In-stream microbial carbon transformation under opposing stresses-drought and sediment transport

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“Resilience determines the persistence of relationships within a system and is a measure of the ability of these systems to absorb changes of state variables, driving variables, and parameters, and still persist.”

-C.S. Holling, 1973-

Mojoj porodici

À ma famille

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ABSTRACT

The mineralization of organic matter (OM) is an important ecosystem service that has come under pressure because of changes in global climate and regional land use. Increased frequency of droughts and higher sediment loads due to erosion together with channel morphology degradation associated with land and channel clearing for urbanization or agriculture, pose new environmental extremes in running waters. In particular, lowland streams in temperate regions may experience reinforced transport of poorly cohesive sand through migratory ripples and also spatial redistribution of sediments due to the channel degradation, resulting in changes of naturally sorted structure of sand and gravel in streambeds towards sand-dominated, homogenized streambed structure. The impact on microbial carbon (C)-transformation from these changes was the main focus of my doctoral thesis. Three impacts were explored (i) the impact of periodic mechanical disturbance associated with ripple migration (ii) the impact of streambed structure homogenization, and (iii) the impact of drought in streambeds with sorted or homogenized sediment structure. I linked the responses of microbial communities to the studied impacts with shading (drought/homogenization) and OM quality (ripple migration), to reveal potential modulating effect of the linked factors for C-transformation. The impact responses and modulating effects on microbial C-transformation were tested using a model system approach, micro- or mesocosms.

In a set of microcosms, I simulated periodic mechanical disturbances similar as they occur during in-stream ripple migration and tested the significance of these disturbances for microbial C-transformation. Thereby, the quantity and quality of the OM in the sandy sediments were varied by the addition of leaves and fish feces to the OM-poor sands prior to subjecting them to periodic disturbances. The results revealed that periodic sediment relocations resulted in significant stress on microbial function and structure of C-transformation. Microbial respiration of the sediment-associated microbial community strongly decreased to a low and similar level, affecting their ability to mineralize particulate OM regardless of its quality. Moreover, these sediment relocations led to a change in composition of microbial community, particularly in the sand with well-bioavailable fish feces.

In a set of experimental flumes, I investigated the importance of the streambed geomorphic complexity (sorted vs homogenized sediment structure) for C-transformation. The focus was on the interaction between benthic and hyporheic microbial processes in C-transformation to better understand the consequences of homogenization of the sediment structure on microbial function in streambeds. This interaction was tested for two levels of light availability (by application of moderate and strong shading and thus different phototrophic production). In both cases equal leaf-litter contents were introduced into the hyporheic zones. The results showed that sediment structure (sorted or homogenized) has a decisive influence on streambed hydraulic patterns which determine connectivity between the benthic and hyporheic zones consequently affecting the quality of stream water. The lower water exchange in homogenized streambeds and thereby reduced supply of freshly produced bioavailable OM from the benthic to the hyporheic zone, curtailed microbial respiration in the latter. The higher microbial respiration in hyporheic zone of sorted streambeds was followed by stronger depletion of leaf-litter associated complex C-compounds in these streams, and this depletion was more pronounced under moderate shading.

The influence of a drought and rewetting phase was tested on C-transformation in streambeds with a sorted or homogenized sediment structure using a flume experiment where one half of the flumes were strongly shaded and the other half moderately shaded. The results showed that streambeds affected by droughts, either with sorted or homogenized sediment structure have a similar function at the first place controlled by shading. Under the moderate shading, the resistant transitional community developed during drought phase along with reduction in its biomass, while there was a continuous reduction in microbial biomass under strong shading without appreciable change in the composition of community. In both microbial communities, C-transformation rate reduced during the drought phase, whereby this reduction was more pronounced under the strong shading. The level of shading dictated the degree of stream C-transformation recovery, but light availability interacted with the sediment structure in terms of recovery of community composition after the resumption of water supply.

Overall, this doctoral thesis was able to show that in sediment transport– and drought-impacted streambeds (i) periodically occurring ripple migration results in decreased C-transformation regardless of the available quality of OM, (ii) homogenization of sorted sediment structure leads to a decrease in microbial C-transformation in the hyporheic zone, which may have a negative impact on the transformation of complex OM sources, such as

leaves, especially under stronger shading, and (iii) interaction between sediment structure and shading leads to a change in the composition of the microbial community and this change was critical for resistance and resilience of C-transformation during drought and after flow resumption.

The findings of this thesis therefore deepen our understanding of the possible consequences of changing ecosystem impacts (sediment transport and droughts) on C-transformation processes in lowland streams in temperate regions. Linking these consequences with in-stream drivers for microbial C-transformation (i.e., light availability and OM quality) is of special significance for the assessment of the resistance and resilience of a stream's structure and function. In view of the expected changes caused by global warming and regional land use, the significance of revealed links for C-transformation plays an important role in the planning and implementation of appropriate measures (e.g. shading by riparian vegetation, strengthening of hydrological and biological connectivity in the habitat, rethinking the ways in which water is distributed between consumptive and environmental needs during periods of drought) to protect stream ecosystems worldwide.

ZUSAMMENFASSUNG

Die Mineralisierung von organischem Material (OM) ist eine wichtige Ökosystemdienstleistung, die durch Veränderungen im globalen Klima oder der regionalen Landnutzung zunehmend unter Druck gerät. Die Zunahme von Trockenphasen und ein erhöhter Sandeintrag durch Erosion, z.B. aus Rodungen und Landwirtschaft, bilden neue Umweltextreme in Fließgewässern. Insbesondere in Tieflandflüssen der gemäßigten Breiten kann es zu verstärktem Sandtransport in wandernden Rippeln und zudem durch die Degradation der Gerinnemorphologie zu Veränderungen der Sedimente auf der Gewässersohle kommen, von einem natürlichen Mix aus sortierten Sand- und Kiesbereichen hin zu einem durchgängig von Sand dominierten, homogenisierten Flussbett. Die Auswirkungen dieser Veränderungen auf die mikrobielle Transformation von Kohlenstoff (C) bilden den Kern meiner Dissertation. Drei Faktoren wurden dabei untersucht: (i) die Auswirkung der periodischen, mechanischen Störung durch wandernde Sandrippel (ii) die Auswirkung einer Homogenisierung der Sedimentlagerung, und (iii) die Auswirkungen von Trockenphasen in Gewässern mit sortierter oder homogener Sedimentlagerung. Dabei war es mein Ziel, den Zusammenhang zwischen den Reaktionen der mikrobiellen Gemeinschaft auf die zu untersuchenden Faktoren wie Beschattung (bei gleichzeitiger Trockenphase bzw. Sedimenthomogenisierung) und OM-Qualität (bei gleichzeitiger mechanischer Störung durch Ripplemigration) und deren regulierenden Effekt auf die C-Transformation aufzudecken. Die Reaktionen und regulierende Auswirkung auf die mikrobielle C-Transformation wurde mit Hilfe eines Modellsystemansatzes aus Mikro- oder Mesokosmen getestet.

In Mikrokosmen wurde die periodische, mechanische Störung simuliert, wie sie in wandernden Sandrippeln auftritt, und die Bedeutung dieser Störung für die mikrobielle C-Transformation untersucht. Dabei wurden Quantität und Qualität des OM in den sandigen Sedimenten durch Zugabe von Laub und Fischfäzes zu den OM-armen Sanden variiert. Die Ergebnisse zeigen, dass die periodischen Sedimentverlagerungen eine erhebliche Störung für die mikrobielle C-Transformation darstellen. Die Respiration der mikrobiellen Gemeinschaft verringert sich unabhängig von der Qualität des OM im Sediment. Die Sedimentverlagerungen führen zudem zu einer veränderten Zusammensetzung der mikrobiellen Gemeinschaft, insbesondere in dem Sediment mit den gut bioverfügbaren Fischfäzes.

In experimentellen Fließrinnen wurde die Bedeutung geomorphologischer Komplexität (sortierte und homogene Sedimentlagerung) für die C-Transformation untersucht. Der Fokus lag auf der Interaktion zwischen benthischen und hyporheischen mikrobiellen Prozessen der C-Transformation, um den Einfluss von Homogenisierung der Sedimentstruktur auf die Leistung der Mikroorganismen besser verstehen zu können. Diese Interaktion wurde bei zwei Lichtintensitäten (durch moderate und starke Beschattung und damit unterschiedlicher benthischer phototropher Produktion) getestet. In beiden Fällen wurde die gleich Menge an Laub in die hyporheische Zone eingebracht. Die Ergebnisse zeigen, dass die Sedimentlagerung (sortiert oder homogen) einen entscheidenden Einfluss auf die hydraulischen Muster im Flussbett hat, welche die Konnektivität zwischen der benthischen und hyporheischen Zone bestimmt und die Qualität des Wassers beinflussen. Der niedrigere Wasseraustausch in einem homogenisierten Flussbett und die dadurch verringerte Zufuhr von frisch gebildetem bioverfügbarem organischem Substrat aus der benthischen Zone, verursacht eine eingeschränkte mikrobielle Respiration im Hyporheal. Auf die höhere mikrobielle Respiration in der hyporheischen Zone sortierter Flussbetten folgte ein stärkerer Rückgang an komplexen C-Verbindungen, assoziiert mit Laub, welcher insbesondere unter moderater Beschattung beobachtet wurde.

Der Einfluss einer Trocken- und Wiedervernässungsphase auf die C-Transformation in einem Flussbett mit homogener bzw. heterogener Sedimentstruktur wurde ebenfalls in Fließrinnen getestet, wobei die eine Hälfte der Rinnen stark, die andere moderat beschattet wurde. Die Ergebnisse zeigen, dass die mikrobielle Aktivität in einem von Trockenheit betroffenen Flussbett mit homogener und mit natürlicher, heterogener Sedimentlagerung ähnlich sind und in erster Linie durch die Beschattung reguliert wird. Bei moderater Beschattung entwickelte sich in der Trockenphase eine resistente mikrobielle Übergangsgemeinschaft, während es bei starker Beschattung zu einer kontinuierlichen Abnahme der mikrobiellen Biomasse kam, ohne dass die Zusammensetzung der Gemeinschaft erkennbar verändert wurde. In beiden mikrobiellen Gemeinschaften verringerte sich die C-Transformationsrate während der Trockenphase, wobei dieser Trend unter moderate Beschattung weniger stark ausfiel. Die Intensität der Beschattung diktiert demnach den Grad der Wiederaufnahme der C-Transformation nach der Wiedervernässung, wobei die Faktoren der Lichtverfügbarkeit und die Sedimentlagerung interagierend auf die wiedergewonnene Gemeinschaftsstruktur wirken.

Diese Arbeit konnte aufzeigen, dass in einem sandigen Flussbett unter den o.g. extremen Umwelteinflüssen (i) periodisch auftretende Rippelmigration zu einer Abnahme der C-Transformation führt, unabhängig von der verfügbaren Qualität des OM, (ii) Homogenisierung von sortierter Sedimentstruktur zu einer Abnahme der mikrobiellen C-Transformation in der hyporheischen Zone führt, was sich negativ auf den Gesamtumsatz von komplexeren OM wie Laub auswirken kann, insbesondere unter stärkerer Beschattung, und (iii) das Zusammenspiel von Sedimentlagerung und Lichtintensität zu einer Änderung führt in der Zusammensetzung der mikrobiellen Gemeinschaft, welche entscheidend für die Resistenz und Resilienz der C-Transformation während Dürre und Wiedervernässungsphasen sind..

Die Erkenntnisse dieser Arbeit vertiefen das Verständnis über die möglichen Folgen veränderter Umwelteinflüsse (Sedimenttransport, Trockenphasen) auf die C-Transformationsprozesse in Tieflandbächen der gemäßigten Breiten. Der Zusammenhang dieser Folgen mit den lokalen Bedingungen (z.B. Lichtzufuhr, OM-Qualität) ist von besonderer Bedeutung für die Beurteilung der Resistenz und Resilienz der Struktur und Funktionsweise von Fließgewässern. In Anbetracht der zu erwartenden Veränderungen durch die globale Klimaerwärmung und Veränderungen der regionalen Landnutzung, spielt die Bedeutung dieser Zusammenhänge eine wichtige Rolle bei der Planung und Durchführung geeigneter Maßnahmen (z.B. eine Beschattung durch Ufervegetation, die Stärkung der hydrologischen und biologischen Verbindungen im Habitat und das Überdenken der Aufteilung zwischen Wasserverbrauch und ökologischen Anforderungen während Trockenperioden), um Fließgewässer weltweit zu schützen.

THESIS OUTLINE

This doctoral thesis is composed of three manuscripts that have either been published or are ready to be submitted for publication in peer-reviewed journals. Each manuscript forms a chapter of the thesis (Chapters II to IV) and contains introduction, methodology, results, discussion, and conclusion sections. In the general introduction (Chapter I), I provide the context for the thesis, the general research aims, and the aims of the individual chapters. In the final discussion (Chapter V), I connect the main findings of the individual chapters and discuss them in a broader context.

Author contributions: **S. Zlatanović (SZ)**, J. Fabian (JF), M. Mutz (MM), B. Woodward (BW), C. Mendoza-Lera (C-ML), K. Premke (KP), G. Singer (GS).

CHAPTER I: General introduction.

CHAPTER II: Zlatanović, Sanja; Fabian, Jenny; Mendoza-Lera, Clara; Woodward, K. Benjamin; Premke, Katrin; Mutz, Michael (2017): Periodic sediment shift in migrating ripples influences benthic microbial activity. In Water Resources Research 53(6), pp. 4741–4755. DOI:10.1002/2017WR020656.

Author contributions: **SZ** and MM designed the experiment, with contributions by JF and KP; SZ performed the research; **SZ** analyzed the data; **SZ** wrote the paper, with contributions by MM, JF, KP, BW, and C-ML.

CHAPTER III: Zlatanović, Sanja; Fabian, Jenny; Singer, Gabriel; Premke, Katrin; Mutz, Michael (forthcoming): Streambed structural complexity influences microbial carbon cycling in shallow hyporheic zone. To be submitted.

Author contributions: **SZ**, MM, JF, and KP designed the experiment; **SZ** and JF performed the research; **SZ** and JF analyzed the data; **SZ** and JF wrote the paper, with contributions by MM, KP, and GS

CHAPTER IV: Zlatanović, Sanja; Fabian, Jenny; Premke, Katrin; Mutz, Michael (2017): Shading and sediment structure effects on stream metabolism resistance and resilience to infrequent droughts. In Science of the Total Environment 621, pp. 1233–1242. DOI: 10.1016/j.scitotenv.2017.10.105.

Author contributions: **SZ** and MM designed the experiment, with contributions by JF and KP; **SZ** performed the research, with contributions by JF; **SZ** analyzed the data; **SZ** wrote the paper, with contributions by MM, JF, and KP.

CHAPTER V: Final discussion and conclusions.

In addition to manuscripts included in the chapters of this doctoral thesis, I also co-authored the following papers, which were derived from the project of this doctoral thesis and have been published or are in preparation for submission for publication in peer-reviewed journals:

Fabian, J.; Zlatanović, S.; Mutz, M.; Premke K. (2017): Fungal–bacterial dynamics and their contribution to terrigenous carbon turnover in relation to organic matter quality. ISME Nature Journal 11 (2), pp. 415–425. DOI:10.1038/ismej.2016.131.

Altenkirch N.; Zlatanović S.; Woodward, B.; Mutz, M.; Molkenthin, F.; Trauth, N. (2016): Untangling hyporheic residence time distributions and whole stream metabolisms using a hydrological process model. In Procedia Engineering, 154. pp. 1071–1078. DOI: 10.1016/j.proeng.2016.07.598.

Fabian, J.; Zlatanović, S.; Mutz, M.; Grossart, H. P.; Van Geldern, R.; Ulrich, A.; Gleixner, G.; Premke, K.: Environmental Control on Microbial Turnover of Leaf Carbon in Streams – Ecological Function of Phototrophic-Heterotrophic Interactions. Submitted to Frontiers in Microbiology, December 2017.

Scheidweiler, D.; Zlatanović, S.; Mendoza-Lera, C.; Mutz, M.: Migrating ripples: significance for composition and function of benthic biofilms. In preparation.

