sediment pore space. We used the following relationship from Mutz et al. (2007) to calculate VWF from the tracer concentration time series:

$$C_s(t) = (C_s[t = 0] - C_{eq})e^{-Qt \frac{V_s + V_p}{V_s \cdot V_p}} + C_{eq}$$

where  $Q$  ( $\text{m}^3 \text{s}^{-1}$ ) is the VWF rate,  $C_s$  ( $\text{mg L}^{-1}$ ) is the tracer concentration in the surface water,  $C_{eq}$  ( $\text{mg L}^{-1}$ ) is the equilibrium tracer concentration,  $t$  is the time,  $V_s$  (L) is the volume of surface water, which can be estimated from the complete mix of uranine and the volume of surface water, and  $V_p$  (L) is the volume of sediment pore water.

### 3.3.4 *Sediment microbial community-associated variables*

We collected sediment samples with polyvinyl chloride (PVC) corers (1.5 cm  $\varnothing$ ) on days 21 and 30. We closed the cores with rubber stoppers to avoid porewater loss during sampling and transport. In level-bed flumes, we sampled sediments from 2 depths (0–2 cm and 2–4 cm) at 3 arbitrarily selected sampling points. We mixed samples from each depth into 1 representative sample per depth and flume. In ripple-bed flumes, we collected sediment cores at 3 randomly selected ripples. We sampled the whole sediment column in each position (stoss face, crest, and lee face) and mixed samples to get a unique sample per flume and position in ripple. We took subsamples from these pooled sediment samples for later estimation of different variables. We fixed 19 mL of sediment with formaldehyde (2%) for later bacterial counts and algal counts and identification. We carried out algal counting and identification by diluting 14 mL of the subsample and agitating it gently before transferring 1 mL of the supernatant to a 10-mL Utermöhl settling chamber. After 24 h, we quantified and measured algal morphotypes (pennate and centrale diatoms, coccale and filamentous green algae; Pohlen et al., 2010) at  $1000\times$  magnification (Nikon Eclipse T1; Nikon, Duesseldorf, Germany; Utermöhl, 1958). We observed  $\geq 400$  individuals (Padisák et al., 1999). When the density was low, we scanned the whole chamber. We diluted 5 mL of the subsample and placed it in an ultrasonic bath to extract bacteria (40% of the maximum power, 2 periods each of 2 min; Gómez et al., 2009). We used 4,6-di-amidino-2-phenylindole (DAPI) staining to estimate bacterial abundance in a separate 5-mL sediment subsample citepNixdorf. We scanned filters under an epifluorescence microscope (Axioskop Zeiss 1000X; Zeiss, Jena, Germany) following the same procedure as for algae. We measured sediment DM from the original sample (dried to a constant mass at  $105^\circ\text{C}$ ). We calculated bacterial biovolume by assuming  $0.1 \mu\text{m}^3 \text{cell}^{-1}$  (Romaní et al., 2009), and we transformed algal morphotype data to algal biovolume with the methods of Hillebrand et al. (1999).

We placed 2 mL of sediment and 8 mL of 90% ethanol in brown glass bottles for chlorophyll *a* analysis. We kept samples cool in a refrigerator and analyzed them within 24 h of collection. We extracted chlorophyll *a* by heating the samples in a water bath (4 min,  $70^\circ\text{C}$ ) and a subsequent ultrasonic treatment (2 min, 40%; Elma Transsonic Digital T790/H, Elma, Singen, Germany). We filtered the extracts (2 filters; MN 619; Macherey-Nagel, Düren, Germany) and measured absorbance at 665 and 750 nm (UV/VIS Spectrometer Lambda

2; Perkin-Elmer, Waltham, Massachusetts).

We measured sediment total organic matter as loss on ignition and reported it as AFDM. We dried 10-mL samples to a constant mass (105°C) and then burned them (4 h, 500°C). We oven-dried 5-g sediment samples for measurement of CaCO<sub>3</sub> content with a calcimeter (Scheibler unit) by treating the samples with 10% HCl. The CO<sub>2</sub> volume produced was equivalent to the quantity of CaCO<sub>3</sub> as %DM. We used pure CaCO<sub>3</sub> as a standard (Kjær, 1968). We estimated the fraction of pore volume occupied per CaCO<sub>3</sub> based on density of calcite (CaCO<sub>3</sub>; 2.711 g cm<sup>-3</sup>).

We measured potential sediment-associated respiration as O<sub>2</sub> consumption by 10 mL of sediment in flow-through columns at a constant temperature (26°C) within 8 h after sampling. The columns were glass syringes (10 mL) sealed at the top with a rubber stopper with an O<sub>2</sub> micro-optode (Microx TX3; Presens, Regensburg, Germany). We also connected control columns filled with axenic sediments to achieve the same porewater volume. We supplied the columns constantly with well-aerated water from the flumes (0.1 mL min<sup>-1</sup>) by a peristaltic pump (Ecoline; ISMATEC, Glattbrugg, Switzerland). We began measurements when the water in the chamber had been completely replaced twice and O<sub>2</sub> consumption rates were stable. We calculated potential sediment-associated respiration from the difference in O<sub>2</sub> between the sample and control columns. We measured sediment DM after the respiration measurements were complete.

We assessed whole-flume metabolism on days 1, 7, 13, 21, and 30 from changes in O<sub>2</sub> in the water column of the flumes. We inhibited gas exchange with the atmosphere during the measurements by sealing each flume from the atmosphere with acrylic glass lids. We lowered the lids by fine screw threads until they touched the water surface without any head space. Gaps between the wall of the flumes and the acrylic glass lids were bridged by rubber disks. Before we started the O<sub>2</sub> time series, we manually removed gas bubbles that accumulated under the disks. We measured changes in water-column dissolved O<sub>2</sub> (DO) with O<sub>2</sub> optodes (Oxy 10 mini; Presens, Regensburg, Germany) at intervals of 30 s. We recorded community respiration (CR) as decline in DO in darkness during a maximum period of 2 h. During this period, we covered the daylight-exposed flumes with an additional opaque tarpaulin to stop primary production. We assessed net primary productivity (NPP) of daylight flumes immediately after measuring CR by recording the increase in DO (maximum period = 2 h) in daylight. We estimated P:R as NPP:CR.

We measured O<sub>2</sub> concentration in the sediment pore water in the flumes around noon on days 1, 7, 13, and 21 with a needle probe micro-optode (Microx TX3; Presens, Regensburg, Germany). We made 3 random measurements per flume. We measured O<sub>2</sub> in the level-bed flumes at 0 to 2.5 cm at 0.5-cm intervals. We measured 3 randomly selected ripples in the

ripple bed at 1-cm depth in the stoss and lee faces and at 2-cm depth in the ripple crest. We observed gas bubbles in the sediment surface of the daylight flumes. We estimated bubble diameter and abundance from high-quality digital photographs (EOS 1000D Digital SLR; Canon, Surrey, UK). We estimated release of bubbles from the sediments with water-filled glass funnels (40 mm  $\varnothing$ ) with the tip closed. We arbitrarily exposed funnels on the sediments of the level-bed flumes for 12 h (day 20). We calculated the volume of gas collected from geometric formulae.

### 3.3.5 Data analysis

We report all values as means and standard deviation. We ran permutation t-test for pair analyses of initial VWF. We estimated VWF reduction rate in the daylight flumes after 13 days with linear regression and compared rates with analysis of covariance (ANCOVA; time as covariate).

We expressed NPP and CR  $\text{m}^{-2}$  sediment surface and standardized CR to 21°C, assuming that relationships between temperature and metabolic rate were exponential according to a  $Q_{10}$  of 2 (The  $Q_{10}$  is defined as the factor by which the metabolic rate increases with a 10°C rise in temperature; Davidson & Janssens 2006). We used 2-way permutation analysis of variance (permANOVA) to test the effect of light and disturbance on physicochemical variables, interstitial  $\text{O}_2$  saturation, CR, NPP, and sediment microbial community-associated variables. We ran 5000 permutations for all permutation analyses (Anderson, 2000). We considered tests significant at  $p < 0.05$ . We ran all statistical analyses in R (version 2.11.1; R Development Core Team, Vienna, Austria).

## 3.4 Results

### 3.4.1 Physicochemical characteristics during the study period

Daylight-exposed flumes had slightly lower mean water temperature and higher pH compared to no-light flumes (permutation ANOVA,  $p_{\text{Temperature}} = 0.026$ ,  $p_{\text{pH}} = 0.011$ ; Table 3.2), whereas conductivity was similar between light conditions or bedform types. The maximal penetration depth of PAR into the sediments was 0.8 to 1.0 cm. Light was reduced 80% at 0.2 cm, and the limit of the photic zone was 0.9 cm. The flow in the flumes was tranquil and laminar (Froude number = 0.054, Reynolds number = 2040), and the shear was insufficient to scour the bed surface and detach the microbial community. Mean rainfall over the entire experiment was low (on average  $0.025 \pm 0.085 \text{ mm h}^{-1}$ ), with the exception of one strong rain event on day 15 when 6 mm were recorded in  $< 2 \text{ h}$ . This event took

place while measuring, so the protective screen was removed from the daylight-exposed flumes. After this event, the entire surface of sediments was covered by craters  $\sim 1$  mm deep generated by individual rain drops.

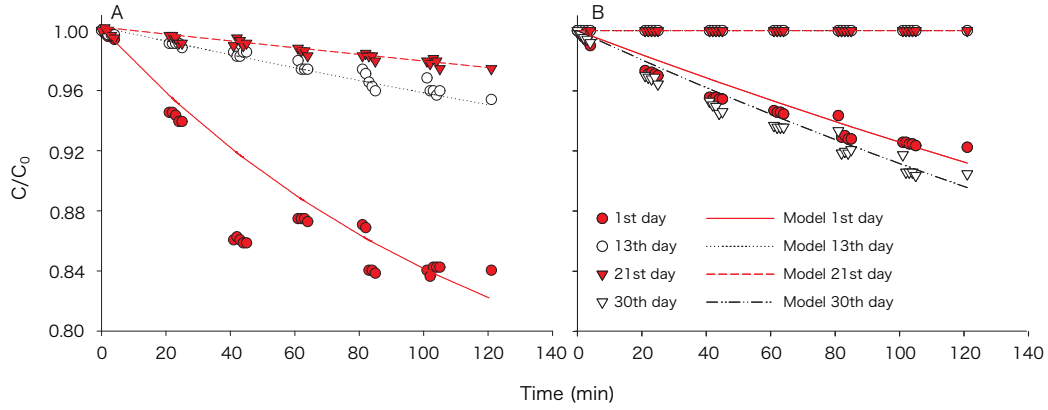
### 3.4.2 *Sediments' microbial community and its activity*

Initial VWF (day 1) was higher in the ripple-bed than in the level-bed flumes (permutation t-test,  $p = 0.016$ ). After 13 days in both level- and ripple-bed daylight-exposed flumes, VWF was disrupted compared with its initial values (Figures 3.1 and 3.2). This disruption was greater in the ripple-bed than in the level-bed flumes (ANCOVA,  $F_{2,13} = 12.21$ ,  $p = 0.001$ ). On day 21, a slight increase in VWF was observed in both bed types (Figure 3.2A, B). VWF was lower under no-light than under daylight conditions but not completely disrupted compared with the initial values. By day 21, VWF was lower in the no-light ripple-bed ( $92.26 \pm 11.50\%$  reduction) than in the level-bed ( $60.69 \pm 26.46\%$  reduction) flumes (Figure 3.2B).

As expected, algal abundance (Table 3.3) was higher in daylight-exposed than in no-light flumes (permANOVA,  $p_{Level} < 0.0001$ ,  $p_{Ripple} < 0.0001$ ). Algae were unevenly distributed in the ripple-bed flumes and in the sediment column of level-bed flumes. Pennate diatom and green coccale were the dominant morphotypes (Table 3.3). Bacterial abundance was higher in ripple-bed flumes under no-light conditions than in daylight (permANOVA,  $p = 0.0068$ ; Table 3.3), but no differences were found between level-bed-flume depth or ripple positions.

Sediment total organic matter was similar in all treatments, and no differences were found among depths or ripple position (stoss face, crest, or lee face; Table 3.3). The fraction of saturated pore space occupied by cells across all treatments was low (Table 3.3), but it was higher in daylight-exposed than in no-light flumes (permANOVA,  $p_{Level} < 0.001$ ,  $p_{Ripple} < 0.001$ ). Ripple position had no effect on the fraction of saturated pore space occupied by cells. The pore volume occupied/cell was higher in the upper 2 cm of sediment than in the 2- to 4-cm layer in level-bed flumes (permANOVA,  $p = 0.0004$ ). The sediments were free of  $\text{CaCO}_3$  on day 1, but it accumulated in the sediments during the experimental period, especially under daylight conditions (permANOVA,  $p_{Level} = 0.014$ ,  $p_{Ripple} = 0.0014$ ; Table 3.3).  $\text{CaCO}_3$  was higher in the upper 2 cm of sediment than in the 2- to 4-cm layer in level-bed flumes (permANOVA,  $p_{Daylight} = 0.029$ ,  $p_{No-light} < 0.001$ ).  $\text{CaCO}_3$  did not differ between sediments from the 3 ripple positions in ripple-bed flumes. In level-bed flumes, the fraction of pore water occupied by  $\text{CaCO}_3$  (Table 3.3) did not differ between light conditions, but a higher fraction of the pore volume was occupied by  $\text{CaCO}_3$  in the upper 2 cm of sediment than in the 2- to 4-cm layer (permANOVA,  $p < 0.001$ ). In ripple-bed

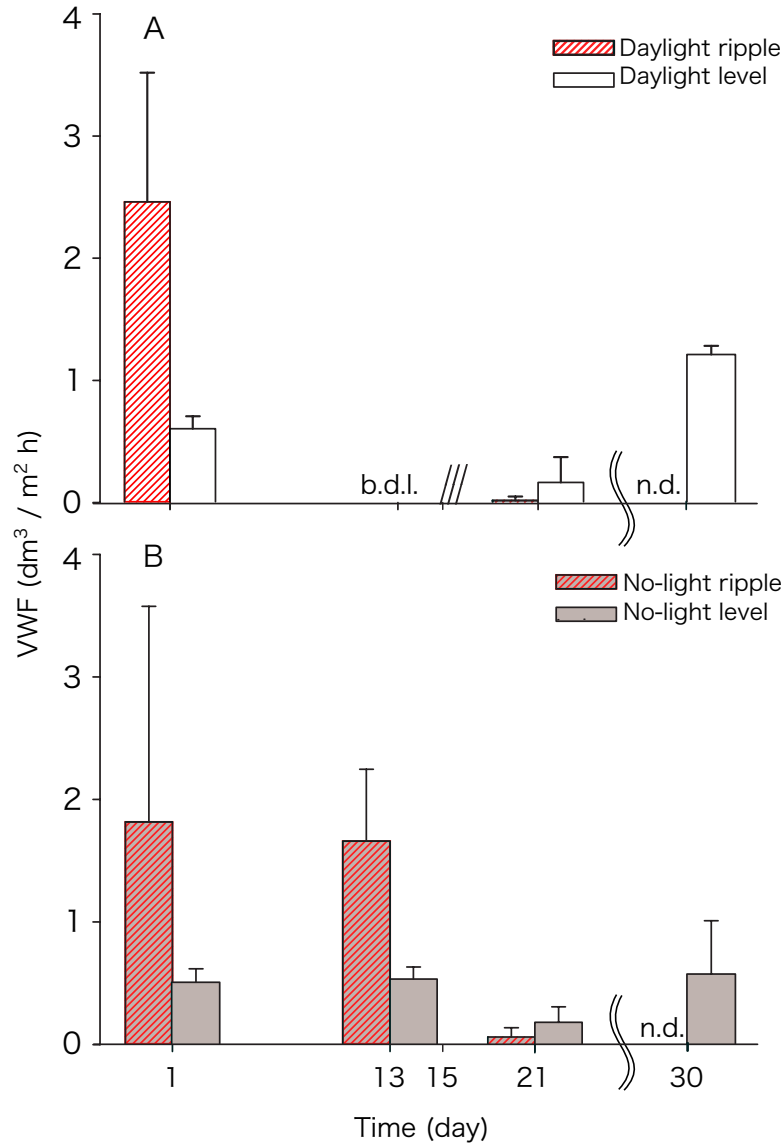
than in the 2- to 4-cm layer (permANOVA,  $p < 0.001$ ). In ripple-bed flumes, the fraction of pore water occupied by  $\text{CaCO}_3$  did not differ between ripple positions, but the fraction of pore water occupied was higher in daylight than in no-light flumes (permANOVA,  $p = 0.005$ ).



**Figure 3.1:** Uranine tracer concentrations in surface water at time  $t$  ( $C_t$ ) relative to uranine tracer concentration when uniformly distributed in surface water ( $C_0$ ) during tracer studies on days 1, 13, 21, and 30 during the experiment in ripple-bed, no-light flumes (A) and in level-bed, daylight flumes (B). Symbols are measured values, and lines show predictions based on the model (Mutz et al., 2007).

On day 1, CR was  $0.054 \text{ mg O}_2 \text{ m}^{-2} \text{ h}^{-1}$ . This value increased to means of  $82.47 \pm 78.025 \text{ mg O}_2 \text{ m}^{-2} \text{ h}^{-1}$  in daylight flumes and  $62.61 \pm 37.90 \text{ mg O}_2 \text{ m}^{-2} \text{ h}^{-1}$  in no-light flumes on day 7, but was highly variable, as can be seen from the high standard deviations (permutation ANOVA,  $p_{\text{Daylight}} < 0.001$ ,  $p_{\text{No-light}} < 0.001$ ). After day 7, CR did not change but remained highly variable. Light condition did not affect CR. In no-light conditions, CR was higher in level-bed than in ripple-bed flumes (permutation ANOVA,  $p = 0.032$ ), but CR did not differ between bedforms in daylight. On the other hand, net primary productivity (NPP) did not differ between level- and ripple-bed flumes, and despite high variability, both bedforms showed the same temporal dynamics. The initial NPP was 0, increased to  $103.26 \pm 4.60 \text{ mg O}_2 \text{ m}^{-2} \text{ h}^{-1}$  on day 13, and decreased to  $56.55 \pm 51.73 \text{ mg O}_2 \text{ m}^{-2} \text{ h}^{-1}$  on day 21. P:R was  $>1$  in ripple- and level-bed flumes ( $7.31 \pm 15.31$  and  $4.55 \pm 5.17$ , respectively).

Initial  $\text{O}_2$  concentration in the sediment pore water was below saturation ( $83.2 \pm 4.6\%$ ). Primary production delivered a surplus of  $\text{O}_2$  to the sediment, and after the first 6 days, the pore water remained oversaturated ( $166.5 \pm 22.2\%$ ) in the daylight flumes. Porewater  $\text{O}_2$  in the no-light flumes remained below saturation ( $79.0 \pm 8.7\%$ ). Porewater  $\text{O}_2$  did not differ between depths or ripple positions.  $\text{O}_2$ -bubble production was observed in the uppermost layer of sediments under daylight conditions. The diameter of the bubbles was  $\leq 1 \text{ mm}$ .



**Figure 3.2:** Mean ( $\pm$  SD,  $n = 4$ ) vertical water flux (VWF) under daylight (A) and no-light conditions (B) in level-bed and ripple-bed flumes. /// indicates a strong rain event on day 15, breaks in the x-axis indicated by curved symbols indicate sediment redeposition in level-bed flumes on day 22. n.d. = no data, b.d.l. = below detection limit ( $\text{VWF} < 0.017 \text{ dm}^3 \text{ m}^{-2} \text{ h}^{-1}$ ).

and  $13.5 \text{ bubbles cm}^{-2}$  were counted on day 20. Approximately 32% of the pore volume in the photic zone was occupied by bubbles when estimated from the bubble diameter. The  $\text{O}_2$ -release rate from the level-bed flumes ranged from  $47.50$  to  $254.45 \text{ cm}^3 \text{ O}_2 \text{ m}^{-2} \text{ h}^{-1}$ . This rate is roughly equivalent to an  $\text{O}_2$  production rate  $104.39 \pm 97.56 \text{ mg O}_2 \text{ m}^{-2} \text{ h}^{-1}$ .

Potential sediment-associated respiration (day 21) in the level-bed flumes was higher in daylight than in no-light conditions (permutation ANOVA,  $p < 0.0001$ ), whereas light condi-



tions did not affect potential sediment-associated respiration in ripple-bed flumes (Table 3.3). In the level-bed flumes, potential sediment-associated respiration was higher in the upper 2 cm of sediment than in the 2- to 4-cm layer of sediment (permutation ANOVA,  $p_{Daylight} < 0.0001$ ,  $p_{No-light} < 0.0001$ ). In the daylight flumes, potential sediment-associated respiration was  $2\times$  greater in the upper 2 cm of sediments than in the 2- to 4-cm layer. In ripple-bed flumes, potential sediment-associated respiration did not differ between the stoss and lee face positions.

### 3.4.3 *Effect of shallow-sediment disturbance*

Sediment redeposition caused an increase in VWF both in no-light and daylight flumes (permutation ANOVA,  $p < 0.001$ , Figures 3.1B and 3.2), whereas total organic matter content in the upper 2 cm did not change (Table 3.3). Neither NPP nor CR were affected (NPP:  $55.0 \pm 8.68 \text{ mg O}_2 \text{ m}^{-2} \text{ h}^{-1}$ ,  $CR_{No-light}$ :  $28.79 \pm 18.95 \text{ mg O}_2 \text{ m}^{-2} \text{ h}^{-1}$ ,  $CR_{Daylight}$ :  $12.54 \pm 1.66 \text{ mg O}_2 \text{ m}^{-2} \text{ h}^{-1}$ ), and P:R remained  $>1$  ( $2.78 \pm 0.72$ ). Sediment-associated potential respiration was not affected by sediment redeposition (Table 3.3), and it remained higher in the upper 2 cm than in the undisturbed 2- to 4-cm sediment layer (permutation ANOVA,  $p < 0.0001$ ).

## 3.5 Discussion

### 3.5.1 *Effect of microbial activity on VWF*

Our first research question was: How do autotrophic and heterotrophic microbial communities affect VWF in realistic simulations of the hydraulic conditions and bedform of lotic ecosystems? Our results demonstrate that VWF can be disrupted by algal and bacterial activity in outdoor flumes simulating sandbed stream conditions. VWF in daylight-exposed flumes was annulled in  $<13$  days, whereas VWF was barely reduced under no-light conditions. However, fluxes  $<0.017 \text{ dm}^3 \text{ m}^{-2} \text{ h}^{-1}$  could not be detected because they were below the detection limit for uranine. This flux corresponds to 0.9% of the initial flux assessed for the ripple-bed and 3% of the flux assessed for the level-bed flumes.

Our results confirm principal findings from simple column experiments with uniform and unidirectional porewater flow in flumes that are more hydraulically complex simulations of lotic systems (Packman et al., 2000; Rehg et al., 2005). Our flumes were similar to natural lotic ecosystems in a semiarid climate — shadeless with high temperatures ( $>20^\circ\text{C}$ ). The slightly higher temperatures measured in the no-light flumes were probably caused by the opaque tarpaulin, which prevented wind refreshment. The daylight flumes were autotrophic

with  $P:R > 1$ , as predicted by Sinsabaugh (1997) for lotic systems in this climate. When CR and NPP values were corrected for  $O_2$  bubbles, they were within the values from natural lotic systems summarized by Townsend et al. (2011).

Flow and bedform, the drivers of VWF (Kasahara et al., 2009), were constant during the experiment. Therefore, the observed reduction in VWF was a result of a decrease in sediment hydraulic conductivity. The lack of differences in conductivity or temperature indicates that physicochemical factors did not cause the observed differences. Thus, VWF disruption resulted from a reduction in pore space caused by the activity of the sediment microbial community. Baveye et al. (1998) pointed out that both pore space and hyporheic exchange can be reduced by biomass, gas production, and precipitation of salts.

Growth of bacterial and algal cells and their extracellular products increased sediment organic matter by day 21. Vandevivere & Baveye (1992b) found that bacterial growth in sand columns of smaller grain size than the particles used in our study (0.065–0.125 mm) caused large reductions in vertical hydraulic conductivity. Vandevivere & Baveye (1992b) showed that bacterial abundances of  $0.5 \times 10^9$  exponentially growing cells  $cm^{-3}$  or  $1.7 \times 10^9$  cells in the stationary phase  $cm^{-3}$  were thresholds for the reduction in vertical hydraulic conductivity. Our abundances (bacteria and algae) were 2 orders of magnitude smaller, and the sand used in the flumes was coarser than in the study by Vandevivere & Baveye (1992b). Therefore, higher abundances would be needed to reduce VWF. Furthermore, the pore space occupied by bacteria and algae in our study was smaller than the volume occupied in Vandevivere & Baveye (1992b; 0.024–0.085  $cm^3$  bacterial cells  $cm^{-3}$  pore volume), except in daylight level-bed flumes, where it was within the range given by Vandevivere & Baveye (1992b). In consequence, the pore space occupied by organisms in our flumes may have affected VWF more in the daylight level-bed flumes than in the no-light flumes. Extracellular polymers forming a biofilm can build a network that stabilizes fine sediments (Yallop et al., 1994). Thus, they can reduce pore space by filling the narrow gaps in the framework where sand grains touch or almost touch each other (Vandevivere & Baveye, 1992a; Baveye et al., 1998). Extracellular polymers may have played a role in the disruption of VWF by creating such a network, but we did not measure them.

The lower abundance of bacteria in the daylight than in no-light flumes was surprising. Gao et al. (2004) reported that bacteria benefitted from the presence of benthic algae. We assume our result probably was caused by underestimation while counting under the microscope because algae and opaque particles can obscure bacteria cells (Lunau et al., 2005).

Gas bubbles in stream sediments have been reported as important to hyporheic processes, but have been little studied. Cuthbert et al. (2010) found  $N_2$  gas, attributed to microbial

denitrification, in up to 30% of sediment pore space. In our study, the high abundance of algae and  $O_2$  oversaturation of the pore water in the uppermost sediments showed that  $O_2$  bubbles formed in the uppermost sediments of the daylight flumes. Orlob & Radhakrishna (1958) reported a 50% decrease of vertical hydraulic conductivity when air bubbles bigger than the pores occupied 12.5% of the pore space in a column experiment with sand similar to the sediment used in our flumes. Bubbles smaller than the pores also can affect the vertical exchange if they adhere to surfaces in the sediments (Baveye et al., 1998) or become trapped in extracellular polymeric secretions (EPS; Battersby et al., 1985). In our flumes, 32% of the pore space in the photic zone was occupied by  $O_2$  bubbles. As predicted by Orlob & Radhakrishna (1958), this quantity of gas bubbles was enough to disrupt the VWF in the daylight flumes.

$CaCO_3$  can block pores (Decho, 2009). The concentration of  $CaCO_3$  in groundwater from the Chicken Creek catchment was  $362 \text{ mg L}^{-1}$  (very hard water; Allan & Castillo 1995). It is unlikely that pure chemical  $CaCO_3$  precipitation occurred in the flumes because such precipitation is favored by alkaline conditions (Barabesi et al., 2007), and pH was  $\sim 6.5$ . However, 2 other processes probably favored  $CaCO_3$  precipitation in our flumes. First, the abundance of microorganisms in both light conditions suggests biologically promoted precipitation that probably is related to extracellular polymers, which act as nucleation agents (Decho, 2009; Dupraz et al., 2009). Second, the level photosynthesis and oversaturation of  $O_2$  point to microbially induced mineralization of  $CaCO_3$ , which occurs when  $CO_2$  partial pressure is reduced by photosynthesis (Dupraz et al., 2009).

Sandy sediments can be clogged by small particles. For example, small amounts of clay in the uppermost layers reduce the permeability of bed sediments (Packman & MacKay, 2003), and bacterial growth and activity in the upper 3 mm can be enough to reduce the vertical hydraulic conductivity of sediment columns (Vandevivere & Baveye, 1992b). In our study, the water supplied to the flumes had no fine particles. Thus, the disruption of VWF in the daylight flumes probably was caused by  $O_2$  bubbles entrapped in the photic zone, algal and bacterial cells, extracellular polymers, and precipitation of  $CaCO_3$  by algae (mainly diatoms and green algae) in the uppermost sediments (Baveye et al., 1998). Reduction of VWF in the no-light flumes probably was caused by bacterial cells, extracellular polymers, and bacterial  $CaCO_3$  precipitation in the uppermost sediments.

**Table 3.3:** Mean ( $\pm$  SD,  $n = 4$ ) algal abundance and proportion of each morphotype, chlorophyll  $a$ , bacterial abundance,  $\text{CaCO}_3$  content, and fraction of pore space occupied by cells and  $\text{CaCO}_3$  in the sediments on day 21. Organic matter content and potential sediment associated respiration in the flumes were measured on day 21 (pre = before surface-sediment redeposition) and day 30 (post = 7 days after redeposition).

Variable	No-light				
	Ripple bed		Level bed		
	Stoss face	Crest	Lee face	0-2cm	2-4cm
Algal abundance (cells $\text{g}^{-1}$ DM) $\times 10^3$	0.16 $\pm$ 0.12	0.047 $\pm$ 0.031	0.067 $\pm$ 0.052	0.12 $\pm$ 0.086	0.17 $\pm$ 0.25
% centrale diatoms	2.6	0	2.0	4.4	1.0
% pennate diatoms	37.3	36.1	39.9	72.3	91.0
% filamentous green algae	18.8	16.2	10.9	7.8	0.7
% coccale green algae	40	45.9	21.1	15.5	7.3
Chlorophyll $a$ ( $\text{mg cm}^{-3}$ )	0.40 $\pm$ 0.31	0.22 $\pm$ 0.29	0.015 $\pm$ 0.031	0.26 $\pm$ 0.523	0.85 $\pm$ 0.75
Bacterial abundance (cells $\text{g}^{-1}$ DM) $\times 10^7$	4.01 $\pm$ 1.78	0.032 $\pm$ 0.037	5.051 $\pm$ 4.90	1.47 $\pm$ 0.38	1.51 $\pm$ 0.47
Proportion cells ( $\text{cm}^3$ cells $\text{m}^{-3}$ pore volume)	0.001 $\pm$ 0.001	0.001 $\pm$ 0.002	0.003 $\pm$ 0.003	0.001 $\pm$ 0.0004	0.001 $\pm$ 0.0005
Total organic matter ( $\text{mg AFDM g}^{-1}$ DM)					
Pre	0.44 $\pm$ 0.10	0.52 $\pm$ 0.067	0.48 $\pm$ 0.087	0.78 $\pm$ 0.22	0.57 $\pm$ 0.091
Post	-	-	-	0.88 $\pm$ 0.066	0.69 $\pm$ 0.049
$\text{CaCO}_3$ ( $\text{mg CaCO}_3 \text{ g}^{-1}$ DM)	0.189 $\pm$ 0.37	0.28 $\pm$ 0.33	0.35 $\pm$ 0.42	0.77 $\pm$ 0.1	0.12 $\pm$ 0.25
Proportion $\text{CO}_3$ ( $\text{cm}^3 \text{CO}_3 \text{ m}^{-3}$ pore volume)	0.002 $\pm$ 0.003	0.002 $\pm$ 0.003	0.003 $\pm$ 0.003	0.006 $\pm$ 0.001	0.001 $\pm$ 0.002
Potential sediment-associated respiration ( $\mu\text{g O}_2 \text{ g}^{-1}$ DM $\text{h}^{-1}$ )					
Pre	0.241 $\pm$ 0.232	0.189 $\pm$ 0.142	0.279 $\pm$ 0.294	0.278 $\pm$ 0.193	0.109 $\pm$ 0.105
Post	-	-	-	0.423 $\pm$ 0.175	0.207 $\pm$ 0.185

Table 3.3: Extended

No-light				
Ripple bed			Level bed	
Stoss face	Crest	Lee face	0–2cm	2–4cm
$477.15 \pm 166.96$	$119.08 \pm 26.61$	$178.31 \pm 51.89$	$466.8 \pm 144.69$	$89.24 \pm 83.42$
0.4	0	0.3	0	0.6
43.6	32.1	40.9	53.8	33.7
8.16	9.8	10.1	7.4	2
47.5	57.5	48.3	38.7	63.7
$1.18 \pm 0.37$	$0.42 \pm 0.28$	$2.097 \pm 0.645$	$6.0 \pm 3.9$	$0.53 \pm 0.50$
$1.35 \pm 0.72$	$1.08 \pm 0.46$	$1.62 \pm 0.55$	$1.24 \pm 0.37$	$1.090 \pm 0.095$
$0.01 \pm 0.009$	$0.008 \pm 0.003$	$0.008 \pm 0.002$	$0.05 \pm 0.02$	$0.02 \pm 0.02$
$0.48 \pm 0.15$	$0.56 \pm 0.097$	$0.65 \pm 0.12$	$0.61 \pm 0.080$	$0.58 \pm 0.18$
-	-	-	$0.69 \pm 0.020$	$0.64 \pm 0.010$
$0.98 \pm 0.24$	$0.50 \pm 0.47$	$1.15 \pm 0.12$	$1.62 \pm 0.37$	0
$0.008 \pm 0.002$	$0.0041 \pm 0.0038$	$0.009 \pm 0.001$	$0.013 \pm 0.003$	$0.000 \pm 0.000$
$0.613 \pm 0.186$	$0.292 \pm 0.157$	$0.459 \pm 0.199$	$1.931 \pm 0.653$	$0.376 \pm 0.131$
-	-	-	$1.758 \pm 0.253$	$0.511 \pm 0.360$

### 3.5.2 Role of bedforms

Our results confirm the significance of the bedform on VWF in unclogged beds (day 1). VWF in the level-bed flumes was similar to that obtained by Mutz et al. (2007) in a flume with a level sand bed, and the initial VWF in the ripple-bed flumes was similar to that simulated numerically by Cardenas et al. (2004) for bedforms. Despite the initially higher VWF in the ripple-bed flumes at the beginning of the experiment, VWF disruption was faster in ripple-bed than in level-bed flumes. Water exchange across a ripple bed is focused on certain locations in the relief by uneven hydraulic head (Cardenas & Wilson, 2006). Water downwells in the stoss face of the ripple, i.e., follows determined hyporheic flow paths and upwells in the lee face. Regarding the ripple dimension generated in the flumes, we estimate a total effective downwelling area of  $0.13 \text{ m}^2$  (30% of flume area). Considering the even hydraulic head at the level bed and assuming equal proportions of down- and upwelling areas, we can estimate that the effective downwelling area in level bed flumes

was 0.22 m<sup>2</sup> (50% of the flume area). Hence, pore-space-reduction phenomena in level-bed systems must occur more homogeneously in a much higher proportion of the bed to cause the same VWF disruption effect as in ripple-bed systems.