Oprei, A., Zlatanović, S.; Mutz, M.: Grazing and drying intensity modulate drought resistance and recovery of shallow hyporheic respiration. In preparation

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LIST OF ABBREVIATIONS AND SYMBOLS

A	Absorbance
AFDM	Ash free dry mass
ANOVA	Analysis of variance
AR	Autotrophic respiration
BCP	Bacterial carbon production
b.d.l.	Below detection limit
C	Carbon
CO_2	Carbon-dioxide
Chl $_a$	Chlorophyll a
CR	Community respiration
Da	Damköler number
DM	Dry mass
DIN	Dissolved inorganic nitrogen
DO	Dissolved oxygen
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
E	East
FAME	Fatty-acid-methyl-ester
F:B	Fungi to bacteria ratio
FI	Freshness index
GPP	Gross primary production
HIX	Humification index
H:A	Heterotroph to autotroph ratio
hz	Hyporheic zone
k	Specific reaction constant
L	Length
LME	Linear mixed effects
n	Sample size
N	North
NEP	Net ecosystem production
OC	Organic carbon
OM	Organic matter
p	p-value, statistical significance
P	Phosphorus

PAR	Photosynthetic active radiation
PLFA	Phospholipid fatty acid
POM	Particulate organic matter
PARAFAC	Parallel Factor Analysis
Q_{10}	Temperature coefficient
SD	Standard deviation
SRP	Soluble reactive phosphorus
T	Temperature
t	Time
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorus
τ	Residence time
u	Pore water velocity
V	Volume
VWF	Vertical water flux

1. General introduction

1.1 Streambeds as habitats for microbial communities and in-stream C-transformations

The benefits that streams offer to humans (e.g., flood control, maintenance of water quality and quantity for drinking or irrigation, and habitats for various plants and animals) are directly derived from the natural biological, geomorphic, and hydrological processes that drive energy flow in streams known as stream metabolism (Hall and Meyer, 1998; Hieber and Gessner, 2002; Tank et al., 2010). Stream metabolism is mostly driven by organic substances that are deposited (e.g., leaf fall) or produced (e.g., photosynthetic activity and metabolic products of fish) in lowland streams and are decomposed by microbial communities (algae, bacteria, and fungi) inhabiting streams (Cotner and Biddanda, 2002; Gessner et al., 2010). The metabolic pathways that organic matter (OM) decomposition undergoes by microorganisms in streams before CO_2 is eventually released to the atmosphere is known as C-transformation and is the major service of stream metabolism. The largest portion of this decomposition takes place in streambeds (Brunke and Gonser, 1997; Battin et al., 2008) where both benthic (commonly dominated by algae) and hyporheic (commonly dominated by bacteria and fungi) microbial communities are responsible for most assimilation and mineralization processes (Lock and Ilynes, 1976). As the decomposition activity of benthic and hyporheic microbial communities is closely related to characteristics that their natural habitat provide (Hall and Meyer, 1998; Hieber and Gessner, 2002; Tank et al., 2010), and given the high variability in streambed characteristics, microbial C-transformation patterns in streams display large variability.

Streambeds are extremely heterogeneous at scales ranging from millimeters to tens of kilometers (Minshall, 1988; Pringle et al., 1988) and are more diverse than any other type of environment. The sizes of relief features (e.g., bed-forms) and the roughness affecting streambed topography, and the arrangement of pore/grain spaces in habitats affecting streambed structure can vary by several orders of magnitude (Schmid, 1999). Effects that variations in habitat topography and structure among streams have on aspects of community structure and population dynamics, such as species diversity, population abundance, dispersal, and the body sizes of resident organisms, are directly linked to the functioning of streams (Poff and Ward, 1992; Ives et al., 1998; Peterson et al., 1998). Moreover, increased velocity and turbulence associated with streams that display greater habitat physical heterogeneity might directly stimulate the metabolic activity of stream microbial communities (Stevenson et

al., 1996; Biggs et al., 1998; Hart and Finelli, 1999), presumably by increasing the delivery rates of nutrients, gasses, or OM resources, and/or by reducing the size of the benthic boundary layer that limits diffusion of these resources to the hyporheic habitats. In parallel, greater physical heterogeneity of habitats induces shifts in dominance in favor of taxa adapted to variable flow conditions (Stevenson et al., 1996; Biggs et al., 1998), which differ in their rates of assimilation and decomposition of OM (Steinman, 1992), hence altering stream C-transformation (Cardinale et al., 2002). The respective relevance of microbial community composition and population dynamics may also shift with time and space, according to seasonal variations in hydrology, which affect stream discharge, and variations in OM input (Acuña et al., 2005). Besides the fact that riparian zones control the quantity and quality of OM input originating from terrestrial surroundings, the variability among these zones control light supply to streams (Steinman, 1992; Hunt et al., 2012), which directly affects variability in community composition as well as quantity and composition of OM related to the phototrophic production of benthic microbial communities (Fisher and Likens, 1973; Webster and Meyer, 1997).

The variability in habitat characteristics continuously shape microbial communities in streambeds, where the complex product of interactions between factors related to the physical habitat type, such as streambed topography and structure, OM quality and light availability, and hydrology, act over different spatial scales, causing variations at the population and individual scales (Schmid, 1999). Consequently, the constant interaction between biological, geomorphic, and hydrological components feeds back on stream metabolism, resulting in C-transformation (Figure 2). However, this interaction is challenged by increasing environmental stressors, sediment transport affecting habitat mobility and structure, and drought. The tight feedback loops between microbial activity and features associated with geomorphic and hydrological change in streambeds are not fully understood in streams affected by the sediment transport and drought. Understanding how and when the physical complexity of streambeds is an aspect of heterogeneity (i.e., the number of distinct habitat or patch types composing stream ecosystems), which likely exerts strong control over ecological processes that maintain the functions of streams (e.g., Pierce and Running, 1995; Gao et al., 2000), is a primary challenge, especially in light of future needs for the preservation and restoration of stream ecosystems under environmental stresses.

1.2 Environmental stressors interacting with streambed microbial community—global change of stream's function as consequence

The alteration of the global environment as a result of human activities has triggered the sixth major extinction event in the history of the biosphere and caused widespread changes in the global distribution of organisms. The role of streams as landscape drainage systems and commonly the main sources of water abstraction (e.g., drinking water and irrigation) makes these ecosystems susceptible to environmental stresses (e.g., sediment transport and droughts) (Harding et al., 1998; Sweeney et al., 2004; Walsh et al., 2005). Given microbial ubiquity but sensitivity to environmental conditions (which selects for communities that are well-adapted to certain conditions), their ecosystem service is ultimately shaped by interactions between their natural habitat characteristics (e.g., shading by the riparian zone and the geomorphic state of the streambed) and environmental stressors (Grimm, 1995). The natural habitat–environmental stressors interactions that shape microbial communities and control their activity vary temporally and spatially as a function of community dynamics produced by stressor-induced changes in habitat heterogeneity (Fisher and Grimm, 1991; Fisher, 1994; Grimm, 1995) and related changes in hydrology (Bridge, 2009). Although previous studies have recognized that physical habitat heterogeneity, and the ways in which its interactions with environmental stressors influence the inhabiting communities, is the main subject of ecological research (Corenblit et al., 2011), the implications for in-stream C-transformation remains unclear (Figure 2).

Lowland streams typically receive elevated loads of sand and are sensitive to its redistribution within the streambed (Wood and Armitage, 1997; Owens et al., 2005; Datry et al., 2015). In-stream sand loads at different spatial and temporal scales (i.e., bed-load) induce pressure fluctuations and changes in hydraulic head gradients; consequently, sandy streambeds are characterized by dynamic and heterogeneous flow path patterns. These patterns are controlled by the topography and sediment arrangement within the streambed structure and are of particular interest, as the resulting difference in water exchange regimes determine the supply and residence time of resources (e.g., OM quantity and quality, dissolved oxygen, and nutrients) critical for associated microbial C-transformation processes. Furthermore, altered habitat characteristics following sand deposition may trigger changes in microbial biomass and community composition in the streambed (Lear and Lewis, 2009; Wang et al., 2011). On the other hand, while changes in streambed topography (e.g., bed-forms presence in streams affected by increased sand loads and consequent sediment transport) drive supply of aforementioned resources and have the potential to increase

microbial activity in the hyporheic habitats, as is often cited for ripples, streambed mobility is known to severely damage or eliminate benthic communities and to reduce their metabolic activities (Holmes et al., 1998; Uehlinger and Naegeli, 1998; Uehlinger, 2000). Ultimately, the absence of flow and streambed desiccation, in streams affected by droughts, severely impact microbial abundance, structure, and activity (Datry et al., 2017). Consequently, the interplay between altered resource supply, sediment stability, and water availability with the natural habitat characteristics (e.g., OM quality and shading) leads to various scenarios whereby microbial processes in streambeds can largely influence stream C-transformation (Figure 1, 2).

Altered in-stream biogeochemical cycles as a consequence of environmental disturbances, e.g., sediment transport and drought, affect stream metabolism resistance and resilience (Findlay, 1995; Langenheder et al., 2006; Allison et al., 2008), subsequently increasing the vulnerability of biodiversity, food security, human health, and water quality. A restored capacity to withstand natural disturbances is widely regarded as an important measure of stream restoration success (Jansson et al., 2005; Palmer et al., 2005; Bond et al., 2005). Despite this knowledge, there is still a lack of mechanistic understanding of how the various factors that influence microbial communities interact and of how to modulate ecosystem C-transformations to support future restoration efforts. Further research is especially needed to fully understand the mechanisms through which physical habitat characteristics influence microbial communities and ecosystem function in C-transformation, which is further challenged by forthcoming global change (Marmonier et al., 2012). Linking changes in streambed mobility, structure, and water availability with OM quality and light availability is particularly important for understanding how and when environmental disturbances inflict stress on microbial structure and function, resulting in changes in reach-scale C-transformation (Figure 1). The aim of this doctoral thesis was to elucidate the influences of streambed physical habitats that have been exposed to environmental stressors on microbial communities, which have profound effects on C-transformation but are not fully understood or have not been tackled previously.

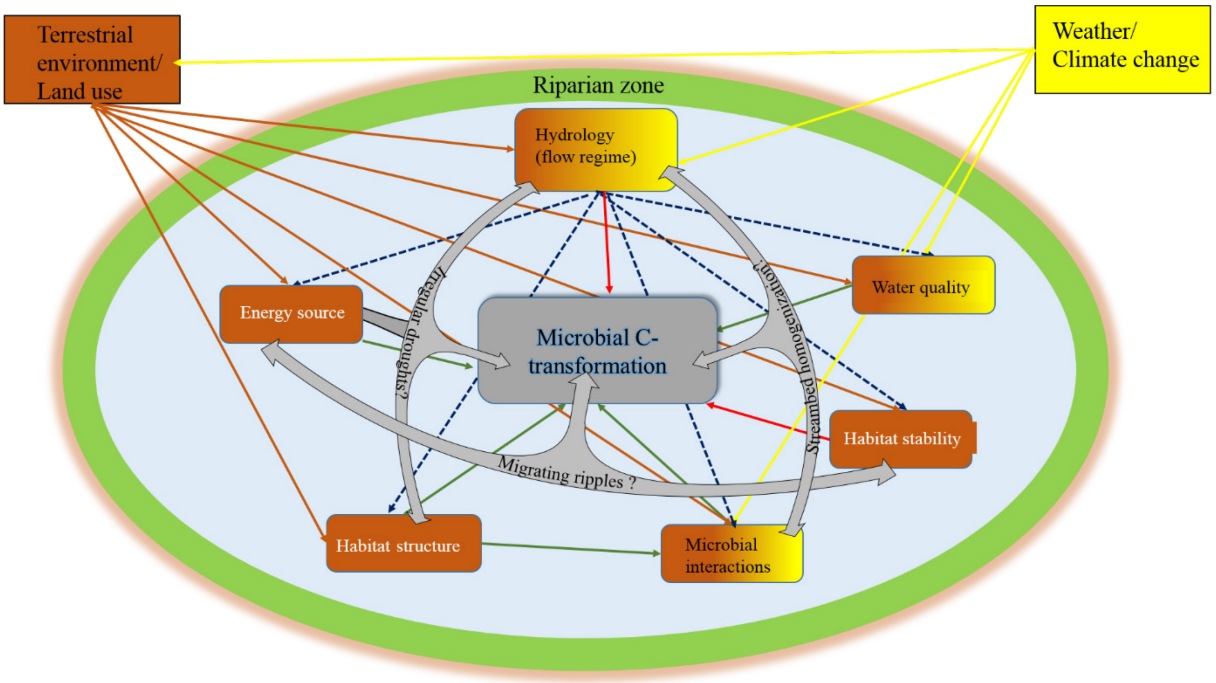

Figure 1 Simplified ecosystem perspective of the in-stream factors (rounded corner boxes) and environmental stressors (rectangular boxes) that influence microbial community and, in turn, C-transformation in streams. Double-ended gray arrows represent the interactions of the studied factors (affected by sediment transport, migrating ripples and streambed homogenization, and/or droughts) and microbial C-transformation. Green and red arrows respectively represent positive and negative influences of in-stream factors on microbial C-transformation. Blue dotted lines represent the direct or indirect influence of hydrology (either vertical water exchange or drought) on microbial C-transformation.

1.2.1 Microbial community and streambed mobility

Due to the strong connection of fluvial networks with the surrounding terrestrial ecosystems, land use affects all major controls on stream metabolism (Bernot et al., 2010). Many human activities, e.g., deforestation, mining, agriculture and urbanization (Hancock, 2002; Owens et al., 2005) increase fine sediment loads to streams (Wood and Armitage, 1997; Owens et al., 2005; Datry et al., 2015). Streambed mobility is common in streams impacted by increased sand loads (Morisawa, 1968; Segura et al., 2011). In streams dominated by sand loads, streambeds are in continuous motion, e.g., periodic sediment shifts in migrating ripples (Bridge, 2009; Segura et al., 2011; O'Connor et al., 2012). The mechanical stress associated with sand grain collision (Bridge, 2003) and the perturbation of biofilm communities (Ward et al., 1998) are two of the basic effects associated with streambed mobility. However, the significance of sediment disturbances during sediment transport for stream heterotrophic metabolism remains unclear (Boynton et al., 1981). Some authors have reported a decrease in stream metabolism after sediment transport events (Uehlinger and Naegeli, 1998), whereas others have reported increased (O'Connor et al., 2012) or unchanged stream metabolism

(Gerull et al., 2012; Mendoza-Lera et al., 2013). Changes in stream metabolism after sediment transport events are attributed to several in-stream features that occur simultaneously with sediment transport and thus contribute to the ultimate response of microbial communities to sediment disturbance. Loss of the community by abrasion or their export to downstream sections (Uehlinger and Naegeli, 1998), flushing/burying of OM (Matthaei et al., 1996; Olsen and Townsend, 2005), or pronounced perfusion of sediments by the surface water during streambed disturbance are some of these confounding features. Hence the previously mentioned disparate findings may be attributed to the inability to provide standardized physical conditions associated with sediment transport, while isolating the transport effect from confounding factors related to seasonal changes in, e.g., organic C quality and quantity (Uehlinger, 2000, 2006; Roberts et al., 2007; Gerull et al., 2012). To develop a mechanistic understanding of the effect of sediment mobility events on C-transformation in streams, I employed microcosms to simulate standardized cycles of sediment disturbance and isolated this effect from confounding factors (Benton et al., 2007). To test the effect of sediment disturbance on microbial communities, I formulated the following research question:

> **Research question 1:** Does periodic sediment disturbances in migrating sand ripples influence the heterotrophic metabolism of sediment-associated microbial communities? Does the proposed effect of periodic sediment disturbance interact with sediment particulate OM quantity and quality? (Chapter II)

1.2.2 Microbial community and habitat geomorphic homogenization

Reflecting their depositional and erosional histories, natural streambeds commonly exhibit textural patches (grain-size facies) that vary both horizontally and vertically (Rice, 1994; Buffington and Montgomery, 1999a, b; Dietrich et al., 2006). Pockets of sand, silt, and organic material of low hydraulic conductivity are common, as are zones of well-sorted gravels and cobbles of high hydraulic conductivity. Each of these is of limited spatial extent, promoting textural heterogeneity with spatially variable distribution of pore size and hydraulic conductivity (Malard et al., 2002) that can drive or enhance hyporheic flow and overall water exchange (Stanford and Ward, 1988; Sophocleous, 1991; Salehin et al., 2004). The effectiveness of near-bed pressure fluctuations in generating hyporheic exchange vary spatially depending on grain-size composition in sediment patches (sorting and percentage of fine material). With the stream regulation the sorting capacity decreases (Wood and Armitage, 1997; Owens et al., 2005; Datry et al., 2015). As the naturally occurring meander-flow is

straighten, the stream becomes limited in small changes in discharges and consequent variation in shear stress after regulation (Powell et al., 2005). Hence poorly cohesive sand becomes easily mobilized and evenly deposited within gravel patches. Consequently, streambeds become dominated by sand characteristics (Buffington and Montgomery, 1999a), which trigger changes in streambed microbial community composition and limits hyporheic exchange from spatial variations in hydraulic conductivity (Powell et al., 2005; Bridge, 2009). This simplified geomorphic structure of natural streambeds (Cummins, 1974; Sweeney, 1992; Minshall et al., 2000), has fundamental hydraulic and biogeochemical implications for community function (Bernot et al., 2010) which together with altered microbial composition, exert significant influence over microbial performance in C-transformation (e.g., O'Connor and Hondzo, 2008; O'Connor et al., 2009; Inoue and Nakamura, 2011). However, the underlying metabolic dynamics of OM decomosition in relation to the sediment structure remain largely unstudied. Nevertheless, in view of the fundamental role of microbial C-transformation in ecosystem functioning (Jones et al., 1995; Mulholland and Hill, 1997; Fellows et al., 2001; Bernot et al., 2010), it is crucial to fully understand how changed streambed hydraulics relate to sediment structure and drive microbial performance in C-transformation in streams. Therefore, to investigate this relationship, I formulated the following research question:

> **Research question 2:** How does structural homogenization of streambeds affect microbial C-transformation? (Chapter III)

1.2.3 Microbial community and drought

In contrast to many other disturbances, which are often of relatively short duration (e.g., days to months in the case of floods and fires) and affect comparatively small areas of the landscape, droughts may last from months to years and impact much larger areas. It is predicted that, with global climate change and increased human withdrawal of water, headwaters in extended temperate regions of the world, including Central Europe, will be subject to more frequent and longer periods of drought (Pohlon et al., 2013a, b). Desiccation has significant impacts on microbial community composition and microbial extracellular enzyme activities, which are key processes in C-transformation in lotic environments. Desiccation and consequent reduction in water availability may lead to decreases in cytoplasmic volume, damage to membranes, proteins, and nucleic acids, and cellular lysis radiation (Qiu and McComb, 1994; Castenholz and Garcia-Pichel, 2000). Consequently, communities exhibit significant decreases in species richness and diversity

as periods of drought increase, suggesting that desiccation is a major stressor selecting for suitably adapted microorganisms (Rothrock and Garcia-Pichel, 2005).