### 3.5.3 *Effect of shallow-sediment disturbances on VWF*

Two shallow-sediment disturbances occurred during the experiment. A strong rain event affected the daylight level-bed flumes, and experimental sediment redeposition of the upper 2 cm affected no-light and daylight level-bed flumes. Strong rain events can detach surface sediments in shallow waters (1-cm water column; Gabet, 2003; Kinnell, 2009). After the rain event (day 15), the entire surface of sediments presented craters of  $\sim 1$ -mm depth generated by individual raindrops. The effect of the rain was stronger in the level-bed (uniform water depth of 1 cm) than in the ripple-bed flume, where water depth ranged from 1 to 3 cm, and the deeper water column absorbed most of the energy of the raindrops.

Our second research question was: How does a shallow-sediment disturbance affect VWF and the microbial community? The experimental sediment disturbance (day 21) offset the clogging in both daylight and no-light flumes. Turning the sediments upside down obviously broke the fine physical structure of the sediments and the microbial biofilms and released the bubbles entrapped in the sediments of the daylight flumes. Considering that the rain affected only the uppermost millimeters of the sediment and slightly restored the VWF and that the bubbles were produced in the photic zone (0–1 cm), we conclude that pore-space blockage took place in these uppermost sediments (0–1 cm), as has been found by other authors (Vandevivere & Baveye, 1992b). After the redeposition in the level-bed flumes, the surface was not leveled again and the slightly irregular surface probably caused the increase in VWF compared to the complete level surface measured at day 1 (Cardenas et al., 2004; Salehin et al., 2004).

The disturbance we applied achieved the goal simulating local disturbance without significant loss of biota and transport in suspension out of the flumes, contrary to the situation during severe floods (Santmire & Leff, 2007). Stability in organic-matter content in sediments before and after a flood also has been found in natural systems (Romaní et al., 1998). However, we cannot distinguish whether the community had recovered to the levels of biomass and activity found prior to the experimental disturbance because of the lag between the disturbance and measurement of VWF, NPP, CR, and sediment-associated variables. The lack of difference in total organic matter and the recovered VWF point to an important role of extracellular polymers, although they were not directly measured. Extracellular polymers can stabilize fine sediments by connecting the voids between grains (Yallop et al., 1994). These polymers created a network within the pores in which precip-

itated  $\text{CaCO}_3$  and  $\text{O}_2$  bubbles were embedded. The shallow-sediment disturbance did not remove the mass of organic matter but probably broke this network of the extracellular polymers, releasing bubbles and opening the pores to increase permeability.

Rehg et al. (2005) predicted that dynamics in the rate and pattern of hyporheic exchange are a result of variations in the rate of transport of particles into the sediments during unstable conditions. We concluded that VWF will vary in time and space because of dynamics of bedforms and flow (Salehin et al., 2004; Genereux et al., 2008), microbial activity, and shallow-sediment disturbances (Biggs, 1996; Battin et al., 2001). Hence, hydraulic conductivity will be fluctuating and variable and VWF will be heterogeneous at habitat and reach scales even in constant flow conditions. This heterogeneity has implications for coupled hyporheic processes (Mulholland et al., 2000). Findlay (1995) proposed a conceptual model of the contribution of the hyporheic zone to stream metabolism based on the relationship between the proportion of total discharge passing through the hyporheic zone and the rate of hyporheic metabolic processes (Claret & Boulton, 2009). Our results suggest that the total discharge passing through the hyporheic zone and, hence, the contribution of the hyporheic zone to ecosystem metabolism (e.g. nutrient cycling, respiration), will vary as a result of VWF dynamics.

VWF dynamics in a streambed will vary depending on grain size. For example, the sediment microbial community in fine sand beds could easily reduce pore space, VWF, and the hyporheic contribution to stream metabolism. Fine material can be biostabilized by the microbial community, so the energy required to erode and disturb the sediment will be high (Droppo, 2001). In coarser sediments, such as gravel, activity of algae and bacteria also can reduce pore space and VWF (Salant, 2011). Excessive input of sand from poor land management is shifting the sediment distribution to a smaller grain size worldwide (Hancock, 2002), and the voids of the sediment framework fraction even in many cobble rivers are now filled up with sands (Blaschke et al., 2003). The input of sands is often combined with eutrophication and increased exposure to light from the removal of riparian trees, so the disruption of the vertical connectivity by the microbial activity shown in our study is probably affecting the integrity of many streams and rivers. Therefore, our results also underline the importance of management strategies against siltation to protect ecosystem integrity and hyporheic contribution to stream and river function.

### 3.6 Conclusion

Overall, our results showed that algae and bacterial activity coupled with bedforms and shallow-sediment disturbances are important factors of the VWF in sandbed streams. VWF

should be seen as heterogeneous and fluctuating in space and time on small scales, overlaying the larger scale and temporal variability of vertical exchange controlled by major flood events and dynamics in groundwater level (Brunke & Gonser, 1997). These results are a step toward a better understanding of the VWF as heterogeneous and dynamic, which has implications for colonization, metabolism and nutrient cycling in the hyporheic zone.

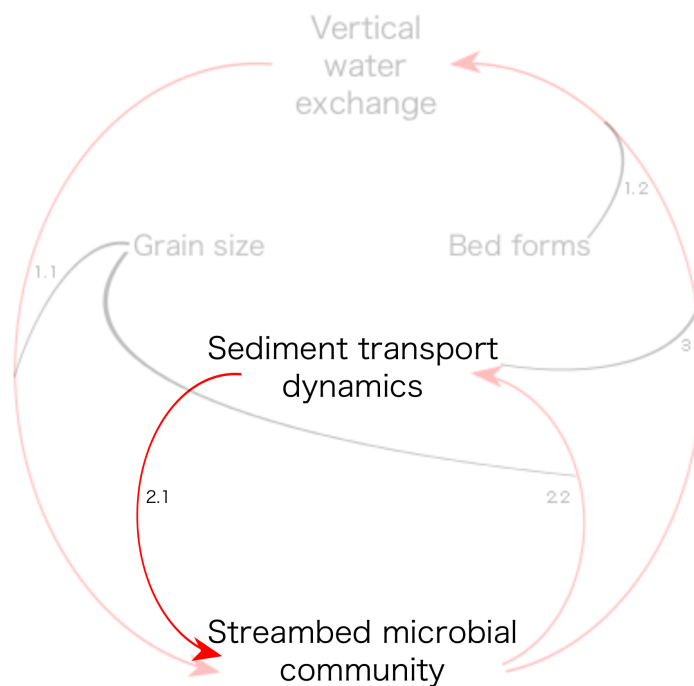
### **3.7 Acknowledgements**

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#### 4. No effect of mechanical stress produced by short-term disturbances on sediment respiration



## 4.1 Abstract

Patches of sandy streambeds are episodically disturbed as a result of small changes in discharge. Due to complex interactions among environmental factors during such changes, the effects of sediment disturbances on streambed heterotrophic metabolism remains unclear. Sediment disturbances are characterized by particle collision. I assessed the effect of mechanical stress associated with a short-term sediment disturbance on sediment respiration by disturbing sandy sediment. To gain knowledge on the effect at different levels of sediment respiration, I sampled undisturbed sediment in winter and late spring from two streams with different riparian vegetation: open-land and forest. To isolate mechanical stress from other factors associated with sediment disturbance (e.g. community loss or changes in advective supply), the samples were placed undisturbed in microcosms and percolated for 85 h. After 45.75 h half of the samples were disturbed, simulating a short-term disturbance, and the immediate and later response (39 h) were observed. Independent from the level of sediment respiration among seasons and riparian vegetations, mechanical stress did not influence it immediately or after 39 h. Thus changes to streambed heterotrophic metabolism after short-term disturbances cannot be attributed to mechanical stress but to other factors such as pore water re-oxygenation or community loss.

## 4.2 Introduction

The microbial community inhabiting bed sediments is of great importance for the heterotrophic metabolism and carbon flux of streams and rivers (Brunke & Gonser, 1997; Battin et al., 2008). Sediment disturbances caused by flow fluctuations are one of the main sources of perturbation of the streambed microbial community (Ward et al., 1998). Sediment disturbances are common in sandy streams as a result of the low cohesiveness of sand (Morisawa, 1968) and spatial heterogeneity in bed shear stress (Segura et al., 2011). Even small changes in discharge can disturb part of the streambed causing short-term sediment disturbance events (hereafter named as short-term disturbance) and patchy bed load transport (Bridge, 2003; Powell et al., 2005).

Still the significance of sediment disturbances for stream heterotrophic metabolism remains unclear (Boynton et al., 1981). There is no conclusive evidence for modifications in the stream heterotrophic metabolism due to episodic disturbances. Uehlinger & Naegeli (1998) reported a decrease in ecosystem metabolism in a prealpine gravel-bed river caused by multiple flow peaks and their associated sediment disturbance over a period of 19 days. In an agricultural stream, O'Connor et al. (2012) observed an increase in ecosystem respiration after a two day storm-driven flow pulse. In a flume experiment, two episodic disturbances events had no effect on overall flume respiration (Gerull et al., 2012).

A short-term disturbance can lead to changes in the sediment microbial community and the heterotrophic metabolism due to: (1) direct mechanical effects: loss of the community by abrasion or export to downstream sections (Uehlinger & Naegeli, 1998) and mixing and/or burying of the community into deeper sediments (Peterson, 1996); (2) modification of the resources: flushing/burying of organic matter (Matthaei et al., 1996; Olsen & Townsend, 2005), altering the sediment permeability (e.g. flushing fine sediments; Olsen & Townsend, 2005) and in turn re-oxygenating the sediments and increasing the supply of solutes and redox partners (Fischer & Pusch, 2001). Even then, disparate findings have been reported. Abundance of ciliates (Risse-Buhl et al., 2014), uptake of glucose (Meyer-Reil, 1978) and content of organic matter were not altered by sediment movement (Naegeli, 1997; Romání et al., 1998). These disparate findings can be attributed to a combination of factors mentioned above. On top of this, the responses of streambed heterotrophic metabolism to a short-term disturbance can vary seasonally (Uehlinger, 2000, 2006; Roberts et al., 2007), and previous studies have shown that sediment respiration also varies throughout the riparian succession (Gerull, 2011). Streambed sediments from early riparian succession such as open-landscape, characterized by graminoids and forbs (Frossard et al., 2013), have lower respiration rates than those from advanced forest stages (Gerull, 2011). The effect of episodic disturbances

can thus be expected to vary seasonally as well as with variations in riparian vegetation. Considering the interactions among all these factors, field studies might not be able to give clear evidence of the influence of a short-term disturbance, if any, on heterotrophic metabolism. On the other hand, microcosm experiments, allow testing those factors one at a time, even if they might be limited in scope and realism (Benton et al., 2007).

A short-term disturbance is characterized by the collision of particles with the resting bed and with each other (Bridge, 2003). Mechanical stress due to such collisions is inherent to the factors influencing the sediment community resulting from episodic disturbances, while other factors site and season dependent. I used percolated microcosms to isolate the mechanical stress factor and addressed the following questions. (1) Does the mechanical stress associated with short-term disturbances influence sediment associated respiration? If so, (2) does the effect of mechanical stress on sediment heterotrophic metabolism vary depending on the level of activity between seasons and throughout the riparian succession?

## 4.3 Methods

### 4.3.1 Field sites

Sediment samples were collected from two low-order oligotrophic sandbed streams with groundwater influence located in Lower Lusatia (eastern Germany; Table 4.1) representing respectively early and advanced riparian vegetation succession: Chicken Creek (CC) and Pohlitzer Mühlenfließ (PM). CC is located in an experimental watershed (Welzow-Süd; lat  $51^{\circ}36'N$ , long  $14^{\circ}16'E$ ), constructed in 2005. A detailed description of the site and stream was given by (Gerwin et al., 2009; Gerull et al., 2011). CC is an early open-landscape stream, with a sandy streambed (Table 4.1), and draining a catchment dominated by grassland vegetation consisting primarily of forbs (*Trifolium arvense*), Canadian horseweed (*Conyza canadensis*), Blueweed (*Echium vulgare*) and Wood small-reed (*Calamagrostis epigejos*), with few emerging trees (*Betula pendula* and *Robinia pseudoacacia*; Zaplata et al., 2011). PM (Table 4.1) is a forested sandbed stream ( $52^{\circ}10'N$ , long  $14^{\circ}35'E$ ) designated as a natural protected area since 2006 (Table 4.1). The catchment is dominated by coniferous plantations, and the riparian vegetation is predominately Alder (*Alnus glutinosa*) and Common Ash (*Fraxinus excelsior*).

### 4.3.2 *Sample collection, percolating system set-up and short-term disturbance performance*

Representative and undisturbed streambed sediment cores ( $n = 30$ ) were collected from portions of the streambed that were free of ripples. Sediment cores were collected three times during three successive weeks for each stream and each season (winter: January–February 2012, in late spring: May–June 2012). The uppermost 5 to 7 cm of the streambed were collected with polyvinyl chloride corers (PVC; 1 cm  $\varnothing$ ) avoiding any disturbance of the sediment structure. Core depth was chosen according to scour and fill depths observed in sandy streams after short-term small discharge changes (Powell et al., 2005; Powers et al., 2009). Each core was transferred immediately under water and without disturbing the sediment structure into glass microcosms (10-mL glass syringes, 1 cm  $\varnothing$ , Fortuna Optima, Poulten & Graf, Werheim, Germany), avoiding any contact with air. The microcosms were kept vertically aligned and submerged in stream water. The upper end of the microcosms was closed with a rubber stopper with a metallic tube (3 mm  $\varnothing$ ) for the outflow of water. The flow of water through the narrow tube towards the atmosphere prevented gas exchange with the microcosm during the operation of the percolation system. At the base of the microcosms, at the needle adaptor side, a mesh disk (200  $\mu\text{m}$  pore size) ensured homogeneous percolation of water along the cores' cross-section. Additionally, parallel replicate ( $n = 30$ ) sediment cores were collected and transferred into HDPE 20-mL vials for initial organic matter determination. The prepared microcosms and the samples for organic matter determination were transported to the lab in darkness and at stream temperature. Approximately 30 L of surface water was collected and transported to the lab for percolating the microcosms. Additional water samples were collected in acid clean PVC bottles for chemical analysis.

The microcosms were connected to the percolation system in a water bath (Julabo, Seelbach, Germany) in darkness and at constant temperature (4°C in winter and 18°C in late spring) within 4 h after sampling. Each microcosm was connected via Tygon tubing to a peristaltic pump (ISMATEC, Glattbrugg, Switzerland) and constantly supplied with well-aerated water from the corresponding stream (bottom to top). Two additional control microcosms with sterilized sediments from each site (500°C, 1 h) were connected to the percolation system 40 mins prior to each measurement. The flow rate in the microcosms was 0.1 mL min<sup>-1</sup> (volumetric flux density or Darcian velocity 7.7 cm h<sup>-1</sup>), and generated a pore water velocity of 34.72 cm h<sup>-1</sup>, which corresponds to intermediate pore water velocities measured *in situ* in sandy sediments of comparable streams (Angermann et al., 2012).

The percolation was operated for a total period of 85 h. In order to determine initial

Table 4.1: Catchment characteristics, physicochemical water descriptors and sediment bulk properties for CC (open-land riparian vegetation) and PM (forested riparian vegetation) during the experimental periods (winter and late spring). Where applicable, values are mean ( $\pm$  SD,  $n = 5$ ), except air temperature is daily mean ( $\pm$  SD,  $n = 60$ ) and its range.

	CC		PM	
	Winter	Late Spring	Winter	Late Spring
Mean discharge ( $\text{L s}^{-1}$ )	0.2	0.007	69.1	40.3
Water depth (cm)	$1 \pm 0.25$	$1 \pm 0.25$	$10 \pm 5.8$	$10.5 \pm 5.9$
Air temperature ( $^{\circ}\text{C}$ )	$-1.0 \pm 7$ ;	$16.4 \pm 5.5$ ;	$-0.5 \pm 6.9$ ;	$16.3 \pm 4.3$ ;
	-21.1–10.6	2.6–30.6	-16.1–9.5	7–24.2
Water temperature ( $^{\circ}\text{C}$ )	$4.87 \pm 0.23$	$17.7 \pm 0.26$	$4.77 \pm 0.25$	$17.93 \pm 0.058$
pH	$8.3 \pm 0.3$	$8.4 \pm 0.2$	$8.1 \pm 0.1$	$8.1 \pm 0.1$
Conductivity ( $\mu\text{S cm}^{-1}$ )	$613.7 \pm 203.9$	$484.3 \pm 22.1$	$265.3 \pm 10.1$	$304.7 \pm 6.4$
$\text{PO}_4\text{-P}$ ( $\text{mg P L}^{-1}$ )	$3.0 \pm 0.46$	$5.43 \pm 0.74$	$5.17 \pm 2.06$	$5.67 \pm 1.08$
$\text{NO}_x\text{-N}$ ( $\text{mg N L}^{-1}$ )	$292.53 \pm 171.48$	$332.93 \pm 64.66$	$417.1 \pm 95.92$	$206.7 \pm 49.95$
$\text{NH}_4\text{-N}$ ( $\text{mg N L}^{-1}$ )	$71.53 \pm 12.18$	$102.5 \pm 65.22$	$64.77 \pm 1.72$	$63.27 \pm 1.03$
DOC ( $\text{mg C L}^{-1}$ )	$8.32 \pm 2.72$	$6.83 \pm 1.87$	$4.98 \pm 2.12$	$4.03 \pm 0.67$
Specific UV absorbance ( $\text{SUVA}_{254}$ ; $\text{L mg}^{-1} \text{DOC m}^{-1}$ )	$0.036 \pm 0.001$	$0.038 \pm 0.0051$	$0.044 \pm 0.01$	$0.065 \pm 0.0094$
Grain size ( $\text{D}_{50}$ ; $\mu\text{m}$ )	$201.4 \pm 4$		$243.26 \pm 23.8$	
Silt content (%DM)		$1.3 \pm 1$	$0.78 \pm 0.038$	
Pore water content (%)	$0.23 \pm 0.038$		$0.23 \pm 0.038$	
OM ( $\text{mg AFDM g}^{-1} \text{DM}$ )	$7.77 \pm 2.66$	$6.68 \pm 1.10$	$3.89 \pm 0.43$	$7.68 \pm 5.14$

acclimation dynamics, sediment associated respiration (hereinafter named as sediment respiration) was determined at 0 h and 24 h. The mechanical stress associated with short-term disturbance was applied to half of the microcosms (disturbed microcosms). In order to determine the immediate response of sediment respiration was measured right before (45.75 h) and after (46 h) the mechanical stress. The mechanical stress consisted of 30 successive stirs of the entire column of each disturbed microcosms with a metallic rod. The collisions of the particles during the disturbance of the microcosms are equivalent to a transport rate of  $0.006 \text{ kg m}^{-1} \text{ s}^{-1}$  during a 20-minutes event (Leopold & Emmett, 1976; Bridge, 2003). By stopping the peristaltic pump during the mixing of the sediment, the mechanical stress was isolated from losses of sediment community or organic matter. Later responses of sediment respiration were determined through measurements at 76 h and 85 h (30 h and 39 h after the mechanical stress).

#### 4.3.3 *Sediment respiration*

Oxygen concentration was measured in the water flowing out of each microcosm with an  $\text{O}_2$  micro-optode (PreSens, Regensburg, Germany) that was inserted through the metallic tube in the rubber stopper. Sediment respiration was calculated as a function of  $\text{O}_2$  consumption as follows:

$$SR = \frac{(O_{2control} - O_{2sample}) \cdot q}{DM}$$

where SR = sediment respiration ( $\mu\text{g O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$ ),  $\text{O}_2$  = oxygen concentration in the outflowing water ( $\mu\text{g O}_2 \text{ L}^{-1}$ ),  $q$  = the flow rate ( $\text{L h}^{-1}$ ), DM = dry mass of the sediment sample (g DM).

#### 4.3.4 *Sediment and water parameters*

Initial sediment organic matter content (OM) for the parallel samples was measured within 4 h from collection, while that for the sediments in the microcosms was measured after the 85 h in the percolation system. OM was determined as loss of ignition and reported as ash-free dry mass (AFDM). Samples were dried to a constant mass ( $105^\circ\text{C}$ ) for dry weight calculation (DM) and then burned (4 h,  $500^\circ\text{C}$ ) for AFDM calculation. Electric conductivity, pH and temperature of the stream water were measured on each sampling day, and daily in the water reservoir supplying the microcosms (WTW, Weilheim, Germany). Water samples for dissolved organic carbon (DOC),  $\text{NO}_x\text{-N}$ , soluble reactive phosphorous (SRP) and ammonia determination were filtered (50 mL; pre-washed  $0.45\text{-}\mu\text{m}$  cellulose acetate filters; Sartorius,



Göttingen, Germany).  $\text{NO}_x\text{-N}$ ,  $\text{NH}_4\text{-N}$  and SRP were measured spectrophotometrically by a segmented flow injection analyzer (PERSTORP Analytical, Rodgau, Germany) and UV/VIS spectrometer (Perkin Elmer, Rodgau, Germany), respectively, according to standard methods (DEV 1976-2009). DOC was measured with a TOC analyzer (Shimadzu, Tokyo, Japan). DOC absorbance ( $A$ ) between 200 and 550 nm was measured spectrophotometrically (Perkin Elmer, Waltham, MA, USA; Helms et al., 2008) in a quartz cuvette at room temperature, and was converted to the absorption coefficient  $a$  ( $\text{cm}^{-1}$ ), following:

$$a = \frac{2.303 \cdot A}{L}$$

where  $L$  is the path length of the cuvette (1 cm). The content of low bioavailability carbon (aromatic molecules; Perdue, 1998), was estimated from DOC-specific UV absorption at 254 nm ( $\text{SUVA}_{254}$ ) and calculated as  $a_{254}$  divided by the DOC concentration (Weishaar et al., 2003).

#### 4.3.5 Data analysis

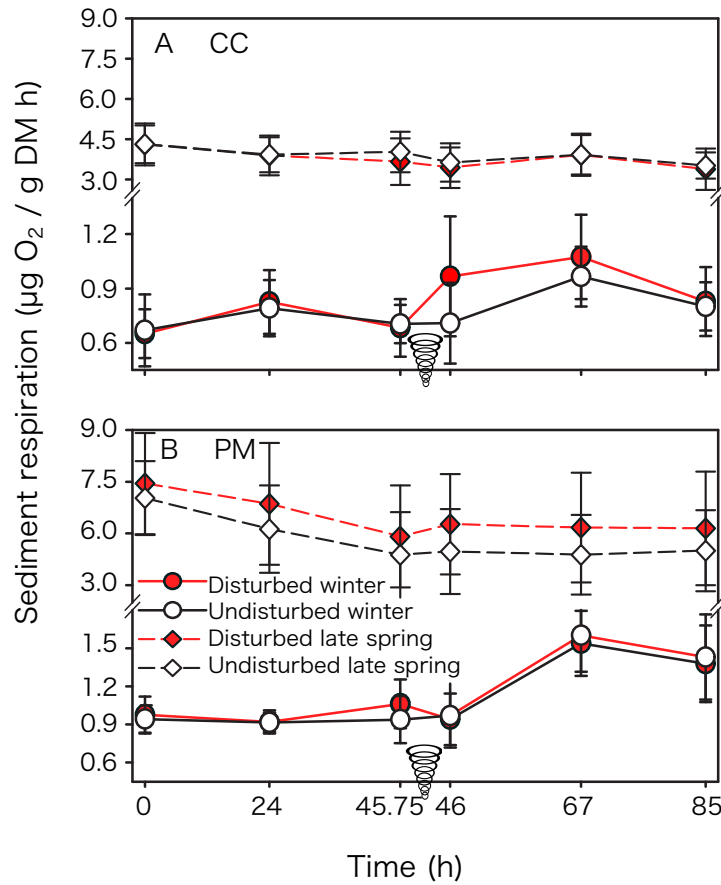
In order to detect seasonal differences associated with riparian succession (Pace Prairie, 2005) sediment respiration is reported standardized at 10°C. This is calculated, assuming that the relationship between temperature and metabolic rate is exponential according to a  $Q_{10}$  of 2 (Davidson & Janssens, 2006). I report all values as means and standard deviation or 95% confidence intervals. We ran two-way permutation analysis of variance (permANOVA) to test for the differences in water physicochemical parameters and OM. A multiple analysis of variance (MANOVA) was used to test for differences in water nutrients, DOC and  $\text{SUVA}_{254}$ . To test differences in sediment respiration we used repeated measures ANOVA (rmANOVA), with time as within-subjects factor, and season, riparian vegetation and treatment (disturbed and undisturbed) as between subjects factors. 5000 permutations were run for all permutation analyses (Anderson, 2001). Tests were considered significant at  $P < 0.05$ . We ran all statistical analyses in R (version 2.11.1; R Development Core Team, 2010).

### 4.4 Results

Physicochemical water descriptors differed between seasons and riparian vegetations (Table 4.1). Dissolved nutrients and pH were similar and conductivity was higher in CC compared to PM (permANOVA, site effect:  $p = 0.002$ ). DOC quantity and bioavailability ( $\text{SUVA}_{254}$ ) between riparian vegetations and seasons did not show a uniform pattern (Table 4.1). In

contrast to CC, PM showed lower concentrations of DOC (permANOVA, site effect:  $p < 0.001$ ), with lower availability ( $\text{SUVA}_{254}$ , permANOVA, riparian vegetation effect:  $p < 0.001$ ). Temperature was similar between riparian succession stages but, as expected, a fourfold temperature increase was observed from winter to late spring (permANOVA, season effect:  $p < 0.001$ ).

During both seasons and for both riparian vegetations, sediment respiration did not vary significantly during the acclimation period (0 h–45.75 h; Figure 4.1). The mechanical stress had no detectable effect on sediment respiration immediately or during the following 39 h (Figures 4.1 and 4.2).



**Figure 4.1:** Dynamics of sediment respiration in winter and late spring from CC (open-land riparian vegetation; a) and PM (forested riparian vegetation; b). Mechanical stress applied to disturbed microcosms (right after measurement at 45.75 h) is represented by the coil at x-axes. Note differences in y-axes between sites. Values are given as mean and error bars represent  $\pm 95\%$  confidence intervals ( $n = 15$ ).

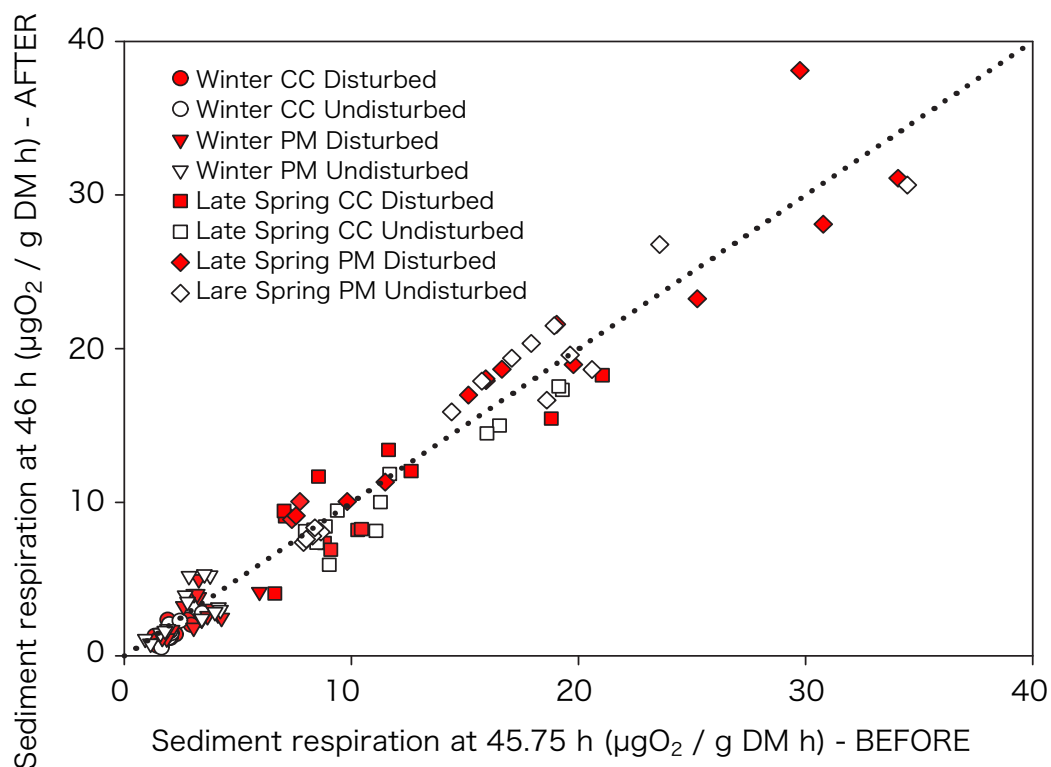
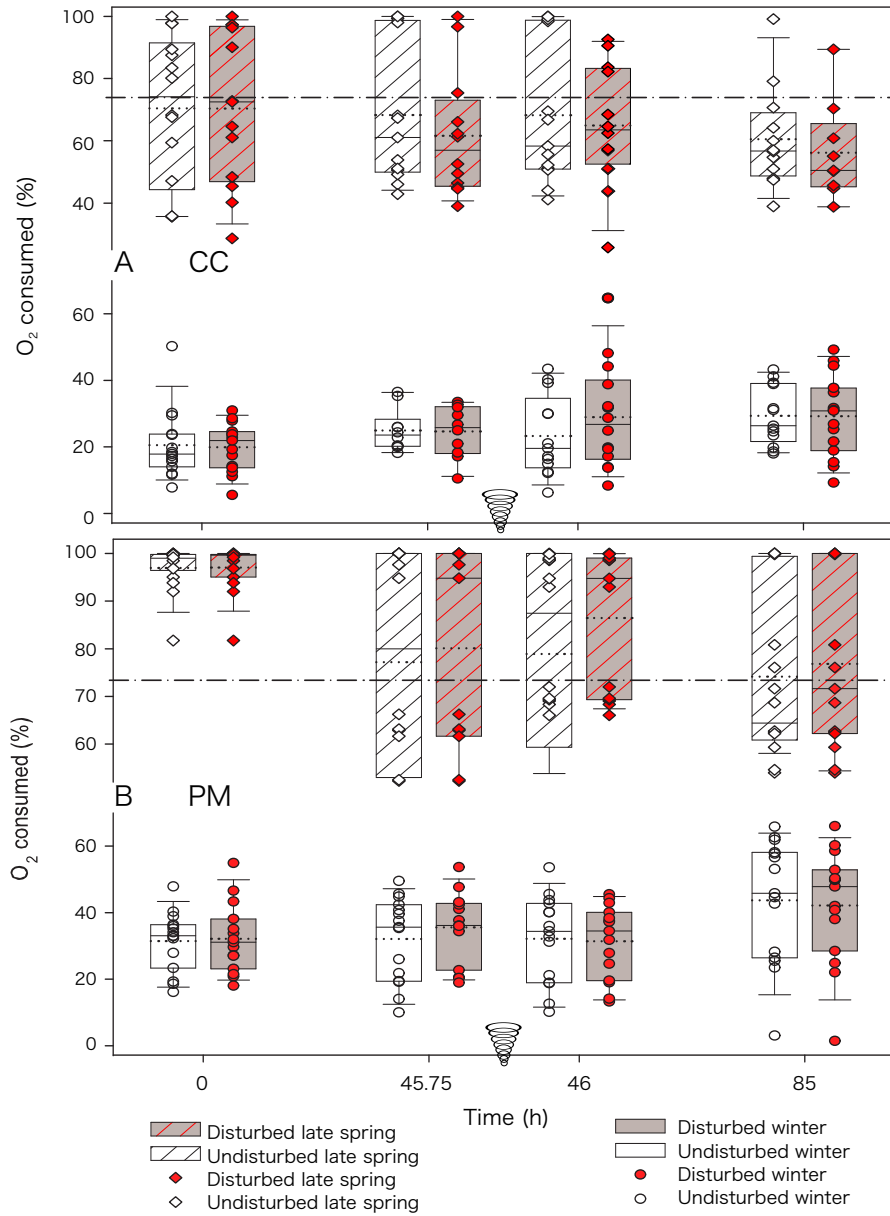


Figure 4.2: Comparison of sediment respiration at 45.75 h and 46 h, for disturbed (solid symbols) and undisturbed (open symbols) microcosms. No changes before and after respiration are given by the 1:1-ratio (dotted line). Values below 1:1 indicate a decrease in sediment respiration from 45.75 h to 46 h, and values above result from an increase in sediment respiration.