While it is commonly known that light availability is a major controlling factor of microbial growth and function during aquatic periods, during drought, direct radiation on streambeds as a consequence of reduced riparian zones, e.g., deforestation causes severe chlorophyll degradation (Timoner et al., 2014) and affects bacterial community functions (Hölker et al., 2015; Wagner et al., 2015) that, in combination with the effects of drought (Timoner at al., 2012), might severely affect stream metabolism. Inundation imposes additional stress on sediment communities, with a sudden return to earlier levels of water availability (Romani and Sabater, 1997; Baldwin and Mitchell, 2000; Belnap et al., 2005). Several lines of evidence suggest that microbial responses to inundation depend on the duration and magnitude of the preceding drought phase, whereby shifted balances within stream metabolism might have caused changes in the C-transformation underlying ecosystem functioning (Acuña et al., 2015). Some authors report elevated CO_2 emission during droughts (Von Schiller et al., 2014), whereas some report severe decreases in stream metabolism (Acuña et al., 2004; Amalfitano et al., 2008; Acuña et al., 2015). A plausible reason for these disparate findings might be the difference in connectivity of deeper hyporheic biofilms that are still in contact with water or from outgassing of CO_2-saturated ground water that becomes connected to the atmosphere once the upper pore-space is aerated (Genereux et al., 2013). Drought is a major disturbance for communities within stream sediments, but the mechanistic effects of desiccation and rewetting on microbial communities in aquatic ecosystems are not well understood, particularly in relation to physical habitat factors (e.g., sediment structure and riparian vegetation). Using standardized drought conditions in mesocosms, the following research question was formulated:

> **Research question 3:** How do shading and sediment structure drive the responses of stream microbial communities to desiccation and rewetting? (Chapter IV)

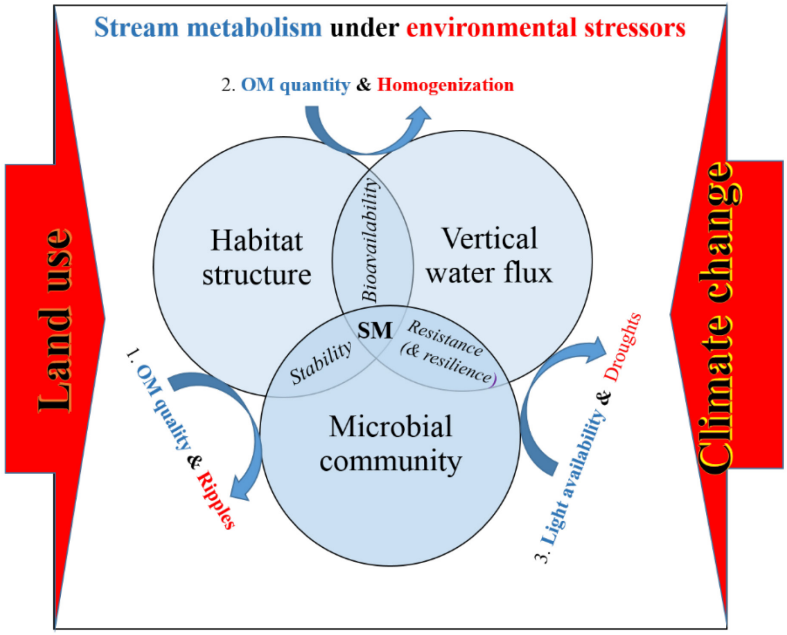

Figure 2 Representation of the studied impacts, ripple migration, streambed homogenization, and irregular droughts affecting the three major components of stream metabolism (SM), biologic (microbial community), geomorphic (habitat structure) and hydrologic (vertical water flux) relevant for in-stream C-transformation (the number represents the research question addressed). Under the external pressures (land-use and climate change), the significance of metabolism drivers such as OM quality and quantity, and light availability is challenged, hence interaction between drivers and pressures ultimately influences C-transformation. As consequence, the ecosystem C-transformation under multiple environmental extremes is modulated through the features such as habitat stability, organic matter (OM) bioavailability, and microbial resistance and resilience.

1.3 Lowland sand–gravel streams

First- and second-order streams account for a large percentage of total stream length (Leopold and Emmett, 1976; Wallace and Eggert, 2009) and drain 60–80% of the surrounding terrestrial landscape (Benda et al., 2005). The high surface-to-volume ratios of low-order streams result in higher sensitivity of lowland streams to large fluctuations in environmental factors within the catchment (Reverey et al., 2016). Close coupling of low-order streams with their riparian and terrestrial environments results in higher rates of stream metabolism than in high-order rivers (Mulholland and Hill, 1997; Alexander et al., 2000; Wondzell, 2011). Changes in regional land use are predicted to especially alter the function of small streams (Cummins, 1974; Sweeney, 1992; Minshall et al., 2000). Altered thresholds of shear stress in regulated streams (Powell et al., 2005) result in highly spatially homogeneous streambeds (Gomi et al., 2002; Gooderham et al., 2007). Increased fluxes of fine sediment in streams (Wood and Armitage, 1997; Owens et al., 2005; Datry et

al., 2015) make streambeds a highly mobile substratum (Morisawa, 1968). Furthermore, flow intermittency has been estimated to account for 69% of the first-order streams below 60° latitude (Raymond et al., 2013). Most predictions agree that the combined effects of irregular climate patterns (Botter et al., 2013; Reynolds et al., 2015) and increasing water abstraction (Barceló and Sabater, 2010; Larned et al., 2010) will cause a shift in many temperate low-order streams, from flow permanence to irregular and unpredictable flow cessation and desiccation (Krysanova et al., 2010). Hence, sandy lowland streams are good models for understanding the significance of sediment transport and droughts for streambed microbial communities and related C-transformation.

1.4 Mechanistic understanding of sediment transport and drought impacts on C-transformation using micro- and mesocosms

Various atmospheric and habitat conditions (e.g. light, temperature, hydraulic and biological connectivity, OM quality) influence C-transformation (Tank et al. 2010; Ziegler and Lyon 2010; Singer et al. 2011) suggesting the importance of spatio-temporal variability of stream habitats for C-transformation patterns on ecosystem scale (Knapp et al., 2017). Hence, previous studies addressing OM dynamics in streams have focused mostly on describing changes in stream's function over time and space (Allan and Castillo, 2007; Mulholland and Webster, 2010; Tank et al., 2010) without taking into account the interrelations between the multiple variables that shape and modulate this change. In contrast, measuring mechanisms that drive changes in stream's function affecting C-transformation and linking this change with explanatory variable is recognized as priority (Acuna et al., 2015) to understand stream's capacity to withstand natural disturbances and support future restoration efforts (Jansson et al., 2005; Palmer et al., 2005; Bond et al., 2005). Yet extensive temporal and spatial scales of ecosystems and hence multitude of variables that influence the function of the streams make them difficult to test mechanistic models (Benton et al., 2007; McCann, 2007), especially with further increase in the complexity of the stream habitat properties driven by changes in sediment mobility, streambed heterogeneity and/or local streambed desiccation. Employment of micro- and mesocosms in experimental manipulation provides a tractable and reproducible approach for testing mechanistic models (Morin, 1998; Huston, 1999, Mendoza-Lera 2015) as they allow the fine-tuning of influential variables and testing the response of microbial structure and function across the time and space. In this sense, experimental manipulations using micro- and mesocosms as model-based approach enables predictions and generalizations of natural ecosystems (Mendoza-Lera 2015). Although, model-based

approach is sometimes perceived as being over-simplistic, insufficiently generalizable, and therefore inappropriate for understanding other, more complex natural phenomena (Drake and Kramer, 2012), the simplicity of micro- and mesocosms makes them especially useful for isolating and testing mechanisms across the scales, that in natural ecosystems could not be controlled for confounding variables (Lawler, 1998; Cadotte et al., 2005; Benton et al., 2007; Drake and Kramer, 2012).

The terms "microcosm" and "mesocosm" used in the context of the present doctoral thesis are defined in the paragraphs below.

A microcosm contains a small (10–50 mL) volume of streambed sediment, disturbed or undisturbed (i.e., stable), in which variables, such as residence time, light regime, OM quantity, and temperature, are controlled. Microcosms thus enable the demonstration of sediment mobility effects on the streambed microbial community. In the two sets of microcosm used, namely stable, vertically positioned columns and rotating, horizontally positioned tubes, small volumes of sediment were positioned and (i) percolated (bottom to top) with artificial stream water and (ii) rotated periodically, allowing pore water to be exchanged at the rate defined by the rotation interval. The streambed microbial community was isolated from the longitudinal and lateral dimensions to allow the detection of its response to the applied sediment disturbance in the absence of confounding factors (Mendoza-Lera 2015).

A mesocosm (i.e., flume) represents a stream (4 × 0.12 × 0.10 m) where water residence time and related vertical water flux (VWF), evaporation, light, temperature, and other meteorological variables, are predefined by the natural environment. VWF is induced by the interaction of the surface water flow (simulated by a recirculating pump) with the sediment bed structure in the flume and the hydraulic conductivity of the sediments, thereby simulating the natural complexity of VWF in the form of a mosaic of down- and upwelling spots connected by semicircular flow paths (Thibodeaux and Boyle, 1987; Salehin et al., 2004; Rehg et al., 2005; Bardini et al., 2013). Furthermore, given their large surface to depth ratio, they enable manipulation of spatial and temporal evaporation rates by variations in surface and subsurface connectivity imposed by streambed structure (Shokri et al. 2010). Mesocosms thus enable the observation of interactions between physical factors (e.g., hydrological exchange and desiccation intensity) and the microbial community, while integrating a larger number of stream ecosystem variables than in a microcosm.

Flumes represent a forested stream section in which riparian inputs of OM are limited to leaf inputs and phototrophic OM production, the content of which is controlled by shading in canopied streams. The flumes used were supplied with oligotrophic groundwater from a local well, which was enriched with nutrients. The upstream input of nutrients and OM was limited to the recirculated water used to supply the flumes. The recirculation mode of flume operation enabled qualitative and quantitative studies of C-transformation and whole-stream metabolism. VWF was limited to the water exchange between the interstitial and surface water, excluding groundwater inputs. Hence, the desiccation process was successfully simulated, as flume setups enabled the exposure of microbial communities to the complexity of natural desiccation processes under standardized conditions while being isolated from confounding factors (e.g., degassing of groundwater during desiccation).

In light of the preceding information and the clarification of the aims of this thesis, the following chapters address each of the aims, showing how the rationale of this study was satisfied.

2. Periodic sediment shift in migrating ripples influences benthic microbial activity

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Key Points:

- The transport-rest cycle during sediment shift with migrating ripples reduces benthic community respiration (CR).
- The reduction of CR in migrating ripples cannot be mitigated with increased particulate organic matter (POM) quality.
- The response of bacterial production and fungal to bacterial biomass ratio to periodic sediment shifts is modulated by POM quality.

2.1 Abstract

Migrating bedforms have high levels of particulate organic matter and high rates of pore water exchange, causing them to be proposed as hot spots of carbon turnover in rivers. Yet, the shifting of sediment sand associated mechanical disturbance within migrating bedforms, such as ripples, may stress and abrade microbial communities, reducing their activity. In a microcosm experiment, we replicated the mechanical disturbances caused by the periodic sediment shift within ripples under oligotrophic conditions. We assessed the effects on fungal and bacterial biomass ratio (F:B), microbial community respiration (CR), and bacterial production (BCP) and compared with stable undisturbed sediments. Interactions between periodic mechanical disturbance and sediment-associated particulate organic matter (POM) were tested by enriching sediments collected from migrating ripples with different qualities of POM (fish feces, leaf litter fragments and no addition treatments). F:B and BCP were affected by an interaction between mechanical disturbance and POM quality. Fish feces enriched sediments showed increased F:B and BCP compared to sediments with lower POM quality and responded with a decrease of F:B and BCP to sediment disturbance. In the other POM treatments F:B and BCP were not affected by disturbance. Microbial respiration was however reduced by mechanical disturbance to similar low activity levels regardless of POM qualities added, whereas fish feces enriched sediment showed short temporary boost of CR. With the worldwide proliferation of migrating sand ripples due to massive catchment erosion, suppressed mineralization of POM will increasingly affect stream metabolism, downstream transport of POM and carbon cycling from reach to catchment scale.

2.2 Introduction

2.2.1 Sediment transport in migrating ripples

While the transport of coarse sediments, such as gravel, in rivers is limited mainly to periods of high flow, the threshold of shear stress for fine sediments, such as sand, can be exceeded at low flow (Uehlinger et al., 2002; Verdonschot, 2001) creating so-called migrating bedforms, ripples and dunes. Migrating ripples, the smaller of these bedforms, have a mean length between 8 and 20 cm and a mean height < 2 cm (Raudkivi, 1997). They develop over a wide range of flows from < 0.2 – 0.6 m/s depth-averaged velocity (Baas, 1999), and occur in small streams to large rivers. The percentage of bed covered by patches of migrating ripples during base flow varies from 5 – 20 % in natural sand bed streams (Mutz et al., 2001) to almost 100 % under heavy catchment erosion (Altmüller and Dettemer, 2006;

Prosser et al., 2001). The wide distribution of catchment erosion (García-Ruiz et al., 2015) makes migrating ripples an increasingly predominant bedform worldwide.

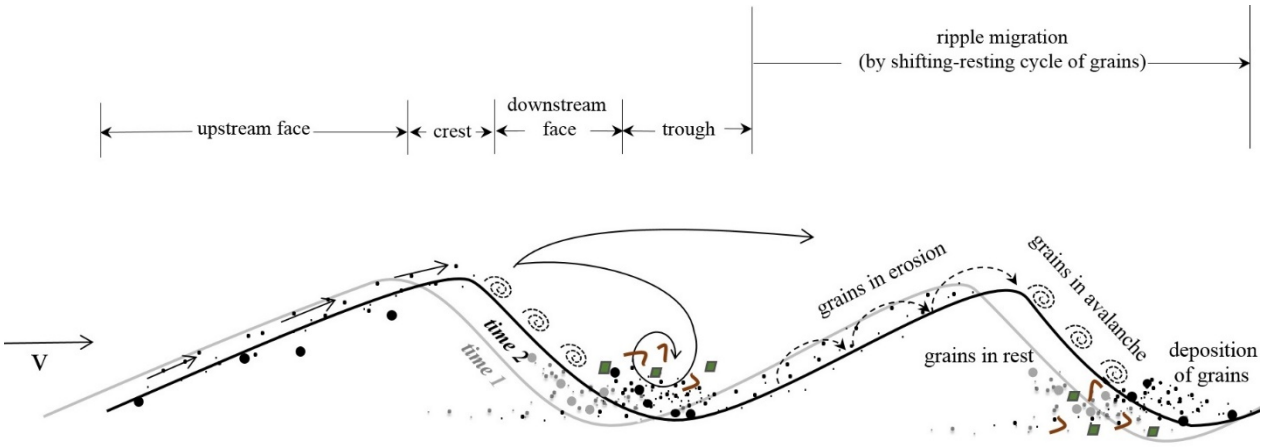

Figure 3 Longitudinal section trough migrating ripples with their geomorphological elements (crest, trough, upstream and downstream face) and geophysical processes (erosion, avalanching and resting of grains). Solid line arrows represent the direction of near bed flow (accelerating parallel to the upstream face, separating from the crest and backflow in the trough area), dashed line arrows and spirals represent the grains movement (eroding from the upstream face and avalanching the downstream face, respectively) resulting in overall sediment shifting (gray and black layers for ripple position at time 1 and time 2, respectively). Green rhomboids and brown cylinders represent the different shapes and distinct qualities of organic matter particulates deposited in trough and worked after deposition by the ripple migration.