Figure 4.1 shows the comparison between sediment respiration right before (45.75 h) and immediately after (46 h) the mechanical stress for both disturbed and undisturbed microcosms; the pattern of change for both the disturbed and undisturbed microcosms was similar. Within 39 h of the application of the mechanical stress, sediment respiration dynamics of the disturbed microcosms were comparable to those for the undisturbed ones (Figure 4.1).

After 85 h in the percolation system, OM did not vary between disturbed and undisturbed microcosms (Winter: CC  $7.24 \pm 3.52 \text{ mg AFDM g}^{-1} \text{ DM}$ , PM  $3.92 \pm 1.23 \text{ mg AFDM g}^{-1} \text{ DM}$ ; late spring: CC  $6.12 \pm 1.97 \text{ mg AFDM g}^{-1} \text{ DM}$ , PM  $10.46 \pm 10.93 \text{ mg AFDM g}^{-1} \text{ DM}$ ) and was not different from initial OM (Table 4.1). In winter, sediment respiration was not different between the two riparian vegetations (Figure 4.1). In late spring, sediment respiration increased  $5.4\times$  in PM and  $4.8\times$  in CC (rmANOVA:  $F_{1,653} = 297.39$ ,  $p < 0.001$ ; Figures 4.1 and 4.3).



**Figure 4.3:** Individual scores and means of the oxygen consumed (%) in the outflowing water in winter (oxygen concentration supplied corresponds to  $12.5 \text{ mg O}_2 \text{ L}^{-1}$ ; A) and late spring (oxygen concentration supplied corresponds to  $9.1 \text{ mg O}_2 \text{ L}^{-1}$ ; B) from CC (open-land riparian vegetation) and PM (forested riparian vegetation). Mechanical stress (right after measurement at 45.75 h) is represented by a coil at x-axes. Consumption up to hypoxia ( $2.5 \text{ mg O}_2 \text{ L}^{-1}$ ; 80% in winter and 73% late spring) is indicated with a dash-dot line. Dots represent single scores. Top and bottom edge of boxes represent the 75<sup>th</sup> and 25<sup>th</sup> percentile, respectively, the line bisecting the boxes represents the median (solid line) and the mean (dotted line) and the ends of the whiskers represent the 90<sup>th</sup> and 10<sup>th</sup> percentile.

Riparian differences resulted in higher respiration rates in PM (rmANOVA:  $F_{1,653} = 21.613$ ,  $p < 0.001$ ). In late spring, at 0 h several microcosms from CC and all from PM consumed more than 90% of the supplied oxygen (Figure 4.3). As the experiment progressed, sediment respiration decreased and fewer microcosms consuming more than 90% of the supplied oxygen were observed (Figure 4.3)

## 4.5 Discussion

Our first research question was: Does mechanical stress associated with short-term disturbances influence sediment associated respiration? Our results show that mechanical stress caused by a short-term disturbance has a negligible effect on sediment respiration. Moreover, the sediment respiration does not vary with the level of activity between riparian vegetations and seasons. Considering the large number of replicates ( $n = 15$ ) employed, any existing differences between treatments should have been detected. As reported for other biological descriptors of the sediment community, 15 replicates should be able to detect a ratio of means equivalent to 1.5 with a power of 80% (Carr & Morin, 2002). Contrary to expectations, sediment respiration was similar for both riparian vegetations during winter, while in late spring, similar to observations made by Gerull et al. (2012), sediment respiration was higher in the advanced forest riparian vegetation (PM) than in early open-land riparian vegetation (CC). High sediment respiration rates in late spring, resulted in a consumption of more than 90% of supplied oxygen. I assume that this was potentially due to respiration during transportation and adaptation to the new advective supply (Boynton et al., 1981; Golde et al., 1994; Higashino, 2012). This assumption is further supported by the reduction of respiration throughout the duration of the experiment. Excluding the microcosms with high oxygen consumption from statistical analysis did not change the conclusion that the mechanical stress had no effect on sediment respiration.

Since the respiration rates were standardized at 10°C, differences in respiration between the two riparian vegetations may depend on other seasonal factors (Pace & Prairie, 2005). Changes in OM, DOC and dissolved nutrients between seasons and riparian vegetations did not support the observed differences in sediment respiration. However, other riparian and season factors, for example the size of the organic matter particles (Bott & Kaplan, 1985; Gerull et al., 2011) and/or the reactivity of the sediment organic material (Nealson & Meyers, 1992) could have played a role in this variation.

Although flow regime and shear stress explain a great part of the variability in stream metabolism (O'Connor et al., 2012), from my results it can be concluded that changes in respiration rates following a short-term disturbance cannot be attributed to mechanical

stress. The collisions among particles of the mechanical stress applied in this study cover a wide range of to short-term disturbances. The duration of short-term disturbances ranges from 3 minutes to several hours (Leopol & Emmett, 1976; Powell et al., 2005), and the rate of sediment transport varies from  $0.000204 \text{ kg m}^{-1} \text{ s}^{-1}$  to  $0.204 \text{ kg m}^{-1} \text{ s}^{-1}$  (Leopold & Emmett, 1976). Thus within this range, the intensity and duration of mechanical stress will not be a factor for sediment respiration. Mechanical stress and its intensity might be a factor for streambed heterotrophic metabolism at higher disturbance frequencies (Hoppe, 1986; Majdi et al., 2011). Therefore any reported change or lack of change in sediment respiration following short-term disturbances possibly need to be attributed to other factors such as loss of community (Uehlinger & Naegeli, 1998), alteration in advective supply of redox partners and nutrients (Fischer & Pusch, 2001; Olsen & Townsend, 2005), and/or re-oxygenation of anoxic zones (Gerull et al., 2012), and their interactions.

All other factors associated with mechanical stress that can potentially influence sediment respiration were excluded in the percolated microcosms. When compared with other studies in which loss of community was excluded, short-term disturbance influenced sediment respiration only in those cases in which sediment pore water was anoxic (Gerull et al., 2012; Mendoza & Mutz, 2013, Chapter 3). Sediment respiration increases in laboratory microcosms after shaking, occurred in those cases when oxygen pore water was depleted (Waksman & Hotchkiss, 1938; Hargrave, 1972; Novitsky, 1983). Depletion of oxygen in pore water and the consequent change in redox conditions are caused by sediment respiration and low advective supply of redox partners and nutrients. In consequence, an increase in advective supply, as well as re-oxygenation or anoxic pore water raises sediment respiration (Boynton et al., 1981; Higashino, 2012). Thus stimulation by short-term disturbances, and possibly overestimation of sediment respiration in disturbed sediment samples (Grimm & Fisher, 1984; Marxsen & Fiebig, 1993; Gerull et al., 2011), may be limited to cases where respiration is limited by insufficient advective supply to molecular diffusion in low permeable sediments (Hargrave, 1972; Novitsky, 1983) or, as observed in some late spring samples of my experiment, to situations where high respiration exceeds the supply of redox partners and nutrients (Boynton et al., 1981).

Hence, while measuring respiration in sandy sediments, unnatural advective supply must be avoided instead of a single mechanical disturbance of sediments. "Undisturbed" sediment respiration measurements by respiration chambers (Grimm & Fisher, 1984; Hedin, 1990) can also over- or underestimate sediment respiration by changing the advective supply. Any element placed in the stream alters the near stream bed pressure gradient and modifies pore water flow and in turn the advective supply (Hedin, 1990; Hutchinson & Webster, 1998), similar to the unnatural flow in respiration chambers (Boynton et al., 1981).

Regarding the second research question on the modulation of effects of mechanical stress with the level of seasonal activity and throughout the riparian succession, my results suggest high respiration rates in late spring could result in anoxic sediments in the streambed, and thus a short-term disturbance would increase sediment respiration, especially in streams with forested riparian zone while in winter or at other periods of low level of activity, short-term disturbances would have a lower, if any, influence on sediment respiration. Measurements of sediment respiration should also take into account the effect of seasonal and riparian vegetation. To summarize, short-term disturbances can influence sediment heterotrophic metabolism mechanically by altering advective supply or re-oxygenating pore water, rather than directly affecting it as a result of the application of mechanical stress.

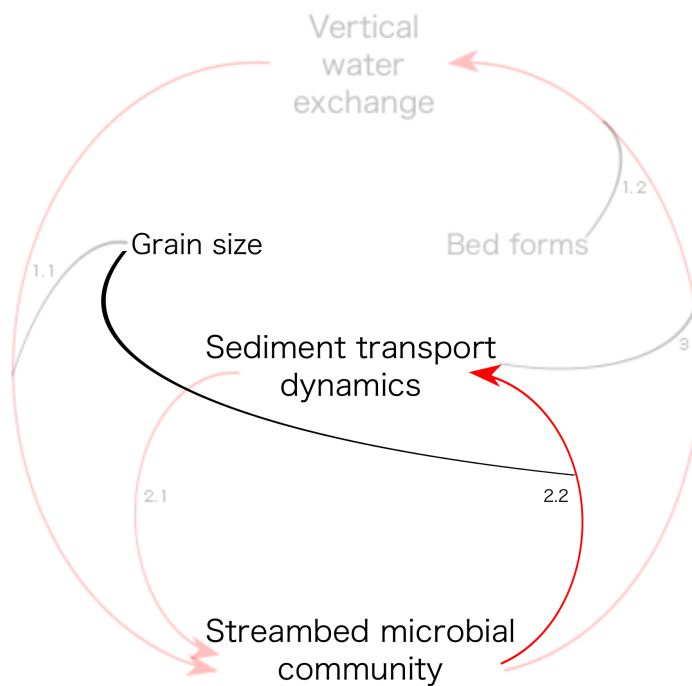
## 4.6 Conclusion

My experiment addressed episodic sediment disturbance as factor for streambed heterotrophic metabolism, and was designed to assess the role of mechanical stress associated with short-term sediment disturbances. A key finding is that short-term mechanical stress (e.g. sediment disturbance or sediment sampling) is not a factor for sediment respiration, and thus other factors are to be taken into account. The observed effects of short-term disturbances seem to rather be attributed to the combination or interplay of the variation in oxygen concentration of the pore water prior to disturbance, which is the result of the level of activity and the advective supply of redox partners and nutrients, and other factors such as loss of community (Uehlinger & Naegeli, 1998), changes in the advective supply (Fischer & Pusch, 2001).

## 4.7 Acknowledgements

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## 5. The algal lift – buoyancy-mediated sediment transport





## 5.1 Abstract

The role of benthic algal mats as biostabilizers of sediments is well-known, however, their potential to lift and transport sediments remains unclear. Under low-flow conditions, matured algae mats may detach from the bed and may lift up sediment, thereby causing disturbance to the uppermost sediment. We tested the potential of algal mats to lift sediments in 12 indoor flumes filled with sand (0.2–0.8 mm), gravel (2–8 mm) or a sand-gravel mixture (25/75% mass). After four weeks, the algal mats covered about 50% of the flumes. Due to the accumulation of oxygen gas bubbles in the mats that developed from high primary production at 4.5 weeks, about half of the algal mats detached from the bed carrying entangled sediments. The area covered by algal mats was similar among sediment types, but the amount of sediment transported was higher for sand and sand-gravel mixture compared to gravel. Our results reveal an algal mat detachment-mediated sediment transport during low-flow periods, with the highest impact for sandy sediments.

## 5.2 Introduction

Benthic production in sand-gravel streams is modulated by the physical characteristics of the streambed. The distribution of sediment grain sizes and sediment disturbances, for instance, influence the biomass, distribution and metabolic activity of benthic microbial communities (Uehlinger et al., 2000; Atkinson et al., 2008; Segura et al., 2011). In reverse, the presence of benthic biofilms and algal mats are known to increase the shear stress needed to entrain the sediments and to reduce the erodibility of cohesive and noncohesive sediment (i.e., biostabilization; Grant & Gust, 1987; Paterson & Daborn, 1991; Krumbein et al., 1994; Yallop et al., 1994; Graba et al., 2010; Vignaga et al., 2013). Biostabilization results from the microbial secretion of extracellular polymeric substances (EPS) that binds sediment particles together (Madsen et al., 1993; Gerbersdorf & Wieprecht, 2015). Such particle-binding can even contribute to the formation of sedimentary structures (Noffke & Krumbein, 1999). Biofilms and algal mats also reduce the streambed permeability by physically occupying the pore space with biomass (cells and EPS) and gas bubbles resulting from metabolic processes, such as methanogenesis (methane; Cuthbert et al., 2010) and primary production (oxygen; Baveye et al., 1998; Mendoza-Lera & Mutz, 2013, Chapter 3).

The development of algal mats and microbial biofilms, in the absence of sediment and flow disturbances, consists of an accrual phase followed by a loss phase (Biggs, 1996). The loss phase is dominated by loss processes through death, emigration, self-generated detachment, and grazing (Biggs, 1996). Self-generated detachment does not only result from death and bacterial degradation (Boulêtreau et al., 2006; Droppo et al., 2007; Graba et al., 2014), but the accumulation of gas in the form of bubbles, such as oxygen or methane, can also be a reason for detachment (Hillebrand, 1983; Fechner-Levy & Hemond, 1996; Gerbersdorf & Wieprecht, 2015). Entrapped gas bubbles increase the buoyancy and, in turn, erodibility of biofilms and algal mats (Sutherland et al., 1998) that may lift from the sediment and eventually form floating mats (Hillebrand, 1983; Cambra & Aboal, 1992; Fechner-Levy & Hemond, 1996). The development of floating mats originating from benthic algal mats has been reported from shallow waters (Hillebrand, 1983), under high nutrient levels (Francoeur et al., 1999), under good light conditions and low-flow in streams (Peterson & Stevenson, 1990), in peatlands (Fechner-Levy & Hemond, 1996), in wave-dominated environments (Droppo et al., 2007), and in lakes (Parker et al., 1982). These floating algal mats differ from metaphyton, which is neither strictly attached to substrata nor truly suspended and originates from true floating algal populations that aggregate among macrophytes and debris (Wetzel, 2001). Although rarely considered, floating algal mats are seasonally prominent features of many freshwater ecosystems (Power, 1990).

Floating mats in freshwater ecosystems have been studied from the perspective of eutrophication, specie composition and insect ecology (e.g. Power, 1990; Simons & van Beem, 1990; Simons, 1994; Sundback & Miles, 2002; Power et al., 2009). The detachment of large seaweeds (kelp) in marine ecosystems was reported to be a small but significant source of sediment transported to beaches that should be considered in sediment budgets for coastal areas (Garden & Smith, 2011). In lotic ecosystems, strong particle-binding processes, detachment and lift from streambed, and drifting and downstream sinking of algal mats and biofilms may also potentially disturb the bed and contribute to the transport of attached sediment particles.

We investigated the potential of algal mats to lift sediments in flumes when detaching from a stream bed as a result of high productivity in a low-flow environment. We compared this for sediments of different grain sizes, ranging from sand (0.2–0.8 mm) to gravel (2–8 mm). We hypothesized that oxygen gas bubbles entrapped in the algal mat as a result of high primary production will increase its buoyancy and cause detachment from the streambed and, consequently, all sediments will be lifted, whereas higher sediment dynamics are expected for sand-beds compared to gravel-beds.

## 5.3 Methods

### 5.3.1 *Experimental setup*

Lowland stream conditions were simulated in 12 flumes located in a greenhouse (Table 5.1). The flumes were supplied with groundwater, enriched with phosphorous to reach oligotrophic conditions, from the experimental site Chicken Creek (Germany, lat 51°36'N, long 14°16'E, 171 m; see Gerwin et al., 2009 for further details) using a peristaltic pump (Watson Marlow, UK). Supply rate during the 37 days of the experiment (26 September to 1 November 2011) was constant at 2.3 L h<sup>-1</sup>. Additional pumps were installed (DDC pumps, Laing, USA) on each flume to partially recirculate the water. The flow conditions simulated were typical of the base-flow conditions of low-energy sand-gravel-bed streams and were well below the threshold for scouring of the bed (Table 5.1).

We divided the set of flumes into three treatments ( $n = 4$ ; Table 5.2) of grain size gravel (2–8 mm), sand (0.2–0.8 mm) and a mixture of both sand and gravel (25/75% mass, respectively). The sediment depth was 4 cm. The initial organic matter content in the washed sediment was  $0.06 \pm 0.04\%$  for sand and was  $0.1 \pm 0.10\%$  for gravel. The flumes were inoculated with a microbial community originating from the streambed of the stream in the Chicken Creek catchment. Twelve sediment samples (500 mL) randomly collected

from the streambed were mixed and suspended in 12 L of the water supplied to the flumes to produce a suspension of the sediment community. We filtered the suspension through a mesh (125  $\mu\text{m}$ ) to avoid macroinvertebrates and to remove larger particulate organic matter. We then inoculated each flume with 1 L of this suspension by adding it carefully to the water column, avoiding any disturbance of the sediment surface.