The form of migrating ripples is typically quasi-triangular parallel to flow and has a gentle upstream face towards a crest followed by a steeper downstream face close to the angle of repose of granules in water (Figure 3). The ripple migration results from the interaction between the near bed flow and the ripple topography through transport of sediment grains (Charru et al., 2013). The flow accelerates along the ripple upstream side and separates from the ripple crest, generating a circulation eddy with backflow and lower speed in the trough to the next ripple. Consequently, grains erode from the uppermost layer of the ripple upstream face, are dragged in a rolling movement to the ripple crest and past the crest avalanche down the downstream face towards the trough. After coming to rest, the grains that are deposited in the trough are covered by following avalanched grains (Figure 3). They remain buried until the eroding upstream face of the migrating ripple approaches the grains' resting position, whereupon the cycle of erosion and transport along the upstream face, avalanching down the downstream face and rest is repeated (Baas, 1999). Hence, at the scale of sediment grains and

the associated biofilm, sediment movement in migrating ripples is a cycle of transport and rest. We define this cycle as periodic sediment shift. The grains' transport phase is in the scale of seconds, while the rest phase ranges from minutes to a few hours, according to ripple size and hydrodynamics (Bridge, 2003; Harvey et al., 2012). While ripples migrate, they retain and bury organic particles from near bed transport and suspension (Harvey et al., 2012) contributing to the retention of particulate organic matter. Coarse and fine organic particles settle in the trough between two ripples due to the reduced velocity in the recirculating eddy, and are then worked into the migrating ripple and released similarly to sand grains. Hence, the whole volume of sand and embedded organic particles experiences the specific transport and resting cycle that can be seen as periodic (mechanical) sediment disturbance and may affect sediment-associated biofilms and their metabolism.

2.2.2 Effect of migrating ripples on microbial communities

A great portion of stream ecosystem respiration takes place in the streambed, including the benthic and hyporheic zones (Naegeli and Uehlinger, 1997). Sediment transport associated with high discharge events is known to impact benthic organisms (e.g., Grimm and Fisher, 1989; Uehlinger, 2000) and reduce sediment-associated metabolism by scour and abrasion (Uehlinger and Naegeli, 1998; Young and Huryn, 1996). However, it is unclear if similar effects occur during periods of low flow in ripple migration. The instability of sediments, and the rolling and avalanching movement of grains during ripple migration probably impact biofilm formation and maintenance. However, the physical forces in migrating ripples are much lower and the way in which sediment grains move in migrating ripples, cyclically avalanching and resting, is also very different to how sediment moves during high discharge events. Therefore, it can be assumed that the influence of migrating sand ripples on sediment communities will be of a lower magnitude compared to the scour of high discharge events.

Migrating bedforms have been reported as hot spots of microbial activity in field observations due to large number of microorganisms (Gillespie, 1982), high oxygen uptake rates (Rutherford et al., 1991) or bacterial production (Fischer et al., 2005). However other factors that may modulate microbial activity were not taken into account, for instance vertical water exchange (Lautz and Fanelli, 2008), variable organic matter quantity and quality (Findlay, 2010), or nutrient availability (e.g., Fischer et al., 2002). Thus, it remains unclear whether migrating ripples can produce hotspots per se or in combination with other factors such as POM quality.

The vertical water exchange across the streambed is generally increased at bedforms (Savant et al., 1987), such as ripples, therefore, they are sites of intensive water exchange between the water column and sediment pore space, irrespective of whether they are stable or migrating. Overlying water enters the sediment pore space through the upstream face and flows out through the downstream face of ripples (Packman and Brooks, 2001; Packman and MacKay, 2003) and in a similar manner to down-welling zones fueling the heterotrophic metabolism. In down-welling zones, where surface water flows into the sediments, microbial metabolism is elevated by a continuous transfer of oxygen, dissolved nutrients and suspended organic particles (particulate organic matter: POM) (Boulton et al., 2010; Harvey et al., 2012). Hence, activity measures in periodic sediment shift of natural streams cannot disentangle the combined effects of increased advective mass transfer from the water column to the sediment biofilms and the mechanical disturbance on the biofilms due to the sediment shifting.

2.2.3 POM as a main factor for microbial metabolism in ripples

One of the most important among all the interacting factors for benthic and hyporheic metabolism is the availability of POM. Aside from its quantity, POM chemical composition and nutritional value (Bonin et al., 2003) control its bioavailability to the sediment microorganisms. Heterotrophic metabolism in low order streams is driven predominantly by POM of terrestrial origin (Marxsen et al., 2006; Vannote et al., 1980). Terrestrial (allochthonous) POM, such as leaf litter that is leached normally on its way before deposited and buried in stream bed, contains high molecular weight carbon (C) compounds and is usually low in nitrogen (N) and phosphorus (P) relative to C (Attermeyer et al., 2013; Mooshammer et al., 2012), assuming it to be refractory for microbial decomposition (Kleber et al., 2011). Other sources of POM however, such as the autochthonous excretion products of fish, are composed of less structurally complex carbon compounds commonly rich in nutrients, therefore can be readily decomposed by the microbial community (Wotton and Malmquist, 2001). To what extent POM bioavailability has an effect on benthic and hyporheic metabolism under the periodic sediment shift in migrating ripples is, to the best of our knowledge, unknown.

2.2.4 Objectives of this study

The aim of this study was to determine i) the influence of periodic sediment disturbance in migrating sand ripples on the heterotrophic metabolism of sediment-associated microbial communities and ii) whether the proposed effect of periodic sediment disturbance

interacts with sediment POM quantity and quality. We used microcosms that allowed the effects of periodic sediment disturbance to be separated from those of pore water exchange. Fungal and bacterial biomass, respiration rate, and bacterial production were assessed as proxies for the response of the microbial community. Based on previous field observation from shifting sediments and the known boost of microbial respiration by organic matter quality and quantity, we hypothesize: (1) Periodic sediment disturbance in migrating sand ripples increases heterotrophic microbial metabolism in the sediments; and (2) similarly to non-migrating sediments, the microbial metabolism in periodically disturbed sediments increases with increasing quantity and quality of sediment organic matter.

2.3 Materials and Methods

2.3.1 Experimental procedures

The top 1 cm of sediments from migrating ripples were collected from a second order lowland sandy stream, D50 of 328 ± 4 µm (Seebach 52°13'08.5"N 14°02'27.8"E, Brandenburg, Germany). Seebach runs mostly through a deciduous forest and has a riparian vegetation dominated by beech (*Fagus sylvatica L.*) and alder (*Alnus glutinosa L.*). After collection, the sediments were submerged immediately in stream water and transported to the laboratory under cool and dark conditions. The sediment collected was wet-sieved with artificial stream water (see below for details) to obtain the grain fraction ranging between 63 µm and 1 mm and to remove invertebrates. The sediment sieved was then maintained in darkness at a temperature observed in the field (10 ºC) and the temperature was increased by 1 ºC each day for 5 days to acclimatize the sediment-associated microbial community. During the acclimation, the sediment was gently turned (twice per h) to maintain *in situ* conditions. Monitoring of oxygen showed little dynamics in the microbial activity during acclimation.

We used autoclaved artificial stream water for sediment incubations to avoid the uncontrolled introduction of dissolved organic C (DOC) and microorganisms in the microcosms. Artificial stream water was prepared from aerated Millipore water containing 0.354 mg C/L and low concentrations of nutrients (32.4 µg NH_4-N/L and 1.5 µg PO_4-P/L) into which a mineral medium was added (oligoelements: 20 mg/L $CaCL_2 \times 2H_2O$, 15 mg/L $MgSO_4 \times 7H_2O$, 20 mg/L $NaHCO_3$ and microelements solution: HCl, $FeCl_2 \times 4H_2O$, $ZnCl_2$, $MnCl_2 \times 4H_2O$, H_3BO_3, $CoCl_2 \times 6H_2O$, $CuCl_2 \times 2H_2O$, $NiCl_2 \times 6H_2O$, $Na_2MoO_4 \times 2H_2O$), modified after *Lehman* (1980).

2.3.2 Experimental design and setup

The effect of periodic sediment disturbance and its interaction with POM quality on microbial communities in ripples was tested in 24 microcosms at 15 ± 2 °C in constant darkness (Figure 4). To ensure the colonization of all types of POM quality, we adjusted the time of the experiment to the colonization of most recalcitrant POM type added. We run the experiment for 23 days based on reports of substantial microbial respiration from oak leaves (*Quercus petraea, (Matt.) Liebl.*) after 17 days of burial in stream sediments (Cornut et al., 2010) and from beech leaves (*Fagus sylvatica, L.*) after 25 days exposure in streams (Martínez et al., 2016). The microcosms were divided into 12 stable and 12 periodically disturbed. The pore water percolation rate in stable treatments was adjusted to match the pore water exchange by the sediment turnover in the periodically disturbed treatments to guarantee identical advective mass transfer (see below). One third of each disturbance treatment (n = 4) was enriched in POM of different quality: i) only initial biofilm and no additional POM augment: POM_{sed} ii) beech leaf litter fragments augment: POM_{bl} and iii) fish feces augment: POM_{ff} (Table 1).

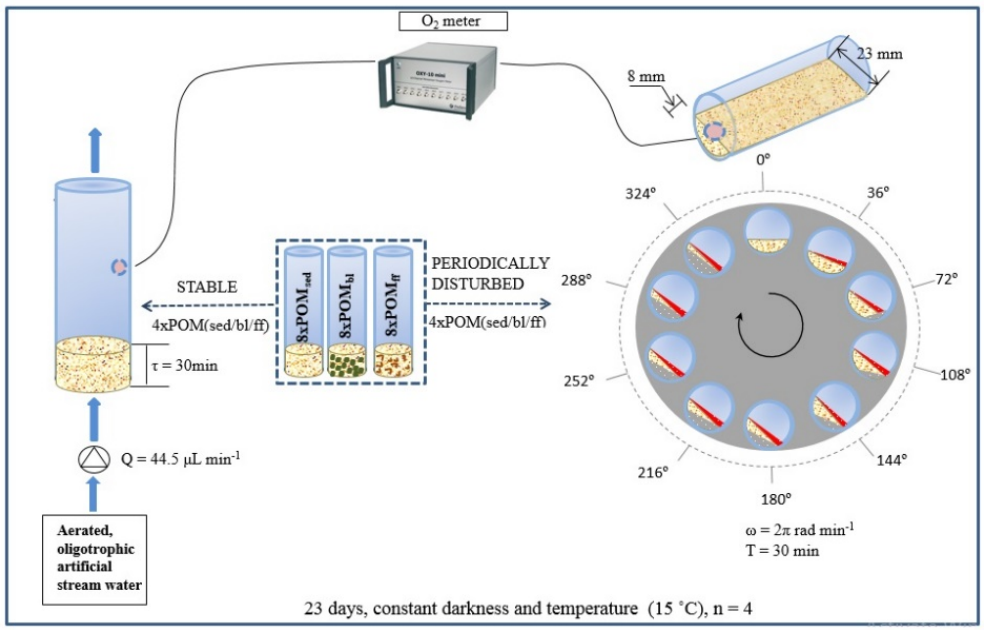

Figure 4 Schematic representation of the experimental setup. Three POM treatments (without POM augment - POM_{sed}, with augment of beech leaves - $POM_{bl,}$ and with augment of fish feces POM_{ff}) exposed to the stable and periodically disturbed environments in two sets of microcosms (vertically positioned flow-through columns and glass tubes horizontally positioned on rotator) and incubated under constant darkness and temperature in the laboratory. Stable sediments provided water residence time (τ) of 30 min while periodically disturbed sediments were rotated every 30 min (period T) with velocity of 1 rpm while shifting the sediments inside the microcosms when the slope of the sediment surface exceeds 30º against horizontal (red dotted triangles). Grey dotted elements represent the sediment grains that are in rest after being exposed to the avalanche during one shifting cycle.

Both POM augments were frozen (-21 °C) and leached for 24 h on thawing in Millipore water prior to the experiment. Leaching corresponded to the *in situ* process where fresh leaves and fish feces experience substantial leaching in the water column when entering the stream or being excreted before sinking to the stream bed and being worked in the sediments. We thus ensured that the effects of periodic sediment disturbance were not masked by the elevated rates of community respiration that an initial leaching of highly bioavailable DOC might have caused.

Table 1 Characteristics of the three POM treatments: POM_{sed} = sediment without POM augment, POM_{bl} = sediment with beech leaf fragments augment, POM_{ff} = sediment with fish feces augment. DM_{sed} is dry mass (DM) of sediment in each microcosm, DM_{POM} is DM of POM added to each microcosm, and C, N and P are percentage of elemental composition of POM added.

POM source	**DM_{sed}** **g**	**DM_{POM}** **mg**	**C** **% DM_{POM}**	**N** **% DM_{POM}**	**P** **% DM_{POM}**	**Organic matter content** **mg DM_{OM}/g DMsed**
POM_{sed}[†]	8.00	/	0.085	0.005	0.002	1.70
POM_{bl}	8.00	63	51.0	0.681	0.05[b]	11.51
POM_{ff}	8.00	68	30.0	4.195	1.5[a]	10.30

[†] *Total iron content determined in sediment was low (0.053 % of DM_{sed}) and its potential for organic matter complexation was considered conservative.*

[a] *Sterner and George*, 2000, [b] *Mooshammer et al.*, 2012.

The organic matter associated with the sediments without augments of POM (POM_{sed}) had a C:N ratio of 23:1. Refractory beech leaves (*Fagus sylvatica L.,* POM_{bl}) that were cut into < 1 mm fragments and had a C:N ratio of 73:1 were added to this sediment at a proportion of 1.2 mg $DM_{POM} \times g^{-1} DM_{sed}$. Similarly, readily bioavailable POM fish feces (*Perca fluviatilis,* POM_{ff}) were added at a proportion of 1.1 mg $DM_{POM} \times g^{-1} DM_{sed}$. The feces were 1 – 2 mm in length and had a C:N ratio of 7:1. POM augments of different qualities (as illustrated by their different C:N ratios) increased sediment organic content by 600 %. Since low nutrient artificial stream water was used during the incubations, sediment nutrient concentrations were controlled mainly by POM augments.

Disturbed sediments

Microcosms for simulation of the mechanical sediment disturbance in migrating ripples were cylindrical glass tubes (2.8 cm in diameter, 6.5 cm in length). Each microcosm was filled with 5.5 cm^3 (≅ 8 g dry mass sediment, DM_{sed}) and 31 – 34 cm^3 of artificial stream water and was then tightly closed avoiding any headspace. The microcosms were positioned horizontally on a rotator that turned them gently for 1 min once around their longitudinal axis. The rotation inclined the surface of the 8-mm-deep sediment volume in the microcosm that reached its critical shear at a slope of about 30° to horizontal, causing the upper sediment layer to start avalanching along the 2.3 cm slope towards the bottom of the microcosm (Figure 4). This avalanching of the upper sediment occurred twice during one rotation, disturbing the entire sediment similarly to the disturbance in migrating ripples during one transport phase. After one rotation, movement was stopped for 29 min to allow the sediments to rest, after which the cycle of rotation and stop, respectively, transport and rest, was repeated. This program simulated the sediment shift and the mechanical disturbance of sand grains and embedded POM in migrating ripples. In order to obtain water and nutrient supply similar to the constantly percolated stable microcosms, water overlaying the sediment was exchanged for well aerated artificial stream water on a daily basis from days 1 to 5 and every second day thereafter till the end of the experiment. This kept oxygen concentrations in the microcosm constantly above critical for the microbial community (> 4 mg L^{-1}). Care was taken to ensure that all particles remained in the microcosm during water extractions. The exchanged water was collected and stored for subsequent quality analysis (see below).

Stable sediments

Glass syringes (10.3 cm in length and 2 cm in diameter, Fortuna Optima, Poulten and Graf, Germany) in an upright position were used as microcosms for stable sediment. The microcosms were filled with 5.5 cm^3 (≅ 8 g dry mass sediment, DMsed)) providing a similar water: sediment ratio as that used in microcosms for periodically disturbed sediments. An additional control microcosm was filled with artificial stream water (hereafter referred to as a blank). Microcosms were tightly closed avoiding any headspace. Each microcosm was individually percolated (bottom-top direction) by a peristaltic pump (IPC, IDEX Health & Science, Wertheim, Germany). Water in all microcosms was supplied from a tank with aerated artificial stream water. Pore residence time was set similar to pore water exchange rate in the periodically disturbed sediments by adjusting a flow rate to 26.7 $cm^3 \times h^{-1}$ (0.85 $cm \times h^{-1}$ Darcian velocity, 3.7 $cm \times h^{-1}$ pore water velocity). The similar rates of pore water exchange

used in this experimental setup allowed the effects of periodic mechanical sediment disturbance on the microbial communities to be isolated when the stable and disturbed treatments were compared. Outflowing water was collected daily for subsequent quality analysis (see below).

2.3.3 Water, sediment and POM chemical analysis

The water collected from stable and disturbed microcosms was pooled on a weekly basis for the duration of the experiment and stored at -21 °C for later analyses of total organic C (TOC), total N (TN) and total P (TP) concentrations according to standard chemical procedures. TOC and TN were analyzed according to DIN EN 1484 on a multi N/C 3100 Analyzer (Jena Analytics). TP was determined photometrically on a UV/VIS-Photometer CARY 1E (VARIAN, Germany) using standard procedures for TP (DIN EN ISO 1189 modified after *Murphy and Riley* (1962)).

Organic matter content in sediments and POM at the beginning of experiment was determined by the loss on ignition method (*Santisteban et al.*, 2004). Dry mass of sediments (DM_{sed}) was determined after the experiment was completed and all metabolic parameters followed in this experiment were normalized on DM_{sed}. Elemental C and N analysis of the sediment and POM at the beginning of the experiment were performed on a CHNS elemental analyzer (Vario EL cube, CN Modus, Elementar Analysensysteme GmbH, Hanau, Germany).

2.3.4 Community respiration

Dissolved oxygen concentration in each microcosms was measured every 30 min that corresponded to the pore water residence time in disturbed and stable treatments, using multichannel microfiber-optic oxygen transmitters (PreSens, Regensburg, Germany). The calculation of community respiration (CR) of both microcosm sets, disturbed and stable, was derived from the mass balance equation applied for specific cases of batch and flow through configurations, respectively, and regarded to the identical pore water residence times. Measurements in the disturbed sediments were conducted immediately after each sediment disturbance (once each 30 mins) when the pore water and the water above the sediment were well mixed.

The CR in these microcosms was registered as the decrease of oxygen concentration through time following equation (1):

$$\boldsymbol{CR} = \left(\frac{\boldsymbol{dC_{treatment}}}{\boldsymbol{dt}}\right) \times \left(\frac{\boldsymbol{V_{water}}}{\boldsymbol{DM_{sed}}}\right) \quad (1)$$

where, $C_{treatment}$ is the oxygen concentration (mg L^{-1}), t is the time (h), V_{water} is the water volume (L) and DMsed is the sediment dry mass (g).

Oxygen measurements in the stable sediments were conducted after each exchange of pore water (once each 30 mins). The rates of CR in the stable microcosms were calculated as the difference in oxygen concentration recorded in the blank and in the treatments, respectively, following equation (2):

$$\boldsymbol{CR} = (\boldsymbol{C_{blank}} - \boldsymbol{C_{treatment}}) \times \left(\frac{\boldsymbol{Q}}{\boldsymbol{DM_{sed}}}\right) \quad (2)$$

where, C_{blank} is the oxygen concentration in the blank microcosm without sediment, $C_{treatment}$ is the oxygen concentration reached in a stable microcosm with sediment (mg L^{-1}), Q is the flow rate of peristaltic pump (L h^{-1}), and DM_{sed} is the sediment dry mass (g). Cumulative respiration over the entire duration of the experiment (cCR) was calculated by numerical integration of the continuous function approximated after interpolation between the 13 measured means of CR (n=4) respective to treatment using R program (*Team R, 2010*).

2.3.5 Bacterial carbon production

Bacterial C production (BCP) from the sediment community was estimated at the end of the experiment. We collected a 0.5 cm^3 sediment subsample from each treatment replicate and one per treatment for control and bacterial protein production determined by sediment incubation at 15 °C based on the incorporation of L-14C-leucine (MP Biomedicals, California, USA, specific activity mCi/mmol) following Buesing and Gessner (2003). We terminated the incubation after one hour by adding TCA (5% w/v final concentration), which was also used to inactivate the controls before leucine addition. Thereafter, samples were sonicated for 1 min at 5–6 W (Elma Transsonic Digital T790/H, Singen Germany) and subsequently processed with multiple washing steps by filtering the samples onto a 0.2 µm polycarbonate Nuclepore Track-Etched Membrane Filter (Whatman, Dassel, Germany), according to Buesing and Gessner (*2003*). We filled 250 µL of the alkaline extract in a 20-mL scintillation vial and added 5 mL scintillation cocktail (Ultima Gold XR, Perkin- Elmer, Downers Grove, Illinois, USA). So prepared solution was radio-assayed. Net DPM

(disintegration per minute) were converted to leucine incorporation according to Buesing and Gessner (*2005*). Subsequently, bacterial carbon production (BCP) was calculated from leucine incorporation assuming 7.3 mol percentage leucine in total protein and a carbon/protein ratio of 0.86 following Simon and Azam (*1989*).

2.3.6 Fungal and bacterial biomass

The presence of viable aquatic fungi and bacteria was determined from phospholipid-derived fatty acid biomarkers (PLFAs) at the beginning and end of the experiment. PLFAs are present in the membranes of all living cells and rapidly degrade to neutral lipids upon cell death (Willers et al., 2015), hence offer sensitive and reproducible measurements for characterizing microbial communities (Boschker and Middelburg, 2002). The PLFAs were measured from 1 g sediment subsamples as fatty acid methyl esters (FAMEs). The lipids were extracted following Bligh and Dyer (1959) and Frostegård et al. (1991), and separated following Kates (1972) and King et al. (1977). After a mild alkaline methanolysis, FAMEs were identified and quantified by gas chromatography–mass spectrometry (Varian CP3800 gas chromatograph coupled to a Saturn 2200 ion trap MS/MS, Varian, Inc., Walnut Creek, CA U.S.A.). Standard nomenclature (Table 2) was used to refer to the PLFAs as described in Boschker et al. (1999) and Steger et al. (2011). Total bacterial biomass was estimated from the summed contents of the bacterial PLFAs identified, while total fungal biomass were attributed to the PLFA cited as specific for fungi (Table 2). The relative presence of two major microbial decomposer groups was defined by calculating the ratio of fungal and bacterial biomass and is described as the F:B ratio in the following.

Table 2 PLFAs detected in this study and their specific microbial group

PLFA	Group	Reference
10Me18:0	Actinobacteria	(*Steger et al.*, 2011)
i16:0	Gram +	
i17:0	Gram +	
a17:0	Gram +	
br16:1	Gram -	
c18:1w7t/5c	Gram -	
cy19:0	Gram -	
a15:0	Heterotrophic bacteria	(*Taipale et al.*, 2015)
18:2w6c	Fungi	(*de Carvalho and Caramujo*, 2014)
C14:0§	General PLFA	(*Boschker et al.*, 2005).
C15:0§	General PLFA	
C16:0§	General PLFA	
c16:1w7c§	General PLFA	
C17:0§	General PLFA	
Sum of bacterial PLFA detected	Bacterial biomass	

§Biomarkers are general PLFA since they can be found in bacteria, cyanobacteria and algae. However, since incubations in this study were carried out in the dark for 23 days and due to the fact that the method measures only viable cells, we assume that these biomarkers are specific only for bacteria and denoted as general bacteria.

2.3.7 Data analysis

We used a two-factorial design to test the effect of periodic sediment disturbance × POM quality augment on the sediment-associated microbial community. We used two-way repeated measures analysis of variance (rmANOVA) to test the effect of period sediment disturbance on CR, with disturbance and POM as fixed factors, followed by Tukey *post hoc* testing (*Zar*, 2010) to test the response of CR. Responses of cCR, BCP, F:B ratios and nutrient concentrations in water (TOC, TN, TP) were tested by two-way ANOVA, with disturbance and POM as fixed factors. The two-way ANOVAs were followed by the conservative Turkey's *post hoc* test to test significant difference between treatments. Each ANOVA was followed by a model validation to check the residuals for normal distribution and homogeneity of variances. Statistical significance of the interaction was tested using a

likelihood-ratio test by comparing the model with and without the interaction and F statistics and p-values were reported. The test was considered significant at p-value less than 0.05. When the interaction was significant, we analyzed parameter response to each factor individually and reported the p-value. All tests were performed using statistical program R (*Team R*, 2010).

2.4 Results

Periodic disturbance reduced CR for all POM qualities (Table 3, Figure 5). The initial CR was comparable in stable and disturbed treatments for all POM qualities except for POM_{ff} in disturbed sediments that had much higher initial CR ($p < 0.001$). The respiration rates in the disturbed sediments decreased from initial levels, then remained constant and virtually identical for all POM treatments (Figure 5). The difference between the POM_{bl} and POM_{sed} treatments respective to disturbance was evident within two days ($p < 0.001$). Despite the higher initial level, the CR of POM_{ff} enriched sediment was lower past day 2 in the disturbed treatment. Also the cumulative respiration for the POM_{ff} treatment calculated for the entire duration of the experiment was lower in disturbed (0.44 mg $O_2 \times g^{-1}DM_{sed}$) compared to stable sediments (0.71 mg $O_2 \times g^{-1}DM_{sed}$), analog to cumulative respiration of the sediment with POM_{bl} in disturbed (0.13 mg $O_2 \times g^{-1}DM_{sed}$) and stable (0.38 mg $O_2 \times g^{-1}DM_{sed}$) treatments (Table 3). By contrast to periodically disturbed treatments, the CR rates in the stable treatments increased clearly during the first days towards a final level that responded to the POM quality, showing higher CR for the POM_{ff} ($p < 0.001$) compared to POM_{bl} and POM_{sed} which were similar.

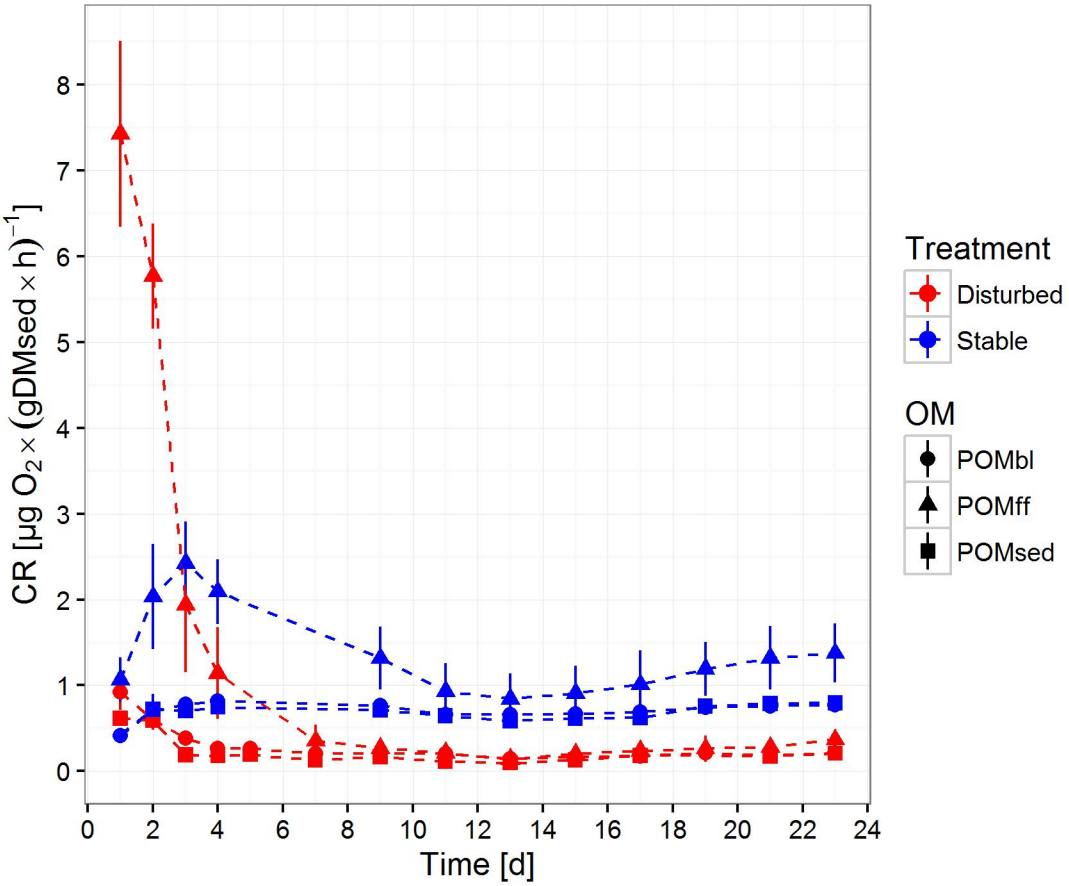

Figure 5 Dynamics of the daily community respiration (CR) rates for disturbed (red filled markers) and stable sediments (blue filled markers), mean (± SD, n = 4). Markers shape show respiration during 23 days for sediment without POM augment (POM_{sed}) and for sediments with the two POM augments (POM_{bl} and POM_{ff}).

The dynamics of CRs for sediments without POM augment and sediments with leaves were similar throughout all 23 days of the experiment in disturbed and stable sediments. Addition of highly bioavailable fish feces, however, caused a pulse of initial respiration rates in disturbed and stable treatments that leveled out after 7 and 11 days, respectively (Figure 5).

Table 3 Effect of sediment disturbance, POM source and their interaction on CR. Two-factorial repeated analysis of variance (rm-ANOVA) for daily rates and two-factorial analysis of variance (2-way ANOVA) for cumulative rates, df = degree of freedom, MS = mean sum of squares, F = statistical test in which test statistics has an F-distribution, p = indicates significant effects at level < 0.05.

	Community respiration (CR)			
	df	**MS**	**F**	**p**
rm-ANOVA, daily CR				
Sediment disturbance	1	4.18	4.93	0.027
POM source	2	30.39	35.87	<0.001
POM source x sediment disturbance	2	2.24	2.65	0.073
Residuals	256	0.85		
2-way ANOVA, cumulative CR				
Sediment disturbance	1	149.5	218.8	<0.0001
POM source	2	488.8	715.5	<0.0001
POM source x sediment disturbance	2	7.2	10.5	<0.001
Residuals	17	0.7		

The addition of POM greatly increased the sediment organic C, and also N and P (Table 1). Hence, we expected release of these into the pore water and the water column that were continually exchanged by oligotrophic artificial stream water. The release of TOC, TN, and TP into the water was similar in the microcosms with sediment disturbance and stable sediment for all POM treatments, indicating similar resource availability in disturbed and stable treatments. Regardless of the disturbance, the release of TOC was similar for sediments with different POM augments. All sediments released nutrients into the pore water modulated by the POM quality (Table 4). The addition of fish feces increased the release of TN and TP compared to the addition of beech leaves and sediment without POM augment ($F_{2,18} = 4.43$, $p = 0.023$ and $F_{2,18} = 23.59$, $p < 0.001$, respectively).

Table 4 Total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP) concentrations in outflow (stable) and exchanged (disturbed) water from microcosms pooled for the entire experiment, mean (± SD, n = 4)

POM source	Sediment disturbance	Water quality parameter		
		TOC	**TN**	**TP**
		mg C/L	**mg N/L**	**µg P/L**
POM_{sed}	Stable	3.62 ± 0.37	0.16 ± 0.03	b.d.l.
	Disturbed	2.05 ± 0.40	0.15 ± 0.02	b.d.l.
POM_{bl}	Stable	3.64 ± 0.18	0.28 ± 0.14	b.d.l.
	Disturbed	2.09 ± 0.45	0.21 ± 0.07	b.d.l.
POM_{ff}	Stable	3.53 ± 0.89	0.39 ± 0.08	10.53 ± 4.50
	Disturbed	2.85 ± 0.40	0.39 ± 0.13	12.00 ± 4.75

b.d.l., below detection limit, TP<6µgP/L

After 23 days, BCP was influenced by periodic sediment disturbance and POM augments ($F_{2,18}$=4.29, p=0.030, Figure 6) whereby sediments with fish feces had a lower BCP in disturbed than in stable sediments (p = 0.027). Sediments with beech leaves had similar BCP in disturbed and stable sediments. The BCP of POM_{ff} in disturbed sediments were 2× higher ($p < 0.001$) and in stable sediments 8× higher ($p < 0.001$) than in POM_{bl} and POM_{sed} which BCPs were similar.