Table 5.1: Flumes, surface flow and water characteristics. Where applicable, values are mean  $\pm$  SD ( $n_{\text{flumes}} = 2$ ,  $n_{\text{water}} = 37$ ).

Flume characteristics		
Lenght (m)		4
Width (m)		0.12
Heigth (m)		0.12
Surface water depth (m)		0.01
Sediment depth (m)		0.04
Surface flow characteristics		
Current velocity ( $\text{m s}^{-1}$ )		$0.029 \pm 0.17$
	Gravel	$1.33 \pm 0.78 / (8.20 \pm 8.88) \times 10^{-2}$
Shear velocity ( $\text{m s}^{-1}$ ) / Shear stress (Pa)	Mixture	$0.16\text{--}2.40 / (2.23\text{--}2.42) \times 10^{-2}$
	Sand	$0.38 \pm 0.22 / (1.80 \pm 1.96) \times 10^{-2}$
Surface water residence time (h)		6
Reynolds number		$296.3 \pm 172.3$
Froude number		$0.095 \pm 0.055$
Water physicochemical characteristics		
$\text{NO}_3\text{--N}$ ( $\mu\text{g N L}^{-1}$ )		$9.2 \pm 10.3$
$\text{NH}_4\text{--N}$ ( $\mu\text{g N L}^{-1}$ )		$11.1 \pm 3.7$
$\text{PO}_4\text{--P}$ ( $\mu\text{g L}^{-1}$ ) <sup>a</sup>		2
SRP ( $\mu\text{g L}^{-1}$ ) <sup>b</sup>		< 0.05
Conductivity ( $\mu\text{S cm}^{-1}$ )		$555.8 \pm 32.1$
pH		$8.2 \pm 0.1$
DOC ( $\text{mg C L}^{-1}$ )		$10.3 \pm 0.9$
Water temperature ( $^{\circ}\text{C}$ )		$18.7 \pm 0.3$

<sup>a</sup> Final concentration of  $\text{PO}_4\text{--P}$  after enrichment.

<sup>b</sup> Values < 0.05 indicate that the concentration was below detection limit.

### 5.3.2 Sediment and surface flow characterization

Hydraulic conductivity ( $K_f$ ,  $\text{cm s}^{-1}$ ) was determined by falling-head tests (soil water permeameter; Eijkelkamp, Giesbeek, The Netherlands) from the same sediment used in the flumes to determine the initial  $K_f$ , and from sediment from each flume at the end of the experiment. The Netherlands). We estimated  $K_f$  of gravel from the permeability ( $\kappa$ ,  $\text{m}^2$ ) as a function of the sediment porosity ( $\phi$ ) and the sediment grain diameter ( $d_s$ ) (Bear, 1988; Boudreau et al., 2005);

$$\kappa = \frac{5.6 \times 10^{-3} \cdot \phi^3 \cdot d_s^2}{(1 - \phi)^2}$$

$$\kappa = K_f \frac{\mu}{\rho \cdot g}$$

where  $\mu$  is the dynamic viscosity of water ( $\text{kg m}^{-1} \text{s}^{-1}$  at  $20^\circ\text{C}$ ),  $\rho$  is the density of water ( $\text{kg m}^{-3}$  at  $20^\circ\text{C}$ ) and  $g$  is acceleration due to gravity ( $\text{m s}^{-2}$ ).

The number of particles per unit g sediment was determined microscopically. Digital pictures of each gravel and sand were taken from 15 replicates of 0.5 g and 0.05 g, respectively. Images were analyzed using ImageJ (Abramoff et al., 2004; Rasband, 1997–2012; Schneider et al., 2012).

The water velocity in the flumes was calculated from the mean travel time of the surface water labeled with a conservative fluorescent tracer (uranine; Fiber-Optic fluorometer; Gotschy Optotechnik, Adnet, Austria). We assessed surface flow velocity of flumes from the breakthrough curves of a pulse injection of conservative fluorescent tracer (uranine) measured by with a field fluorometer (uranine; Gotschy Optotechnik, Adnet, Austria). We calculated the Froude number ( $Fr$ ) as

$$Fr = \frac{v}{(g \cdot d)^{0.5}}$$

where  $v$  is the mean surface-water velocity ( $\text{m s}^{-1}$ ), and  $d$  is the flow depth (m).

We calculated Reynolds number ( $Re$ ) as

$$Re = \frac{v \cdot L}{\nu}$$

$L$  is the mean flow depth (m), and  $\nu$  is the kinematic viscosity ( $\text{m}^2 \text{s}^{-1}$  at  $20^\circ\text{C}$ ).

Shear stress ( $\tau_0$ ) was estimated from shear velocity ( $U_*$ ,  $\text{m s}^{-1}$ ).

$$U_* = \sqrt{\frac{\tau_0}{\rho}}$$

Table 5.2: Sediment characteristics. Where applicable, values are mean  $\pm$  SD ( $n = 5$ , except number of particles  $n = 15$ ).

	Gravel	Mixture	Sand
Sediment grain size (mm)	2–8	0.2–8	0.2–0.8
Hydraulic conductivity ( $\text{cm s}^{-1}$ )	0.77	$0.004 \pm 0.0019$	$0.0062 \pm 0.0025$
Porosity	$0.18 \pm 0.004$	$0.18 \pm 0.005$	$0.20 \pm 0.005$
Num of particles $\text{g}^{-1}$ sed	$5.14 \pm 2.6$	$2,005.73 \pm 706.1$	$2,969.56 \pm 125.44$

Shear velocities ( $U_*$ ) were calculated following Wilcock (1996):

$$\frac{U}{U_*} = \frac{1}{\kappa} \ln \frac{z}{z_0}$$

where  $\kappa$  is von Karman’s constant (taken to be 0.40),  $z$  (m) is the bed roughness length corresponding to  $U = 0$ ,  $z_0$  (m) is the height above the bed estimated from the particle size.

### 5.3.3 *Water physicochemical characteristics*

We measured the water temperatures in the flumes continuously (HOBO; Onset, Bourne, Massachusetts). Water-column conductivity and pH were measured weekly (WTW, Weilheim, Germany). We collected and filtered ( $0.45 \mu\text{m}$  cellulose acetate filters; Sartorius, Göttingen, Germany) water samples for analysis of dissolved organic carbon (DOC),  $\text{NO}_x\text{-N}$ ,  $\text{NH}_4\text{-N}$ , and soluble reactive phosphorous (SRP) every five days. We measured  $\text{NO}_x\text{-N}$ ,  $\text{NH}_4\text{-N}$ , and SRP spectrophotometrically with a segmented flow injection analyzer (PERSTORP Analytical, Rodgau, Germany) and UV/VIS spectrometer (Perkin Elmer, Rodgau, Germany), according to standard methods (DEV, 1976-2009). The DOC was measured with a total organic carbon analyzer (Shimadzu, Tokyo, Japan).

### 5.3.4 *Algal mat development, lift and characterization*

A multichannel fluorescence probe with a glass fiber extension and a shielding against environmental light (Aberle et al., 2006; bbe-Moldenke, Schwentinental, Germany) was used to survey the Chlorophyll  $a$  (Chl  $a$ ) concentration at the sediment surface of the flumes four times during the experiment. The default calibration was used. Measurements were carried out at five randomly chosen sites in each flume on dates without macroscopically detectable (obvious) growth of filamentous algae. When visible growth of patches of filamentous algal

mats started, Chl *a* was measured at three randomly chosen positions of the algal mats and at three randomly chosen positions without mats (hereafter called benthic biofilm).

Whole-flume metabolism was measured at days 5, 16, 30, and 37. We sealed the water surface gastight and without any headspace against the atmosphere with acrylic glass lids; any gas exchange through the gap between the walls of the flumes and the lids was avoided by using rubber insulation strips. We measured the change in water-column oxygen concentration over time at 30 s intervals with oxygen optodes (PreSens, Regensburg, Germany). Community respiration (CR) was recorded as the decline in oxygen concentration after having the flumes covered with an additional black (opaque tarpaulin) foil for a maximum time of 2 h. Subsequently, gross production was determined by removing the tarpaulin and measuring the change in oxygen concentration (max. 2 h). Net primary productivity (NPP) was calculated as the difference between gross production and CR. Oxygen saturation in surface water and the uppermost 1 cm of the sediments (photic zone; Mendoza-Lera & Mutz, 2013, Chapter 3) was measured at days 2, 16, 30, and 35 around noontime with a needle probe microoptode (PreSens, Regensburg, Germany). Three random sites were measured per flume at 0.5 cm intervals at a depth of 0 to 1 cm. When production started to peak in the progress of the colonization, oxygen gas bubbles were produced at the sediment surface; oxygen production and release in form of oxygen gas bubbles were measured at days 25 to 27. The release rate per sediment surface ( $\text{m}^2$ ) was estimated by sampling the oxygen produced in glass funnels (40 mm  $\varnothing$ ) with a closed tip, completely filled with water from the flume and arbitrarily located on the sediment surface. We measured 9 out of 12 flumes (3 per sediment type) over a period of 24 h, changing the funnels after 12 h to differentiate between day and night conditions.

After the detachment at day 32, the floating mats remained in the flumes to enable observation of their dynamics for five additional days. At day 37, the floating mats were collected from each flume, including the entangled sediment. The mats of each flume were dried in paper, and about 0.2 g of material was frozen for later Chl *a* determination. The rest of the material was dried at 60°C for 24 h and then at 105°C for 16 h, and their dry mass (DM) was subsequently weighed. Later, they were combusted at 500°C (3 h) to obtain organic matter (OM) as ash-free dry mass (AFDM). The samples were then combusted at 900°C 2 h; Heiri et al., 2001) to obtain the actual content of sediment free of carbonates in the mats. The Chl *a* was extracted in ethanol (90%) with glass beads (1 mm diameter, max intensity 30 s). The mixture was kept at 70°C for 4 min and then placed in an ultrasonic bath at medium intensity (45% of the maximum power) for 2 min. Within 2 h after extraction, the extracts were cleared by filtering through paper filters (Macherey-Nagel Germany). The Chl *a* concentration in the cleared extract was measured according to DIN

38412-L16. Closeup areal pictures of all flumes were taken (Canon, Surrey, UK) at day 32 to assess the surface coverage of the algae and the extent of the floating algal mats. ArcGIS (ESRI 2009) was used to quantify the algal coverage from the pictures after georeferencing.

### 5.3.5 *Data analysis*

We reported all values as means and standard deviation. We expressed NPP and CR per  $\text{m}^{-2}$  sediment surface and standardized CR to  $21^{\circ}\text{C}$ , assuming that relationships between temperature and metabolic rate were exponential according to a  $Q_{10}$  of 2 (Davidson & Janssens, 2006).

We tested the differences between sediment types by permutation analysis of variance (permANOVA; R-package *lmPerm* 1.1-2). We ran 5000 permutations for all permutation analyses (Anderson, 2001). Post hoc tests were carried out with Tukey's HSD (R-package *agricolae* 1.2-0). Data were transformed ( $\log(x+1)$ ) when necessary. We considered tests significant at  $p < 0.05$ . We ran all statistical analyses in R (version 2.11.1; R Development Core Team, 2010).

## 5.4 Results

### 5.4.1 *Physicochemical characteristics*

The characteristics of the water supplied to the flumes did not vary throughout the experiment and the flumes were oligotrophic (Table 5.1; Baldy et al., 2007). The water temperature in the sand flumes was lower than in the gravel and mixed sand-gravel sediment flumes (permANOVA:  $p = 0.025$ ), but the differences were less than  $0.41^{\circ}\text{C}$  on average. The flow in the flumes was tranquil and laminar (Table 5.1), and the shear stress was well below critical threshold to entrain sand and gravel (0.1 Pa and 0.5 Pa, respectively; Bridge, 2003) and insufficient to scour the bed surface and detach the benthic community.

### 5.4.2 *Algal mat development and detachment*

The colonization and development of algal mats was similar for all sediment types (Figure 5.1). After 17 days, the entire surface of the flumes was visually covered by algae and the mats started to develop. After 31 days, about 50% of the surface of the sand and gravel flumes ( $0.218 \pm 0.05 \text{ m}^2$ ; Figure 5.1) and about 70% of the sediment mixture flume was covered with mats that accounted for more than 80% of the Chl *a* in the bed of the flumes. During the night hours from day 31 to 32, most of the algal mats detached from the flume



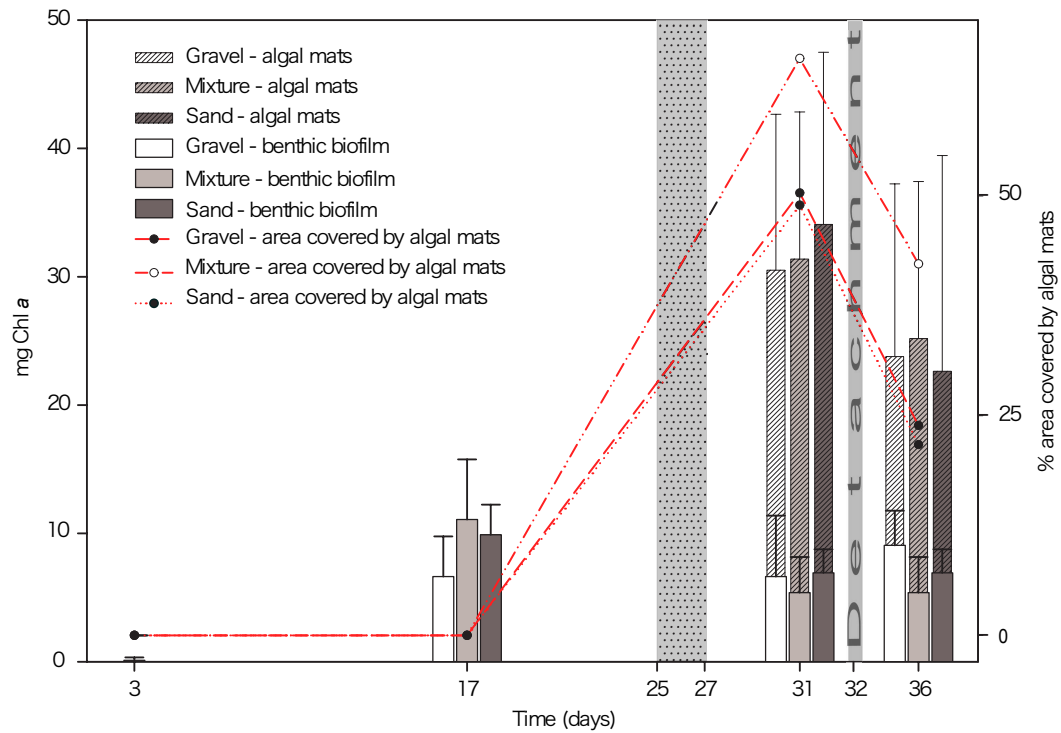


Figure 5.1: Total amount of Chl *a* contained in the benthic biofilm and the algal mats for each sediment type (left y-axes) determined by the fluorescence probe. Average proportion of total flume area ( $0.44 \text{ m}^2$ ) covered by algal mats (right y-axes). The period of intense oxygen bubble release is indicated between days 25 and 27. The detachment is indicated at day 32 (mean  $\pm$  SD,  $n = 4$ ).

bed and formed floating mats. The area covered by mats that detached (hereafter called detached area) was similar for all sediment types ( $0.114 \pm 0.036 \text{ m}^2$ ) and reduced the area covered by algal mats by about  $50 \pm 17\%$  (Figure 5.1; permANOVA:  $p = 0.0006$ ). From day 32 till day 37, the floating mats remained buoyant in the flumes and did not sink. During those five days at the flow velocity of the flumes, the floating mats that also carried entangled sediments would have drifted up to 12 km downstream.

Although there were no signs of algal or biofilm growth at day 5 (Figure 5.1), the community inhabiting the flumes was metabolically active (Figure 5.2). Independently of the sediment type, CR increased about fivefold and NPP about eightfold from day 5 to day 16 and did not change thereafter until the removal of the lifted portions of mats from the flume at day 37, when both CR and NPP returned to the rates measured at day 5 (permANOVA:  $p_{CR} < 0.0001$  and  $p_{NPP} < 0.0001$ ; Figure 5.2).

On average, the surface water was oversaturated with oxygen ( $113.4 \pm 14.3\% \text{ O}_2$ ). No differences in oxygen saturation were observed between surface water and the pore water

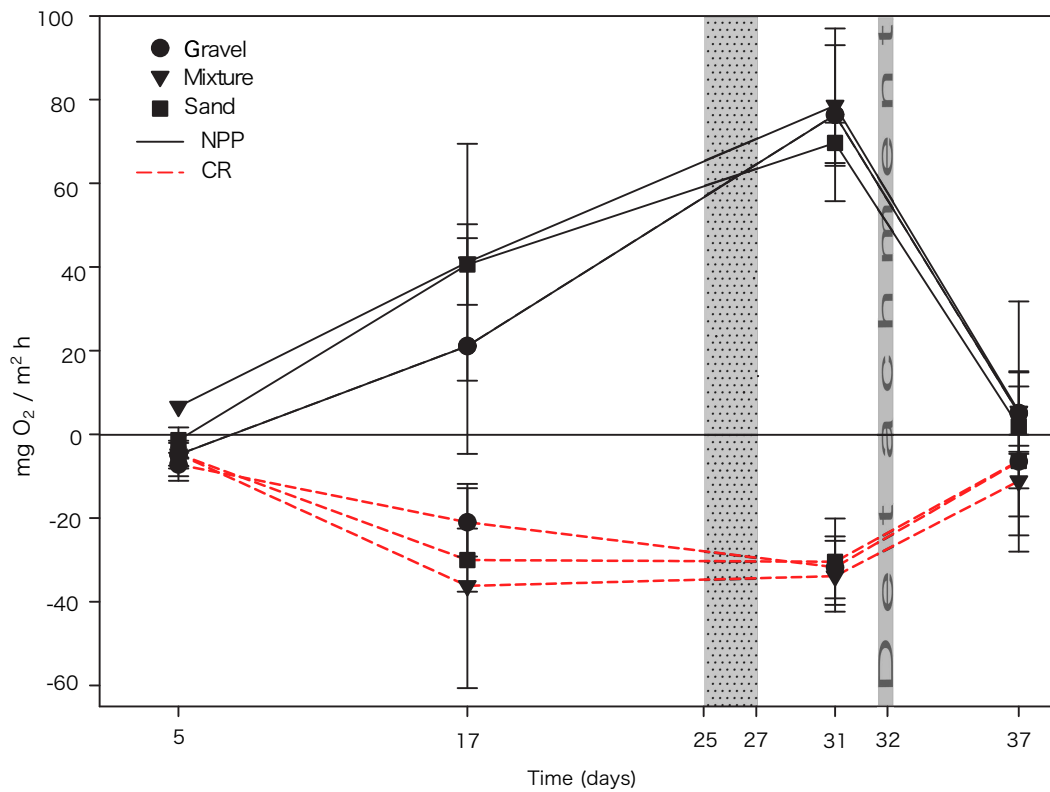


Figure 5.2: Rates of community respiration (CR) and net primary productivity (NPP) standardized at 21 °C. Area between days 25 and 27 indicates the period of intense oxygen bubble release. The detachment is indicated at day 32 (mean  $\pm$  SD,  $n = 4$ ).

in the uppermost 1 cm of the sediments; from day 2 till day 35, oxygen saturation in the surface water increased from  $94.2 \pm 1.3\%$  to  $114.7 \pm 4.1\%$ . The O<sub>2</sub> supersaturation resulted in gas bubbles dominated by O<sub>2</sub>. From day 25 to 27, production and release of O<sub>2</sub> bubbles by algal mats was  $91.1 \pm 5.9 \text{ mL O}_2 \text{ h}^{-1} \text{ m}^{-2}$  for all sediment types, which corresponded to an O<sub>2</sub> production rate of  $121.1 \pm 6.5 \text{ mg O}_2 \text{ h}^{-1} \text{ m}^{-2}$ . Oxygen release in the form of bubbles was twofold higher during the day ( $161.8 \pm 14.1 \text{ mg O}_2 \text{ h}^{-1} \text{ m}^{-2}$ ) than during the night ( $75.1 \pm 13.7 \text{ mg O}_2 \text{ h}^{-1} \text{ m}^{-2}$ ; permANOVA:  $p < 0.001$ ).

A different volume of gas was needed to increase the buoyancy and detach the algal mats depending on the sediment types (Table 5.3; permANOVA:  $p = 0.0134$ ); the mixture was similar to sand and gravel, but algal mats from sand needed about twofold more gas to detach than those developing in gravel. Based on the NPP measured at day 30, the volume of oxygen needed to detach the mats in the entire flumes would have been produced in  $0.36 \pm 0.233 \text{ h}$ . From day 30 to 32, the difference in temperature between day and night was not significantly different from other days. The amount of Chl *a* and OM per unit of detached

area did not vary between sediment types (Figure 5.1 and Table 5.3). More than twice the mass of Chl *a* and of OM was needed to detach a unit of gravel compared to sandy sediments (Table 5.3; permANOVA:  $p_{Chl\ a} < 0.0001$ ,  $p_{OM} = 0.0014$ ). We calculated the potential bed area disturbed by the detachment assuming that the sediment entangled in the floating mats was removed from the uppermost layer of sediment grains. The area of entangled sediment was more than two orders of magnitude larger in the sand-beds than in the gravel-beds and mixed sand-gravel-beds (Table 5.2; permANOVA:  $p = 0.00004$ ).

## 5.5 Discussion

Our results show that algal mats can develop on noncohesive sediments and, as a result of high primary production, detach to form floating mats in shallow low-energy oligotrophic streams with good light conditions and no grazer pressure. Proliferation of algal mats is commonly attributed to high nutrient levels that allow them to escape grazer control (Francoeur et al., 1999). Our results show that in the absence of grazer pressure, the algal mats can develop independently of the trophic state of the stream (Power et al., 2009). However, the timing of the sloughing of algal mats is, of course, related to net production controlled by the trophic state. In our study, the algal mats detached 32 days after inoculation independently of the sediment type. In agreement with Romaní et al. (2014), we considered that the algal mats mature after 28 days. In previous studies, detachment of algal mats in eutrophic systems started after 22 days (Hillebrand, 1983), and autotrophic biofilm developing in cohesive sediments detached after 9 days (Droppo et al., 2007). Biggs (1996) presented an idealized accrual curve in which loss phase due to detachment occurred when the mats were mature and the biomass reached a maximum (peak biomass). Graba et al. (2010) reported peak biomass of algal mats at 32 g AFDM  $m^{-2}$ , which is similar to the organic matter detached from the mixture- and gravel-bed flumes in our experiment.

The detachment in this study was buoyancy-mediated by the accumulation of oxygen bubbles in the algal mats resulting from high primary production; these findings match previous observations (Hillebrand, 1983; Cambra and Aboal, 1992; Sutherland et al., 1998; Droppo et al., 2007). Due to the supersaturation of oxygen as a result of primary production, oxygen gas bubbles resulted spontaneously nucleated in imperfections of the sediments and filaments (Hemmingsen, 1977), and detached when the adhesive forces were overcome by their buoyancy, in a similar way to the detachment of the algal mats. Two main processes occurred to detach the algal mats and lift sediments: (1) supersaturation, which caused the formation of oxygen bubbles, and (2) the strong filamentous matrix characteristic of mature algal mats to retain the bubbles from individually lifting.

Table 5.3: Characteristics of the floating algal mats. Where applicable, values are mean  $\pm$  SD ( $n = 4$ ).

	Gravel	Mixture	Sand
Chl $a$ (mg Chl $a$ g <sup>-1</sup> sed)	1.16 $\pm$ 0.201	0.52 $\pm$ 0.16	0.31 $\pm$ 0.09
Chl $a$ (mg Chl $a$ m <sup>-2</sup> )	15.61 $\pm$ 2.44	24.58 $\pm$ 5.79	31.24 $\pm$ 20.14
OM (mg AFDM g <sup>-1</sup> sed)	0.17 $\pm$ 0.03	0.062 $\pm$ 0.03	0.025 $\pm$ 0.014
OM (mg AFDM m <sup>-2</sup> )	33.78 $\pm$ 12.0	33.79 $\pm$ 29.9	12.20 $\pm$ 2.83
Entangled sediment (g m <sup>-2</sup> )	41.0 $\pm$ 19.1	100.0 $\pm$ 76.8	153.6 $\pm$ 147.1
Transported particles (n <sup>o</sup> m <sup>-2</sup> )	144.2 $\pm$ 76.0	187,224 $\pm$ 138,338	433,407 $\pm$ 426,196
Disturbed area (m <sup>2</sup> )	(2.05 $\pm$ 0.81) $\times 10^{-4}$	(2.02 $\pm$ 0.94) $\times 10^{-3}$	(4.95 $\pm$ 2.01) $\times 10^{-3}$
Disturbed area (%)	0.19 $\pm$ 0.103	2.57 $\pm$ 1.9	5.6 $\pm$ 5.58
Volume of gas oxygen (mL O <sub>2</sub> mat <sup>-1</sup> )	1.88 $\pm$ 0.52	3.06 $\pm$ 1.10	5.30 $\pm$ 1.86

In previous studies, in the same flumes and with similar sandy sediments, no algal or sediment detachment was observed in spite of the high NPP and high density of gas bubbles in the sediment (Mendoza-Lera & Mutz, 2013; Chapter 3). In this previous experiment, dense benthic algal mats did not develop and, due to the lack of a filamentous matrix, gas bubbles were released from the pores when a certain volume was reached. This process, however, did not cause the detachment of any biofilm. We attribute this different behavior to the differences in phosphorous and light intensity. As observed by Hill et al. (2009), algal biomass reaches a maximum around  $100 \mu\text{E m}^{-2} \text{ s}^{-1}$ ; when phosphorous concentration is low, algal biomass decreases when light intensities increase ( $>100 \mu\text{E m}^{-2} \text{ s}^{-1}$ ), while at higher phosphorous concentrations, increases in light intensity do not influence algal biomass. In the present study, the water had  $2 \mu\text{g PO}_4\text{-P L}^{-1}$  and the light intensity was  $240 \mu\text{E m}^{-2} \text{ s}^{-1}$ , while the water from Mendoza-Lera & Mutz (2013, Chapter 3) had  $< 0.5 \mu\text{g PO}_4\text{-P L}^{-1}$  and the light intensity was  $1200 \mu\text{E m}^{-2} \text{ s}^{-1}$ . Jørgensen et al. (1983) reported that oxygen gas bubbles had a lifetime of  $< 1 \text{ h}$  before they grew to a size where the buoyancy made them detach from the sediment. In our study, the high release of oxygen bubbles previous to the detachment and the short time required to produce the volume needed to detach the algal mats took about seven days to accumulate in the mats enough to detach them. Changes in temperature or pressure could have favored the lift of the mats at day 32, however, daily oscillations in temperature on day 32 were not different from daily oscillations since the start of the development of the algal mats. Thus, the development of a dense network of filamentous algae in a mature stage seems to be a factor for detachment rather than the level of primary production and the generation of oxygen bubbles.

Our results suggest that the mats can remain drifting for more than five days and, hence, could potentially drift several kilometers downstream. However, floating mats tend to accumulate at shallow stream banks and in low gradient downstream reaches at much shorter distances (Power et al., 2009). This process causes export and heterogeneous distributions of nutrients and autochthonous particulate organic matter. In fact, floating masses of filamentous algae in the western part of the Netherlands have even been used as fertilizer (Hillebrand, 1983).

Removing the floating mats from the flumes of this study significantly reduced CR and NPP. Similar spatiotemporal changes in streambed metabolism are caused by sediment disturbance events related to increased flow (Gerull et al., 2012; Mendoza-Lera & Mutz, 2013, Chapter 3). Therefore, in addition to this well-recognized process, the heterogeneity in streambed metabolism will be driven by buoyancy-mediated detachment and drift during low-flow periods with high primary production.

Due to the high heterogeneity in the entangled sediments in sand and mixture, no

differences were detected in the amount of entangled sediments between sediment types. However, the general trend indicated that as the mean particle size was reduced (gravel > mixture > sand), the amount of entangled sediment was increased. We attribute this heterogeneity to differences in the distribution of the filament matrix within the sediment. Differences in the volume of oxygen needed to detach the mats suggest that the potential to transport sediment resulted from the particle size, rather than from the total mass of entangled sediment. Thus, algal mats developing on sandy sediment had a higher potential to transport sediment.

A fourfold increase in base flow ( $0.05 \text{ m}^3 \text{ s}^{-1}$ ) in a sandy stream for 40–120 min resulted in  $0.0124 \text{ m}^3 \text{ m}^{-2}$  of sediment scoured and transported downstream (Powell et al., 2005; Powell et al., 2007). In our flumes, buoyancy-mediated sediment transport represented less than 1.5% of the sediment transport reported in their studies. Therefore, the amount of transported sediment by buoyancy-mediated detachment and drift might be of low significance from a sediment transport balance perspective. However, it may be significant during low-flow periods for sediment transport to downstream sites and it certainly contributes to the heterogeneity and dynamics of metabolism at the streambed: shallow disturbances of the streambed sediments (2 cm), increased vertical water flux due to the disruption of EPS and the creation of irregularities in the bed surface (Mendoza-Lera & Mutz, 2013, Chapter 3). The detachment of algal mats increased irregularities in the streambed by (1) creating glades or streambed clearing, and (2) by removing the uppermost layer of sediment particles differently among sediment types (i.e., entangled area). Streambed metabolism will, therefore, vary not only as a result of physical loss of algal mats as mentioned before. Small changes in the streambed surface can alter the pressure gradient over the streambed and increase vertical exchange (Mendoza-Lera & Mutz, 2013, Chapter 3), with consequences for the streambed and hyporheic zone heterotrophic metabolism (Boulton et al., 1998). In low-energy sand-bed environments during low-flow periods, such as litoral zones (Sundbäck, 1994) and lowland streams, bed heterogeneity and streambed metabolism will also be affected by the detachment of algal mats. The buoyancy-mediated detachment and drift should be considered to accurately model biofilm and algal biomass (Boulêtreau et al., 2006), metabolism and sediment transport at low-flow.

## 5.6 Conclusion

Our experiment confirms that in low-flow and even oligotrophic conditions, algal mats developed that detached and caused disruption of the upper sediment layers. Lifted algal mats and sediments were transported downstream to sedimentation sites. This process can

affect cohesive (Droppo et al., 2007) as well as noncohesive sediments, including gravel beds. Under low-flow conditions, progressive accumulation of oxygen bubbles in the dense network of algal mats, generated by a high primary production rate, resulted in buoyancy-mediated sediment transport. The particle entrapment in floating mats was shown to be stronger for sand-beds compared to gravel-beds, consequently, fine-grained areas of the streambed were shown to have a higher potential for this type of biogenic sediment dynamics.

## 5.7 Acknowledgements

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## 6. Final discussion and conclusions

The present dissertation aimed to study the reciprocal influences of streambed hydrogeomorphology and the microbial community (Figure 1.2). The influences comprise a portion of the interactions (Figure 6.1) occurring in the streambed which, in turn, modulate the streambed metabolism and nutrient cycling. The single research questions (section 1.3.1) were separately discussed in the previous chapters. Here, I discuss the implications of the influences studied in the broader context (conclusions in margin notes).