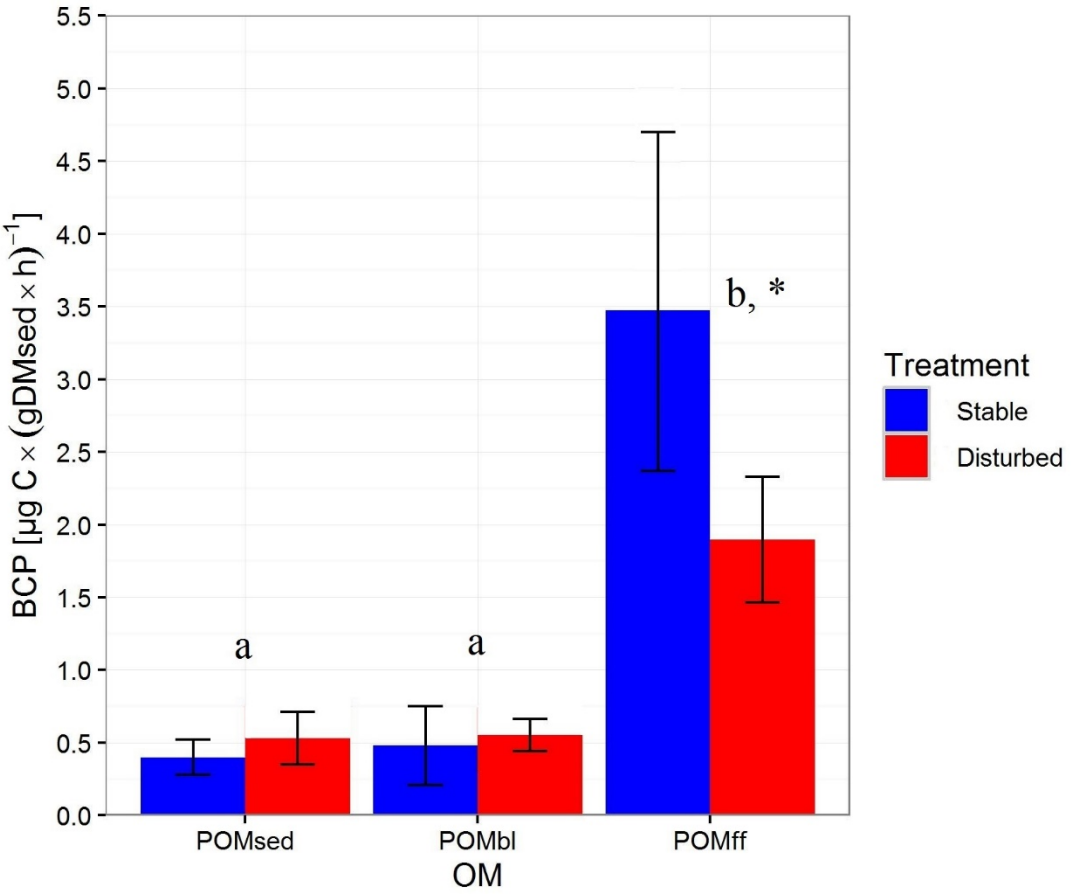

Figure 6 Bacterial carbon production (BCP) for disturbed (red bars) and stable (blue bars) treatments for sediment without POM augment (POM_{sed}) and for sediments with the two POM augments (POM_{bl} and POM_{ff}) after 23 days, n = 4. Asterisk denotes significant differences between the stable and disturbed treatments and letters (a, b) denote significant differences between the POM augments.

The addition of fish feces increased the ratio of fungi to bacteria (F:B), while the addition of beech leaves did not change the F:B ratio compared to sediment without POM augment ($F_{2,2}$ = 43.20, p = 0.02, Figure 7). After 23 days of exposition, all disturbed sediments had a similar F:B ratio regardless of POM quality (Figure 7), while stable sediments with fish feces had a higher F:B ratio than sediments with beech leaves ($p < 0.001$). The F:B ratio in sediment with fish feces was lower in disturbed than in stable sediments ($p = 0.021$).

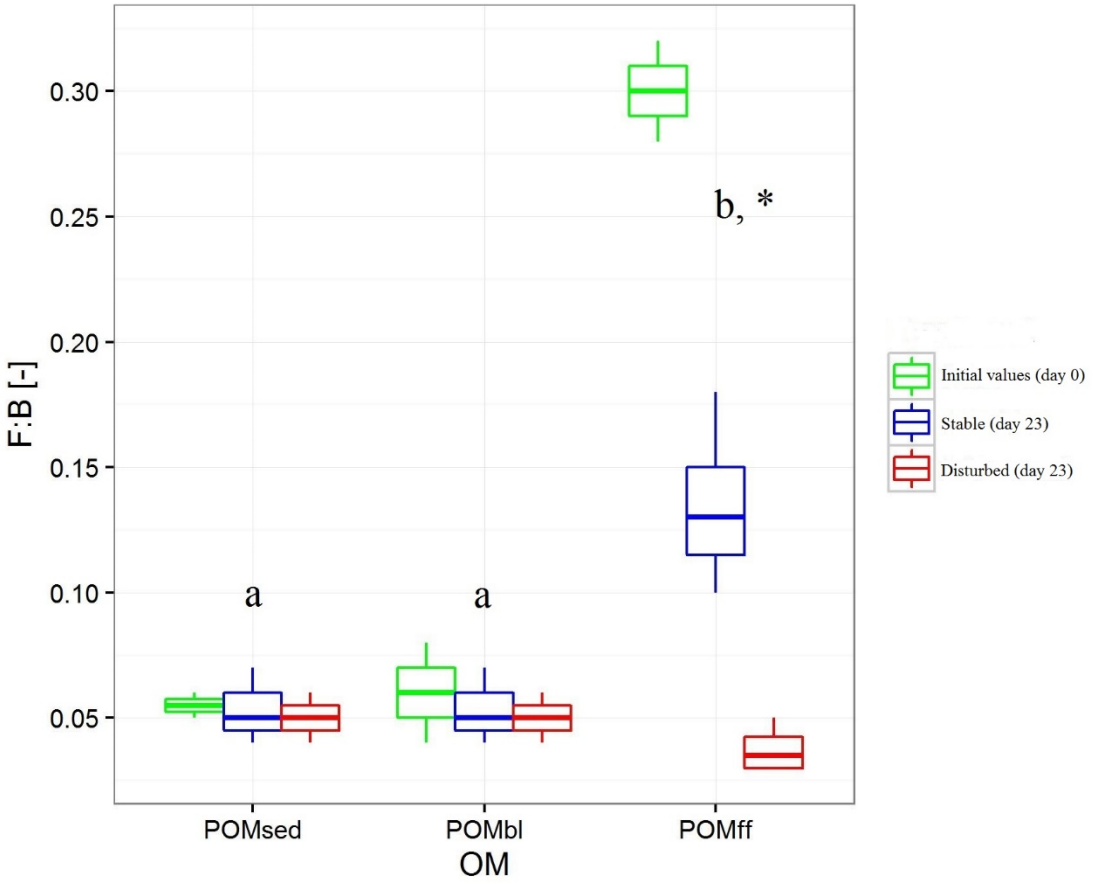

Figure 7 Fungi to bacteria ratio (F:B) in sediment, where bacterial biomass was calculated as the sum of PLFA biomarkers specific for actinobacteria, gram+, gram-, heterotrophic and general bacteria and related to PLFA specific for fungal biomass. Boxes show F:B data of initial values measured for each sediments augmented with POM of different quality before subjected to stable or disturbed conditions (green boxes), and sediments after 23 days of exposition to disturbed (red boxes) and stable treatment (blue boxes), (n = 4). Boxes are grouped according to POM augment; without POM augment (POM_{sed}), with augment of beech leaves (POM_{bl}) and fish feces (POM_{ff}). Asterisk denotes significant differences between the stable and disturbed treatments and letters (a, b) denote significant differences between the POM treatments.

2.5 Discussion and Conclusions

2.5.1 Effects of periodic sediment disturbance on microbial activity

Periodic sediment disturbance reduced CR

Based on previous field studies that proposed shifting sands as hot spots of microbial activity (Fischer et al., 2002; Fischer et al., 2005; Rutherford et al., 1991; Rutherford et al., 1993), our first hypothesis was that periodic sediment disturbance in migrating ripples increases heterotrophic microbial metabolism in the sediments. However, our results contrast with this suggestion. The comparison of periodically disturbed and stable sediments under identical pore water exchange shows that the periodic mechanical disturbance of sand grains, as in migrating ripples, curbs CR.

The CR rates in periodically disturbed sediments averaged 0.25 μg O_2×(g DM_{sed} × h)$^{-1}$ and are comparable with respiration rates found in a sandy oligotrophic forested stream after sediment scour by flood 0.32 μg O_2 × (g DM_{sed} × h)$^{-1}$ (Romani et al., 1998). As sediment particles roll along the upstream face and avalanche down the downstream face of a sand ripple (Figure 3), collisions and abrasion between the sand particles occur which likely stress and damage microbial biofilms. In comparison to high flow events that mobilize the streambed and are known to severely damage or eliminate benthic communities and reduce metabolic activities (Holmes et al., 1998; Uehlinger, 2000; Uehlinger and Naegeli, 1998), the forces of collision and abrasion in sand are lower compared to those at high flows because of the small mass of sand grains and the comparably low flow causing sand ripple migration (Baas, 2003). Gerull et al. (2012) found that a single disturbance of a sand bed in flumes did not affect respiration of the microbial community. The sediment in these flumes was disturbed once only by mixing the upper 1 cm of the sand bed, generating a mechanical disturbance that was similar in depth and nature to the disturbance during one cycle of sediment shift in migrating sand ripples. These findings together with the initial dynamics of CR observed in our experiment indicate that numerous shifting–resting cycles are needed to fully curb the activity of the sand microbial community. The final low level of CR was observed after 3 days (equivalent to 146 shifting–resting cycles) for sand without POM augment and sand with augment of beech leaves, and after 7 days (336 shifting–resting cycles) for sand augmented with fish feces (Figure 5). The cumulative effect of repeated moderate disturbances in migrating ripples is plausible, since the 29 min of rest within a single cycle of transport and rest is too short to allow significant compensation of cell damage by microbial growth and turnover time which scale in the range of several hours to several days (Sinsabaugh

et al., 2015). Hence, the specific periodic disturbance in migrating ripples is needed to cumulate to the full functional curb observed. The proximity of fungi and bacteria in biofilm matrix improves the transfer of mineralized nutrients (Arnon et al., 2010; Blenkinsopp and Lock, 1994; Lock et al., 1984). Thus, disruption of biofilm architecture by sediment mixing may have negative effects on biofilm beyond the mechanical damage of the cells affecting their functions. In nutrient-poor environments, such as oligotrophic sand-bed streams, the maintenance and functionality of biomass relies on tight nutrient-recycling pathways within biofilms (Mulholland et al., 1991), which are not quickly reestablished during resting period following periodic sediment disturbance (Peterson, 1996), as observed in disturbed sediments with fish feces (Figure 7).

Although leaf litter and fish feces are of contrasting quality and differ in their bioavailability for microbial decomposition (e.g., Attermeyer et al., 2014; Mayor et al., 2012), the addition of POM was unable to increase CR in the periodically disturbed sediments to the levels found in the stable sediments. Moreover, CR was curbed to same level after 7 days regardless of POM quantity and its bioavailability (Figure 5). Therefore, our second hypothesis that the microbial metabolism in periodically disturbed sediments increases with increasing quantity and quality of sediment organic matter similarly to stable sediments must be rejected.

Surprisingly, beech leaves did not increase CR when mixed in the stable sediments compared to sediments without POM addition throughout the 23 days of the experiment. After freezing, the beech leaves were only sparsely colonized. Measurement of the CR of the initial leaf fragments in a suspension of artificial water exhibited low activity in the order of 2 µg O_2 × (g leaf fragments × h)$^{-1}$. Hence, leaf addition had initially increased CR in the microcosms by approximately only 0.01 µg O_2 × (g DM_{sed} × h)$^{-1}$, less than the variability among the replicates of the sediment without POM augment. Considerable microbial respiration is reported to be associated to beech leaves after 25 days of exposition in streams (Martínez et al., 2016) and from recalcitrant oak leaves (Quercus petraea, (Mattus) Liebl.) after 17 days of burial in stream sediments (Cornut et al., 2010). Therefore, we expected colonization and increasing respiration of the leaves added within the duration of the experiment. However, the use of the sterile artificial stream water may have impeded fungal colonization of the beech leaves. This is in accordance with the findings of Danger et al. (2012), who reported the fungal inoculum from a sediment being less efficient and the hyporheic environment being less favorable for fungal development compared to the benthic

zone. Immobilization of nutrients from the surrounding water is essential for the initial activity and growth of microorganisms associated to low quality leaf litter such as beech (C:N 73:1) (Cross et al., 2005; Mehring et al., 2015). Since our experiment was conducted with oligotrophic artificial stream water, competitive demand for nutrients among sediment biofilm and the organisms associated with the added beech leaves may have limited the overall CR. Moreover, leaf litter commonly contains C compounds of high molecular weight and complexity (i.e., lignin) and release phenolic compounds (Baldwin, 1987). These can inhibit decomposition through inactivation of enzymes (Benoit and Starkey, 1968).

Other than leaves, the addition of fish feces affected overall activity clearly and modulated the response to periodic disturbance in migrating ripples. Sediments augmented with fish feces showed a higher initial CR and while full curb of CR in the other two treatments was observed past day 3, CR in the treatment with feces declined for 7 days until low constant level was observed. Fish feces used in this experiment had a low C:N ratio (7:1), which is shown to be a favorable substrate with respect to microbial demands for growth (Yoshimura et al., 2008) and reflected in the higher activity in the stable sediments with feces. Opposite to beech leaf fragments, fish feces had already been colonized by millions of microorganisms that pass through the fish gut (Wotton, 1994), and these reacted rapidly after unfreezing and leaching, as shown by the initial pulses in CR of both disturbed and stable treatments and mineralization of the POM (Carroll et al., 2012). The high initial activity in both disturbed and stable treatments and the decay of the initial pulse in stable treatment reflect the rapid exploitation of the readily bioavailable compounds during the first days of the experiment (Hagrave, 1972) by resident and sediment-associated microorganisms that colonize fish feces. In the first two days of the experiment the POM_{ff} treatment showed higher activity in the disturbed than in the stable sediments opposing the general findings. We assume that the high activity during this initial period may have been caused by the fining of the feces strings in the shifting sands that destructed their physical structure and made them accessible to faster colonization and decomposition. The relative longer time till the full curb of the activity in the POM_{ff} treatment might have been due to respiration of easily bioavailable compounds likely released during the first days and potentially counteracting the curbing effect of the sediment disturbance. However, as in the other disturbed POM treatments CR continuously decreased during the first days of the experiment to identical low activity level.

The initial boost of activity in the disturbed POM_{ff} treatment must have consumed more resources (bioavailable organic carbon and nutrients) compared to the stable treatment. Still, succeeding resource scarcity can be excluded, since the cumulative respiration calculated for the entire duration of the experiment was lower in the disturbed compared to the stable POM_{ff} treatment (Table 3) showing that bioavailable OM in disturbed POM_{ff} treatment was not fully utilized. Further, the losses of nutrients and TOC to the water were similar in disturbed and stable treatments. Hence, decrease and curb of CR in the disturbed POM_{ff} treatment must be caused by cumulative effects of the periodic sediment disturbances as in the other POM treatments.

2.5.2 Periodic sediment disturbance effect on BCP depends on POM bioavailability

While the reduction in CR was observed for all POM augments, the BCP showed interesting differences. In sediments POM_{sed} or POM_{bl} BCP remained similar after 23 days under periodic sediment disturbance and stable treatment, while in the POM_{ff} sediments disturbance also curbed BCP after the same time period (Figure 6). Decoupling of CR and BCP in aquatic systems is common, whereas the nature of organic substrates and the availability of nutrients determine BCP and its relation to CR (Del Gorgio and Cole, 1998; Stelzer et al., 2003). When the supply of bioavailable organic matter is low or refractory, as it was in the treatments without POM augment and beech leaf augment, respectively, the organic matter will be used primarily for maintenance of energy requirements rather than for biomass production (Russell and Cook, 1995). Furthermore, the production of new bacterial biomass under oligotrophic conditions is also largely reduced compared to CR (Del Giorgio and Cole, 1998). Since we used low concentrations of DOC and oligotrophic water in our experiment, the POM augment to the sediments was an essential source of both organic matter and nutrients (Table 4), and both constraints for BCP were present in the treatments without POM augment and the refractory beech leaf augment. This explains the low and similar level of BCP in the stable and disturbed sediments of these treatments in which BCP was probably already limited to low values without disturbance. Findings from Fisher et al. (2002) in sediments of the River Spree further support that production is strongly correlated with the amount and quality of POM. These authors reported lower production in deeper sediments (mean 0.36-1.2 µg C × $(cm^3 sed \times h)^{-1}$) and coincided with the lower BCP values found in POM_{sed} and POM_{bl} sediments of this study (mean 0.59 µg C × $(DM_{sed} \times h)^{-1}$ (0.86 µg C × $(cm^3 sed \times h)^{-1}$).