### 6.1 Streambed microbial community can influence its physical habitat

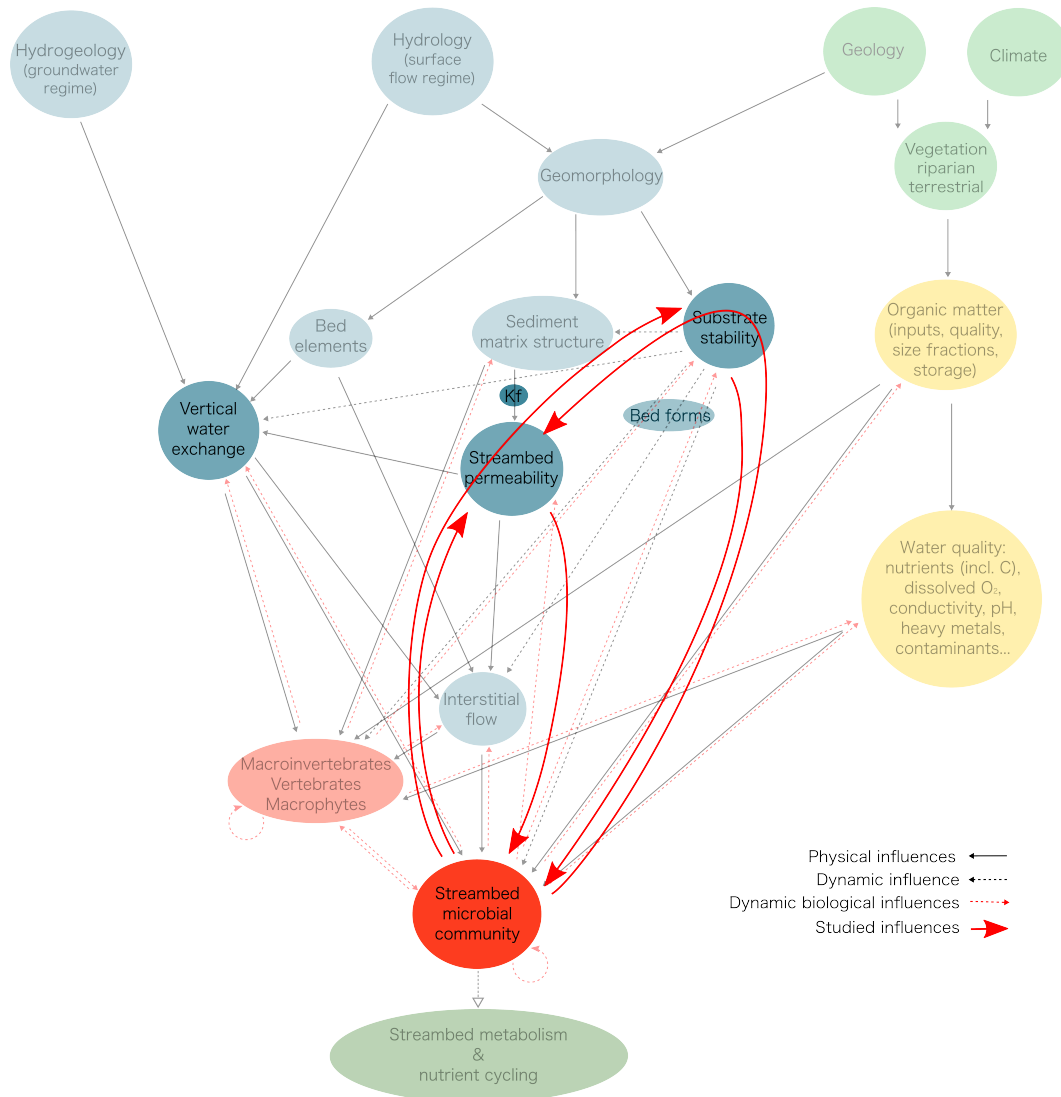
One of the novel findings of the present dissertation is the potential of the microbial community to influence its physical habitat by means of gaseous end products. Streambed sediments are several orders of magnitude larger than the inhabiting microorganisms; as a consequence, it is very unlikely that the microbial community influences the sediment matrix directly and, in turn, the VWE across the streambed. Previous studies, however, showed that the microbial community in sandy sediments can influence the VWE by means of metabolic products such as extracellular polymers (Vandevivere & Baveye, 1992; Nogaro et al., 2006). Biogenic gas bubbles originating from denitrification and methanogenesis have been reported to influence streambed permeability (e.g. Baveye et al., 1998; Boudreau et al., 2005; Istok et al., 2007; Cuthbert et al., 2010). It is generally assumed that oxygen gas bubbles originating from primary production are rapidly consumed and are not significant for VWE (e.g. Baveye et al., 1998; Gerbersdorf & Wieprecht, 2015). In contrast to this general assumption, the flume experiments revealed that gas bubbles originating from high primary production can reduce pore space and in turn block VWE as well (Chapter 3) and, consequently, influence sediment transport (Chapter 5).

Entrapment of gas bubbles in the dense filament network of algal mats subsequently caused lifting and transport of algal mats and entangled sediment particles (Chapter 5).

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Biogenic gas bubbles mediated the influence of the microbial community on its physical habitat.

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**Figure 6.1:** Significance of the influences studied (Figure 1.2; thicker red lines) within the simplified ecosystem perspective represented in Figure 1.1. Those lines crossing a factor indicate interactions of several factors. Influences with a dynamic character are indicated by dotted lines. Blue bubbles correspond to geomorphological factors, red bubbles correspond to the streambed community, and the orange ones correspond to organic matter and water quality. Kf = hydraulic conductivity. Adapted from Boulton et al. (2010) and Brunke & Gonser (1997).

This, to the best of my knowledge, is a novel mechanism of sediment transport occurring during low-flow periods which has not been regarded until now. Detachment of algal mats as a result of high primary production (Hillebrand, 1983; Cambra & Aboal, 1992; Fechner-Levy & Hemond, 1996) or methane (Fechner-Levy & Hemond, 1996) has been reported previously, but not observed as a sediment transport mechanism. Buoyancy-mediated sediment transport is of small significance from a sediment transport perspective. Assuming

that, similar to the observations made in the flumes, half of the surface covered by algal mats will be lifted, about  $0.032 \text{ kg m}^{-2}$  of sediment can potentially be transported downstream during low-flow periods lasting longer than 32 days.

## 6.2 Reciprocal influences among the factors studied

As discussed in Chapter 4, the significance of sediment transport dynamics (i.e., short-term sediment disturbance) for stream metabolism is a function of the VWE. In areas with higher VWE, resulting from the presence of bed forms, such as ripples, or coarser grain size resulting from different patterns of sediment transport (Bridge, 2003; Cardenas et al., 2008; Segura et al., 2011), sediment respiration will be less affected by sediment transport events. This is because the advective supply of redox partners and nutrients maintains the microbial community under “well-oxygenated conditions.” Therefore, re-oxygenation of pore water with surface water does not result in increased respiration. However, other factors such as season or riparian vegetation, influence sediment microbial respiration and can also potentially mask the interrelation between the VWE and sediment transport.

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The significance of sediment transport dynamics for streambed metabolism is a function of the VWE.

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In Chapter 3, the VWE was annulled by the microbial community in sandy flumes with differing VWE (in level- and ripple-bed flumes). Thus, the influence of the microbial community can interact with sediment transport events and increase sediment respiration independently of the expected VWE based on streambed hydrogeomorphology. Although the immediate effect of a short-term disturbance on streambed metabolism was not assessed in Chapter 3, the gas-mediated clogging did not cause a decrease in oxygen concentration in the pore water. The presence of oxygen bubbles trapped in the pores can maintain the mass transfer of oxygen from the gas bubbles when the VWE is blocked. Hence, the influence of the microbial community on the VWE that reduces the exchange of water between the streambed and the surface water does not necessarily influence the mass transfer of oxygen. Flume experiments also revealed that sediment transport influenced the VWE. In Chapter 3, a short-term sediment disturbance released the entrapped gas bubbles. This not only restored but also increased the VWE by creating irregularities in the flume bed, which, with the surface water flow, altered the pressure pattern (Thiboudeaux & Boyle, 1987; Cardenas et al., 2004; Salehin et al., 2004). Hence, sediment dynamics are a source of spatiotemporal VWE heterogeneity.

Fisher & Grimm (1991) proposed a conceptual model in which “control” of the ecosystem function varies spatiotemporally as a function of the disturbance regime (floods and drying; Grimm, 1995). In the present study, I considered short-term sediment disturbances that did not cause drastic changes in the system and whose influence varied as a function of the VWE.

Thus my results supplement the conceptual model proposed by Fisher & Grimm (1991) by adding that between drastic disturbance events, such as floods and drying, the VWE is the primary physical template shaping streambed microbial communities and that it drives the ecosystem function. Moreover, the influence of short-term sediment disturbances depends firstly on the VWE, and secondly, on the influence of the microbial community on VWE.

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VWE is the primary physical template for streambed microbial communities.

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Previous research suggested that, assuming constant metabolic rates, differences in streambed metabolism resulted from heterogeneity in the VWE and interstitial flows (Findlay, 1995). The results obtained in Chapter 2 indicate that the metabolic rates depend on the advective supply of electron acceptors and nutrients. Therefore, streambed metabolism is controlled by VWE at different scales. This observation suggest that in agreement with recent research that highlighted the fractal relationship between geomorphology, bed elements and VWE across a wide range of scales (from microhabitat to continents) (Wörman et al., 2007), the hydromorphological controls of the microbial community and ecosystem function will also have a similar fractal relationship.

Since changes in the VWE will alter the function and potentially the structure of the microbial community and, in turn, ecosystem functions, the results reinforce the need to understand the significance of increased fine sediment input into streams for ecosystem services (Datry et al., 2014). In my dissertation, I focused on oxic sediments, but the VWE is also a factor for anaerobic organisms and their metabolism. The presence of anaerobic areas of the sediments depends on the VWE and streambed permeability (Baker et al., 2000). Low VWE or high groundwater inflow favours anoxic conditions and anaerobic biogeochemical processes, such as denitrification or fermentation, that play an important role in nutrient cycling (Brunke & Gonser, 1997; Nogaro et al., 2010, 2013; Datry et al., 2014). Management strategies should orient towards increasing the VWE heterogeneity, since ecosystem services will be fully achieved when the streambed covers a wide range of VWE that maintain different metabolic processes. In fact, spatial and temporal heterogeneity in the VWE contribute significantly to streambed metabolism (Cardinale et al., 2002).

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The microbial community as a stochastic source of VWE heterogeneity.

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As suggested by Grimm (1995), all the interactions studied point out that the influence of the microbial community on its physical habitat has a stochastic character whose occurrence is determined by factors such as light, trophic state or temperature. The stochastic character of microbial influences occurs to an extent that can temporarily mask some of the expected patterns of the microbial community according to its physical habitat. The production of biogenic gas bubbles is highly dependent on the local temperature. Not only metabolic rates and, thus, gas production vary with the temperature (Davidson & Janssens, 2006), but also

the oxygen saturation (Martens & Val Klump, 1980) and, with it, the gas-transfer between bubbles and water. Thus, the reduction of the VWE or the buoyancy-mediated sediment transport will vary with the temperature and seasons and not only as a result of season or temperature-dependent changes in metabolic rates. Moreover, as shown in Chapter 2, other stochastic factors play a role in the interactions between the microbial community and VWE. After 13 days under high sunlight intensity, the VWE was annulled by the microbial community (Figure 3.2). A strong rain event in combination with the shallow surface water in the flumes partially released the bubbles trapped in the pore space and slightly increased the VWE. The influence of stochastic factors is, thus, punctual and spatially heterogeneous. It happens in certain areas at a given time for a short period as a result of the combination of several factors, which, in turn, determine its significance for the ecosystem. Within the same stream reach, for instance, buoyancy-mediated transport and its associated changes in metabolism will be limited to shaded areas of the streams during low-flow periods that last at least six weeks (Chapter 5), whereas the biogenic gas mediated reduction of the VWE will occur in those shallow areas that experience intense sunlight (Chapter 3). Buoyancy-mediated sediment transport will be of significance for the ecosystem during low-flow periods and low light intensity. According to my results from Chapter 5, during these periods, algal mats cover at least 50% of the streambed surface (Chapter 5; Figure 5.1) and about 25% of the surface covered by algal mats can detach, transporting sediment downstream. The detachment and transport downstream reduces stream metabolism and can increase the the VWE by altering the streambed relief and, consequently, the pressure pattern over the streambed (Thiboudeaux & Boyle, 1987; Cardenas et al., 2004; Salehin et al., 2004). Additionally, heterogeneous distribution of patches where the VWE has been annulled by means of gas can increase the VWE in adjacent areas, as observed by low permeability patches within more permeable environments (Ward et al., 2011). The spatial extent of biogenic gas-mediated VWE reduction will be of significance in open land streams during low-flow periods. Previous studies have reported that organisms are sources of spatial heterogeneity of the physical habitat (e.g. Turner, 2005; Battin et al., 2007). The results presented show that microbial communities in lotic ecosystems are not only a source of spatial heterogeneity, but together with sediment transport dynamics, they represent a stochastic source of spatiotemporal heterogeneity of the VWE.

The flume experiments presented in Chapters 3 and 5 received their inoculum from the same origin (Chicken Creek). The main differences in the experiments were light intensity and phosphorous, leading to different development and structure of the microbial community. Another difference between the experiments was the high wind in Chicken Creek in

Chapter 3 compared with the greenhouse in Chapter 5. However, no disturbance of the bed of the flumes was observed as a result of turbulences caused by the wind over the water. The differences in light and phosphorous determined the composition of the microbial community and, in turn, its influence on the physical habitat. In Chapter 3, the autotrophic microbial community was dominated by coccale green algae and pennate diatoms (Table 3.3), and did not develop mats. On the other hand, the bed of the flumes in Chapter 5 was dominated by filamentous green algae that formed algal mats. Although diversity in microbial assemblage structure is often associated with spatiotemporal heterogeneity of the physical habitat (Febria et al., 2012; Nogaro et al., 2013) or trophic state (Smith, 2003), to the best of my knowledge few studies compared the influence of different microbial communities on the physical habitat. A gap exists between the diversity and function of microbial assemblages (Tringe et al., 2005; Marmonier et al., 2012; Nogaro et al., 2013). My results indicate that this gap of knowledge also includes the influence of the microbial community on its physical habitat as a function of microbial community structure.

### 6.3 On the significance of results obtained with micro- and mesocosms

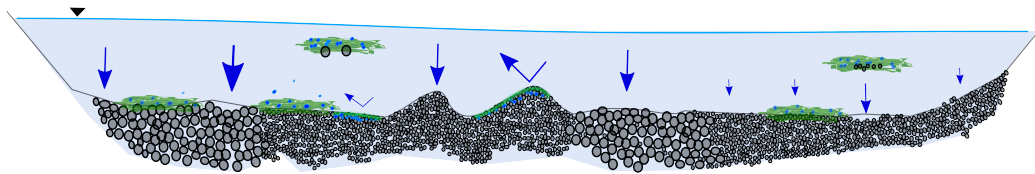
As indicated in the introduction (see section 1.5), model systems have allowed me to detect and elucidate mechanisms by which the microbial community and its physical habitat interact and those which, in some contexts, give rise to a phenomenon such as buoyancy-mediated sediment transport (Glennan, 1996; Machamer et al., 2000; Drake & Kramer, 2012). Of course, my results do not represent accurately what happens in the stream but this is not because of simplicity. It is rather a result of interactions and synergies among factors that result in spatiotemporal heterogeneity of the reciprocal influences. Model systems cannot adequately disentangle the set of possible causal relations because of the nature's uncontrollable complexity (Cadotte et al., 2005). I determined in the experiments performed what can happen in the ecosystem in a specific context (see also Drake & Kramer, 2012). A good example of such interactions among factors is given in Chapter 2. The influence of grain size on the microbial community was established as a function of colonisable area per unit of sediment and the hydraulic conductivity of the sediment. The results showed that advective supply of redox partners and nutrients was the major factor for the structure and function of the microbial community. However, the interaction of both colonisable area and hydraulic conductivity resulted in similar microbial community structure and function among grain sizes. More area in finer sediment compensated the high abundance of algae

and bacteria and metabolism activity in coarser sediment as a result of higher mass transfer (Figure 2.5). Therefore, interactions between factors, specifically in such a stochastic framework, can lead to unclear and unpredictable patterns. The use of model systems can, therefore, help to overcome the limitations imposed by stochasticity.

## 6.4 Summary

In sum, the results presented in this dissertation using model systems (micro- and mesocosm) mimicking sandy lowland streams indicate that, within the complex pattern of interactions occurring in the streambed, the VWE dominates the influence of hydrogeomorphology on the microbial community and, in turn, on streambed metabolism and nutrient cycling. Within this primary physical template defined by the VWE, the microbial community does not only carry out those ecosystem functions, but it also punctually influences streambed hydrogeomorphology (Figure 6.2), increasing its heterogeneity and, thus, ecosystem functions.

These findings have implications for understanding the causal and modulating mechanisms underlying streambed metabolism and nutrient cycling and, therefore, for ecosystem management and river conservation. They suggest that a more comprehensive understanding including biological-physical interactions will provide the basis to fully understand and predict ecosystem functions.



**Figure 6.2:** Schematic cross-section of a lowland sand-gravelbed stream representing VWE heterogeneity caused by the sediment transport dynamics (ripples) and microbial community. Influences studied in Chapters 2, 3, and 5 are represented. Blue arrows represent the VWE, and the magnitude of the VWE is indicated by the size of the arrows. Broken arrows represent reduction or blockage of the VWE by the microbial community.





# List of abbreviations

AFDM	Ash-free dry mass	p	p-value, statistical significance
ANCOVA	Analysis of covariance	P	Phosphorous
ANOVA	Analysis of variance	PAR	Photosynthetic active radiation
b.d.l.	Below detection limit	permANOVA	Permutational ANOVA
°C	Degree celsius	PM	Pohlitzer Mühlenfließ
C	Carbon	P:R	ratio of production and respiration <i>in sensu</i> NPP:GR
CC	Chicken creek	N	North
Chl <i>a</i>	Chlorophyll <i>a</i>	Q <sub>10</sub>	Temperature coefficient
CR	Community respiration	rmANOVA	repeated measurements ANOVA
DAPI	4,6-di-amidino-2-phenylindole	SD	Standard deviatio
DM	Dry mass	SRP	Soluble reactive phosphorous
DOC	Dissolved organic carbon	SUVA	Specific ultraviolet absorbance
<i>E</i>	East	TOC	Total organic carbon
EPS	Extracellular polymeric sub- stances	VWE	Vertical Water Exchane
Kf	Hydraulic conductivity	VWF	Vertical Water Flux
lat	Latitude		
long	Longitude		
m a.s.l.	Meters above see level		
MANOVA	Multivariate analysis of variace		
<i>n</i>	Sample size		
N	Nitrogen		
n.d.	No data		
NPP	Nett primary productivity		
OM	Organic matter		



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