The addition of fish feces that provided readily bioavailable organic matter and nutrients supported substantial BCP in the stable sediments (Figure 6). Under these conditions, BCP was reduced by the periodic sediment disturbance, similar to the CR. The negative effect of sediment disturbance on BCP supports the assumptions of Hubas et al. (2007), who proposed that instability and sediment erosion in marine sediments caused by the wave action and the tidal cycle negatively influenced bacterial biomass production. Mean values of BCP, 3.5 µg C× $(DM_{sed}× h)^{-1}$ (5.1 µg C× $(cm^3sed× h)^{-1}$ in stable and 1.9 µg C× $(DM_{sed}× h)^{-1}$ (2.8µgC× $(cm^3sed× h)^{-1}$ in disturbed sediments with fish feces augment coincided with BCPs measured by Fischer et al. (2001) in the in the upper layer of stratified sediments (5.41 µg C× $(cm^3sed* h)^{-1}$) and sediments exposed to the regular shifting (2.40 µg C× $(cm^3sed× h)^{-1}$) collected in the River Spree in October (mean temperature 13.5 ºC).

2.5.3 Effects of periodic sediment disturbance on fungal and bacterial biomass

The addition of fish feces increased the initial ratio of fungal to bacterial biomass (F:B), while the addition of beech leaves did not change the F:B ratio when compared to sediment without POM augment (Figure 7). For the treatments POM_{bl} and POM_{sed} F:B did not change during the 23 days and were similar in stable and disturbed sediments, analog to BCP. This again supports the potential limitation of growth for fungi and bacteria without disturbance by low availability of nutrients (Gulis et al., 2003, Suberkropp and Chauvet, 1995) and poor fungal inoculum in the POM_{bl} treatment as discussed in the previous section.

The initially high F:B in POM_{ff} was unexpected. The feces had been collected from fishes fed on Chironomid larvae that, as indicated by high F:B ratio in fresh POM_{ff}, likely were parasitized by fungi (De Souza et al. 2014). Presence of a fungal PLFA in fish feces resulting from ingested 'fungal-infected' Chironomid larvae has been reported (Bärlocher, 1981). Moreover, addition of fish feces provided readily bioavailable organic matter and nutrients that supported a substantial bacterial decomposer community. However, similar to CR and BCP, periodic disturbance reduced F:B in sediments with fish feces. This is not surprising given that beside the significant difference in their metabolic requirements and cellular capabilities (Mille-Lindblom and Tranvik, 2003) fungal and bacterial decomposers differ in their size and morphology (Baldy et al., 1995). The size and typical filamentous structure of most fungal groups together with the relative lower growth rates (Bardgett et al., 1999; Attermeyer et al., 2013) make aquatic fungi more sensitive to periodic mechanical disturbance than bacteria.

2.5.4 Relation of the microcosm results to ripples in streams

The microcosms used in this study allowed the investigation of periodic mechanical disturbance, at levels similar to those found in natural sand ripples, and further enabled effects of these disturbances on microbial metabolism to be investigated for different POM qualities found in ripples. During one rotation in our microcosms, the top layers of the 8-mm-deep sediment continuously avalanched over the deeper layers, milling and disturbing the entire sediment in a similar way to the disturbance in migrating ripples during one shifting phase. Grains were exposed to the avalanche over the side length of 2.3 cm when the critical angle of repose was reached (≈ 30°), shifting the sediments with velocity of 0.7 mm s^{-1}, which is well within the range of 0.06 to 1 mm s^{-1} propagation velocity observed for ripples in flumes (Baas et al., 2011; Storms et al., 1999) and in streams (Sukhodolov et al., 2006). The similarity in ripple dimension and in turn the similarity in the forces of the gravity driven sediment shift that occurred in our experimental device and at the downstream face of migrating ripples let us assume that the biofilms experience similar disturbance intensity, forces and abrasion as that found in natural streams.

Opposite to our findings, observational studies in several mobile-bed rivers have found appreciable microbial activity in shifting sediment: high concentrations of adenosine-triphosphate (Hickey, 1985), large numbers of microorganisms (Gillespie, 1982), bacterial production (Fischer et al., 2005) and high oxygen uptake rates (Rutherford et al., 1991). It is not clear, however, whether the shifting sands investigated in literature have been ripples, as the sediments were described as shifting, but geomorphology of these bedforms has not been reported. Only in the study by Rutherford et al. (1991) was denoted that the bedforms for the river studied were dunes. High oxygen uptake rates found in this study may therefore be explained by comparably lower disturbance frequencies (≈ 1 h^{-1}) for the dunes with wavelengths ranging between 1 and 8 m (Rutherford et al., 1993). The shorter wave length of ripples in streams result in a higher frequency of disturbance, e.g. of 2 h^{-1} as used in our experiment. Moreover, the river studied in Rutherford et al. (1991, 1993), was impacted by heavy sewage outflow that created a very special situation, high concentrations of nutrients and bioavailable DOC and high redox demand in the sediment. Since oxygen is provided from the surface stream, biofilm might even benefit from sediment disturbance that prevents overgrowing of sediment pores in heavily polluted situation. In polluted and eutrophic conditions pores of stable sediments can be blocked by algal and bacterial cells and by their metabolic products, such as extracellular polymers or gas bubbles (Baveye et al., 1998; Mendoza-Lera and Mutz, 2013). Drastic limitation of metabolism by interruption of advective

mass transfer between the water column and stable sediments can be consequence (Nogaro et al. 2010; Naval et al. 2011). This might make migrating dunes and ripples that are well perfused by river water (Elliott and Brooks, 1997; Packmann and Brooks, 2001) relative hotspots in polluted streams, despite the curb of the metabolic function by the mechanical stress. Hence in a stream bed, metabolism is of course balanced by disturbing effects of migrating ripples shown in this study and advective mass transfer that might be limiting in stable sediments under eutrophic conditions.

2.5.5 Conclusions

Our laboratory study is the first experimental investigation on the effect of periodic disturbance on sediment metabolism in migrating sand ripples. It reveals that migration of ripples modifies sediment associated C-sequestration. Periodic sediment shift in migrating ripples results in significant disturbance on the microbial functions, decreasing the sediment associated microbial community respiration (CR) and the ability to mineralize allochthonous particulate organic matter (POM). The results refine previous suggestions which proposed shifting sands as general hotspot of microbial activity in rivers and indicate inference with POM quality. For highly bioavailable autochthonous fish feces (POM_{ff}) a temporary initial boost of CR was observed assumable as consequence of mechanical fining of the feces strings when first worked in migrating ripples. Yet, the strong general effect of C quality on its turnover commonly observed in river ecosystems does not account after longer exposition in migrating ripples. In the time scale of several days the cumulative disturbances associated with periodic sediment shift decrease and curb CR for all POM qualities to identical low level.

The present study is of great significance in the light of the increasing worldwide input of sand in streams and rivers (Datry et al., 2014) promoting strong proliferation of migrating ripples. Substantial increase of migrating ripples will modulate mineralization and downstream transport of POM in rivers with likely implications on fluxes of organic carbon and associated nutrients through rivers from reach to catchment scale. Our laboratory experiment focused on the physical conditions in migrating sand ripples. Since the observed disturbance effect has cumulative character, the significance of disturbance with lower periodicity e.g. during dune migrations should be investigated. Future studies should further address the response of autotrophic microorganisms to periodic sediment shift under natural light regime. Such knowledge is needed to possibly transfer and upscale the disturbance effects to field conditions and estimate consequences on catchment scale.

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3. Streambed structural complexity influences microbial carbon cycling in shallow hyporheic zone

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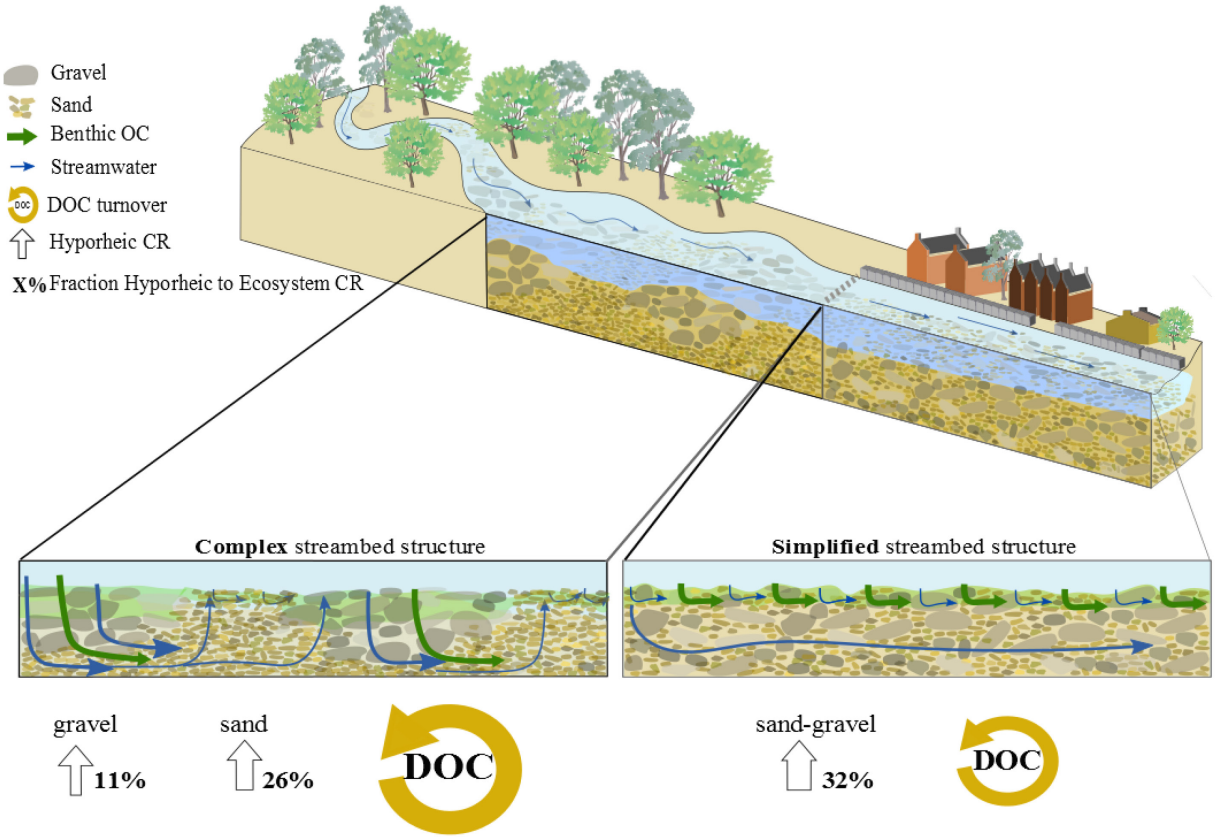

3.1 Graphical abstract

Microbial processes in the hyporheic zone of streambeds significantly contribute to stream ecosystem functions by actively metabolizing and transforming organic carbon (OC) and nutrients. However, their ultimate contribution to processes at ecosystem scale relates to streambed hydraulics that significantly varies according to the spatial complexity of streambed structure. In 16 experimental outdoor streams, we manipulated the complexity of streambed structure through a sorted versus a mixed arrangement of gravel and sand in the streambed and studied how accompanying streambed hydraulics mediate hyporheic microbial activities in resulting habitat types. Additionally, we manipulated photoautotrophic activities by two levels of light. Factorial crossing of streambed structure and light enabled us to reveal how streambed hydraulics interact with benthic activity in shaping hyporheic activity. Patterns in microbial carbon respiration and net ecosystem production imply that the metabolism of hyporheic communities was promoted in sorted sediments by an increased connectivity of hyporheic and benthic processes in the streambed. Thereby, our findings underline that increasing complexity in streambed structure facilitated a larger vertical and horizontal supply of highly bioavailable dissolved organic carbon (DOC) through well-conducting gravel to poor-conducting sand habitat compartment. Associated variations in DOC quality indicate that microbial processing of stream DOC was promoted in sorted streambeds and thus indicate a shift in microbial function towards higher DOC transformation with increasing complexity. On the contrary, in a simplified structure of mixed streambeds, microbial processes in the hyporheic zone remain largely disconnected from benthic processes resulting in lower microbial activities. Overall, our results improve our understanding about the complex interaction of microbial communities with major factors of their physicochemical environment, habitat structure and resource quality, that largely determines their ultimate function in stream ecosystems.

3.2 Introduction

Microbial transformation of organic carbon (OC) drives energy flow and nutrient cycling in stream ecosystems and is thus fundamental for their functioning (Cole and Caraco, 2001; Hotchkiss et al., 2014). While the light-exposed benthic surface of the streambed is the most active site for the accumulation and transformation of photosynthetically produced OC (Battin et al., 2016), most OC mineralization driven by microbial activities occurs in the hyporheic zone of the streambed (Jones and Holmes, 1996; Mulholland and Deangelis, 2000).

Indeed, microbial metabolism of OC in hyporheic sediments may markedly influence OC processing at the ecosystem scale (Battin et al., 2003).

The hyporheic zone comprises the interstitial space of the bed sediments that is hydrologically linked to the open stream channel (Findlay, 1995). Previous research suggests a link between hydrology and microbial activity in hyporheic sediments (Battin et al., 2003). Within streambeds, variations in carbon (C) cycling were attributed to spatial and temporal variations in streambed hydraulic conductivity (e.g. Gantzer et al., 1988; Mutz and Rohde, 2003; Lowell et al., 2009). In a porous medium like sediment, hydraulic conductivity, which translates to permeability for solutes, is largely defined by the sediment grain-size distribution but also by the spatial arrangement of grain sizes in the streambed, e.g. patchy versus a homogenously mixed grain sizes (Bear, 1972). The actual hyporheic flux, residence time distribution and geometries of flow paths (interactively understood as the "hyporheic flow regime") are defined by variations in pressure at the sediment–water interface, by streambed morphology and surface roughness, and by the spatial arrangement of sediment compartments differing in their porosity in the streambed (Stewardson et al., 2016). Indeed, streambeds are characterized by diverse channel morphology and commonly exhibit high structural complexity created by a grain-size arrangement that varies both vertically and horizontally at within-reach scale (e.g. Buffington and Montgomery, 1999b, 1999a). Naturally, streambeds provide a more or less sorted grain size arrangement with different dominant sediment fractions in several habitats (Frostick et al., 1984; Lisle, 1989; Sear, 1993): poor-conducting habitats of sand or silt occur next to well-conducting habitats of gravel and cobbles (Bridge, 2009; Powell et al., 2005). Such a complex streambed structure favors up and down welling of water across the sediment surface, and hence supports dynamic mixing and exchange of surface and streambed pore water (Malard et al. 2002, Salehin et al. 2004). This flow of stream water across the streambed was shown to drive the mass transfer of solutes (nutrients, carbon and electron acceptors) to the microbial community and thus controls the microbial activities of hyporheic sediments (Battin, 2000; Higashino et al., 2009).

Activity of hyporheic microbial communities and their influence on ecosystem functioning further strongly depends on the bioavailability and quantity of OC, besides the availability of redox partners and additional nutrients (Findlay et al., 1993, Boulton et al., 1998; Kaplan and Newbold, 2000). On the benthic surface, microbial activity is dominated by phototrophs that add highly bioavailable OC to the ecosystem (Battin et al., 2016). In addition to this labile OC, streambeds receive a substantial amount of particulate and dissolved OC

from their terrestrial catchment, e.g. in the form of falling leaves, woody debris and eroded soil constituents. These terrestrial OC subsidies differ in their chemical composition with respect to molecular structure and nutrient content from in-situ produced, phototrophic OC (Cole et al., 1982; Guillemette and del Giorgio, 2011). Accordingly, streambeds face a diverse mixture of OC substrates of different quality (Bodmer et al., 2016), and often there are steep compositional gradients in space with benthic streambed areas forming an interface between the overlying surface water and the hyporheic zone. Accordingly, a strong supply of benthic DOC into the subsurface of the streambed has been hypothesized to promote microbial activity in hyporheic sediments (Findlay et al 1993, Muhlholland and Hill, 1997). Consequently, streambed structure, which controls the hydraulic coupling of benthic and hyporheic zones, also has implications for microbial activities via controlling DOC supply. However, microbial activity itself feeds back onto the qualitative composition of stream DOC; streambed morphology thus appears to be an ultimate control on OC cycling (Battin et al., 2003).

Anthropogenic activities, such as river regulation and channel clearance largely reduce the variability of flow and shear stress. These ongoing activities simplify streambeds from a spatially sorted to a more homogenously mixed arrangement of sediment grain-sizes. The resulting decreases in both, habitat diversity and solute supply to the hyporheic zone is considered to have profound consequences for microbial function in ecosystem processes (Findlay et al., 1993, Soulsby et al., 2001). Thus, it is of great ecological importance to understand how alterations in streambed structural complexity affect microbial C cycling in freshwater ecosystems. Several studies investigated how changes in streambed hydraulics influence microbial activity (e.g Belnap et al., 2005; Ceola et al., 2014; Mendoza-Lera et al., 2017) but only few focused on potential implications for microbial OC transformations (Singer et al., 2010; Battin et al., 2003; Perujo et al., 2017). The present study evaluates how microbial OC transformation in stream ecosystems relates to the structural complexity of the streambed and associated hydraulic connectivity between a stream´s benthic and hyporheic zones.

In 16 experimental outdoor streams, we applied two levels of structural complexity by manipulating the spatial arrangement of sandy and gravel sediment (sorted vs. mixed), while keeping grain-size distribution constant (50% of each sediment fractions, gravel and sand, was used in both sorted and mixed arrangement). This was done to provide contrasting streambed hydraulics in respect to the connection of streams' benthic and hyporheic

streambed zones. Additionally, we manipulated photoautotrophic activities by two levels of light availability. In this respect, we evolved whether resultant effects of streambed hydraulics on microbial activities in the hyporheic zone relate to associated shifts in the supply with benthic phototrophic OC of high quality, besides oxygen and other redox partners. Furthermore, we looked at how interactions between streambed hydraulics and hyporheic microbial processes are reflected in optical characteristics of the whole-stream DOC pool as proxies for related underlying shifts in microbial function and DOC transformation. In this respect, we hypothesize that structural simplification of streambeds will reduce the supply of hyporheic communities with high quality C from the benthic zone and in turn lead to a lower contribution of hyporheic microbial processes to whole-stream OC cycling.

3.3 Material and Methods

3.3.1 Experimental set-up

16 outdoor experimental streams (400×12×8cm) were filled with a 3 cm layer of gravel (2 - 10 mm grain size, d50 = 4.75 mm) and sandy sediment grains (0.2-2 mm grain size, d50=0.57 mm). Sediments were rinsed with 10 % hydrochloric acid prior to use to avoid uncontrolled input of microorganisms. Two levels of sediment structure were generated by variation of the spatial arrangement of sand and gravel in the streambed (Figure 8, 8x sorted vs. 8x mixed streambed structures). A sorted streambed was generated from 8 consecutive 45 cm long blocks of sand or gravel, hereafter referred to as spatially complex streambed structure. Further, a mixed streambed was generated from a homogenous mixture of both sand and gravel (50:50 vol %), hereafter referred to as spatially simplified streambed structure. 31.25 g C_{Leaf} m^{-2} of beech leaf fragments were homogenously mixed into streambeds as OC source of terrestrial origin. In addition, two levels of benthic phototrophic OC production were generated through variations in light availability, shaded versus ambient light conditions, which caused low and high phototrophic activities as discussed in detail by Zlatanović et al., 2017. Shaded light conditions were generated by placing a layer of black nets (grid size 1.29 x 1.13 mm) 20 cm above experimental stream, resulting in a mean reduction of the light intensity to 54±8 % of ambient conditions. The combination of two levels of streambed structure with the two light conditions resulted in 4 experimental treatments, each in replicates of four: 1) Sorted + Ambient light, 2) Mixed + Ambient light, 3) Sorted+ Shaded light, 4) Mixed + Shaded light.

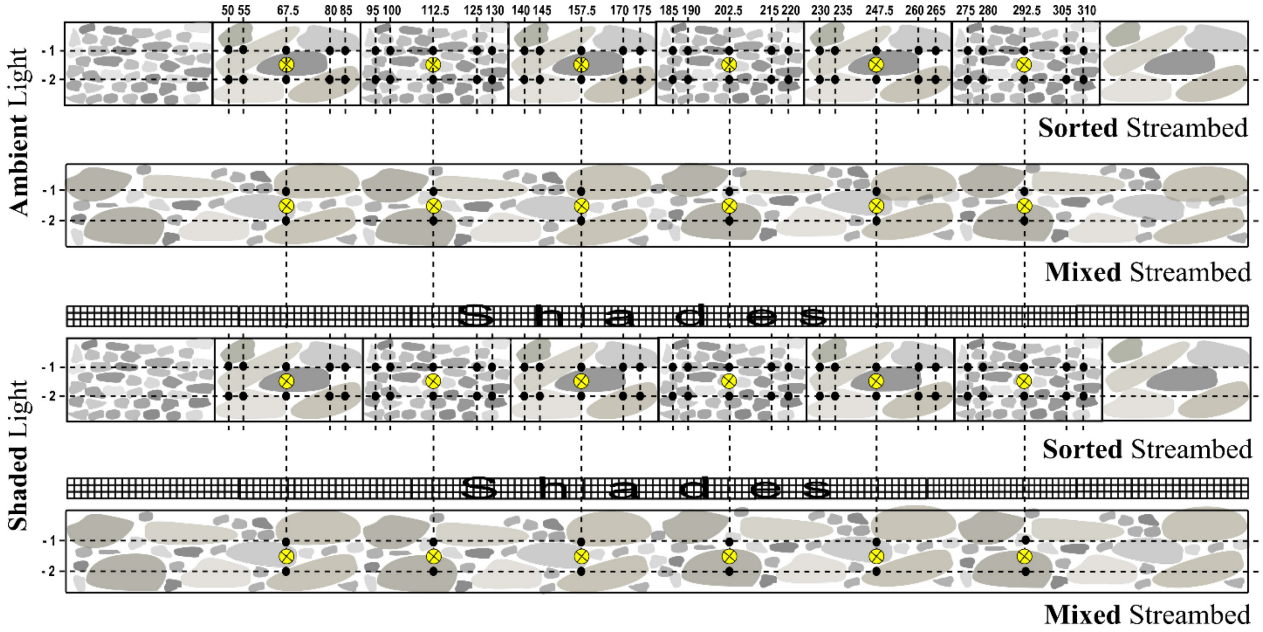

Figure 8 Overview of treatments (streambed structure x light conditions) applied to streams and of measurements for profiling the interstitial concentrations of O_2 (black dots) and the microbial activities in the hyporheic zone (yellow circles). Numbers give depth and length in flow direction in cm.

Groundwater from a local well was re-circulated through each experimental streambed at a surface water depth of 2.8 ± 0.2 cm (~20 L per experimental stream) and at a constant mean water velocity of 2.54 ± 0.03 cm s^{-1} (Pumps by EHEIM GmbH & Co.KG, Germany). Prior to use, the groundwater water was enriched 1.0 mg L^{-1} $N\text{-}NO_3^-$, + 0.2 mg L^{-1} $P\text{-}PO_4^{3-}$, + 0.3ml L^{-1} SL10, (Lehman 1980) to prevent nutrient limitation of microbial growth. Streams were inoculated with a microbial community from a perennial first order stream with a forested catchment (Waldbach, Germany, 52°16'N and 14°03'E). The inoculum was generated from the suspension of randomly collected sediment, litter and woody particles in well-aerated stream water for 48 hours under outdoor conditions. Thereafter, the inoculum was sieved through a 125 µm mesh to exclude macroinvertebrates and larger particles of organic matter. Subsequently, a subsample of the filtered inoculum was added to each experimental stream to achieve a starting concentration of 16 mg TOC L^{-1}. Microbial activities in hyporheic sediments and streambed profiles of interstitial O_2 concentrations were measured at the end of the experiment; 6 weeks after inoculation with microbial communities. In-stream ecosystem activities, absorbance and fluorescence of dissolved organic matter (DOC quality) and biomass of benthic photoautotrophs were measured weekly throughout the experimental period.

3.3.2 Microbial activities

The 'ecosystem' microbial activity refers the total activity of each stream and was revealed from day-night dynamics in concentration of O_2 dissolved in the surface water (DO). During measurements, streams were sealed gas tight with acrylic glass lids (without headspace) to prevent the exchange of DO with atmospheric O_2. DO was measured every 30 minutes for 24 hours using multichannel fiber oxygen optodes (Oxy 10 mini; Presens, Regensburg, Germany). Diurnal DO pattern was converted to metabolic rates for carbon respiration (CR) and net ecosystem production (NEP) according to Odum (1956) as described in (Zlatanović et al., 2017). In addition, we estimated microbial activities for the different sediment textures (sand, gravel, sand-gravel), and zones (benthic, hyporheic) hereafter referred to as 'habitat-specific' and 'zone-specific' microbial activity to reveal the contribution of each habitat and zone to the whole stream metabolism. Habitat-specific CR in the hyporheic zone, $hz\ CR_{habitat}$, was calculated from DO dynamics in the interstitial space of the respective sediment type. An oxygen optode (diameter: 5mm, precision 0.03% DO) was carefully placed in the center of the each gravel and sand block in sorted streambed and at the corresponding equidistant position in the mixed structure (Figure 8) at a depth of 1.5 cm below the sediment surface. Changes in DO concentration were monitored every 5 minutes for 1 h, during which the pumps and thus surface water and pore water flow was stopped to prevent an interference of measurements with oxygen supplied with advective flow from the surface. Further, limitation of DO measurements to a period of 1 h prevented interference of measurements with O_2 diffusion from the surface water (order of magnitude 10^{-5} cm^2/s at 20ºC). Variations in hyporheic habitat DO were converted to CR following a similar procedure as for ecosystem microbial CR. Volumetric hyporheic CR was transformed to CR per surface area covered by each of the habitat type (sand, gravel, sand-gravel) after excluding the volume that corresponds to photic zone (Equation 1, also Table 1).

$$hz\ CR_{habitat} = \frac{dDO}{dt} \times \frac{V_{hz}}{A_{habitat}} \quad (1)$$

where DO is dissolved oxygen concentration (mg L^{-1}), t is time (h), V_{hz} is pore water volume (L) in the hyporheic zone, and $A_{habitat}$ is the surface area (m^2). $hz\ CR_{Habitat}$ was expressed as C equivalent using a respiratory quotient of 0.85 (Del Giorgio and Peters, 1994) and standardized to 20ºC using a temperature coefficient Q_{10} of 2.

Habitat specific NEP was calculated following sequence of equations 2-6:

$$Ecosystem\ GPP = EcosystemNEP + EcosystemCR \quad (2)$$

$$GPP_{habitat} = \frac{(Biomass_{habitat})}{Biomass_{Ecosytem}} \times Ecosystem\ GPP \quad (3)$$

$$AR_f = \frac{(Ecosystem\ CR - hz\ CR_{Ecosystem})}{Ecosystem\ GPP} \quad (4)$$

$$Benthic\ CR_{habitat} = AR_f \times GPP_{habitat} \quad (5)$$

where gross primary production (GPP, mg C m^{-2} h^{-1}), *ecosystem* and *habitat* specific, were calculated following equation 2 and 3, respectively. Periphytic biomass ($Biomass_{habitat}$, $Biomass_{Ecosystem}$, mg C m^{-2}) was determined from chlorophyll *a* assuming 40 mg C per µg chlorophyll. Chlorophyll *a* was measured on the sediment surface using an in vivo fluorometer (Bentho Torch, bbe-Moldaenke, Germany) as described by Zlatanović et al., (2017). The fraction of benthic CR (AR_f) on ecosystem GPP was calculated for each stream according to equation 4 and then multiplied with the habitat GPP to reveal habitat specific benthic CR according to equation 5 similar as in McCallister and del Giorgio, 2008. Ultimately, the habitat specific NEP (mg C m^{-2} h^{-1}) was calculated from equation 6:

$$NEP_{habitat} = GPP_{habitat} - Benthic\ CR_{habitat} - hz\ CR_{habitat} \quad (6)$$

3.3.3 Streambed oxygen (DO) profiles

In the sorted streambeds, vertical and longitudinal profiles of DO were measured (Figure 9). An overview on the exact measurement points in streambed is given in Figure 8. DO was measured with a needle-type optode microsensor (0.9 mm diameter; Microx TX3, Presens GmbH, Regensburg, Germany) protected inside a 20-cm long steel tube. Before measurements, the tube was carefully placed into the sediment at a defined depth and 50 – 100 µl pore water was drawn into the tube with a syringe connected to the upper end of the tube. Additionally, temperature profiles needed to correct oxygen data were measured immediately after each oxygen profile by inserting an electronic temperature sensor directly into the sediment and taking readings at identical depths as DO measurements.

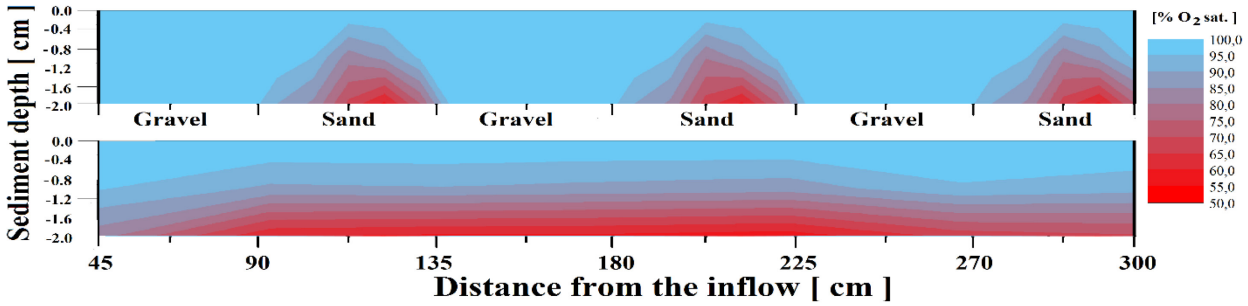

Figure 9 Contour map of sediment oxygen saturation (% O_2 sat.) in stream bed, generated from oxygen measurements in subsurface of sorted (up) and mixed (below) streambeds (see figure 8). O_2 was measured in vertical direction every cm from the surface to the bottom and along flow path from the inflow to the outflow of the stream. All values are given in cm.

3.3.4 Streambed hydraulics

Surface water velocity was calculated based on mean travel time of the conservative fluorescent tracer uranine sodium fluorescein ($C_20H_{10}O_5Na_2$). A small pulse of a highly concentrated tracer solution was injected at the up-stream site of the experimental stream. The tracer concentration was measured directly in the water column at the opposite end of the experimental stream by an online fluorometer (Fiber-Optic fluorometer; Gotschy Optotechnik, Adnet, Austria). Multiple breakthrough curves were recorded from single uranine pulse additions to minimize uncertainty for the velocity estimate.

After the uranine was homogeneously mixed in the surface water (approx. after 15 min) down welling of tracer into the bed and upwelling of tracer-free water out of the bed resulted in a measurable decline in tracer concentration in the surface water. From this decline the vertical water flux (VWF) was determined as described in Mutz et al. 2007. Briefly, the measured tracer concentration time series (every 15 min for approx. 5 h, Figure 13) in the surface water were used to fit the exponential model given in the equation 7:

$$C_S(t) = (C_S|t=0| - C_{eq}) \times e^{-\frac{Q_t \times (V_S + V_p)}{V_S \times V_p}} \times C_{eq} \qquad (7)$$

where Q_t (L h^{-1}) is the VWF, C_S (mg L^{-1}) is the tracer concentration in the surface water, C_{eq} (mg L^{-1}) is the equilibrium tracer concentration, t is the time, V_s (L) is the volume of surface water and V_p (L) is the volume of sediment pore water. Obtained Q_t was expressed per m^2 of whole streambed area and is an average VWF across all habitat types. However, due to fast exchange of pore water in the well-conducting gravel within the first 15 minutes, the

exchange in the gravel habitats cannot accurately be assessed. Hence the flux measured in sorted stream bed represents mostly the exchange in the sand habitats and is a clear underestimate of the overall VWF in the sorted stream bed.

The pore water velocity *u* was measured in each sediment type using high sensitivity heat pulse sensors (HPS, reported HPS application limits are 1.0×10^{-5} m/s for low flow conditions (Angermann et al., 2012). Three HPS were placed 5cm downstream of a single heat point source in the experimental stream's cross-sections to measure the temperature breakthrough curves and assess lateral pore water movement as well as stream-wise pore water velocity (Altenkirch et al., 2016). A data analysis routine was applied to calculate the effective pore water velocity *u* from three individual *u* obtained in the stream-wise and 2 lateral directions (Lewandowski et al., 2011).

3.3.5 Sediment reactivity potential

The Damköhler number (Da) can be applied as a scalable metric of biogeochemical transformation and was shown to explain variation in the capacity of the stream to remove or retain carbon and nutrients (Ocampo et al., 2006; Oldham et al., 2013). Precisely, Da summarizes the reactivity (carbon transformation capacity) of microbial biofilms taking into consideration biofilm activity shaped by their biomass and composition, and solute supply (carbon transport capacity) driven by conductivity of habitat type (Fogler, 2005). The dimensionless Da is defined as the ratio between the median hyporheic residence time, namely τ_{50}, and a characteristic reaction time of a biogeochemical process (Abbott et al., 2016) and was calculated according to equation 8-10:

$$\frac{d(DO)}{dt} = k \times DO \tag{8}$$

$$\tau_{50} = \frac{L}{u} \quad . \tag{9}$$

$$Da = k \times \tau_{50} \tag{10}$$

where *k* is the specific reaction rate constant assuming aerobic respiration as the first-order reaction (Oldham et al., 2013, Vieweg et al., 2017). *k* is calculated from the measured habitat specific decrease of DO in the hyporheic zone of each textural type. Additionally, τ_{50} was calculated for each habitat type, i.e. sand and gravel (sorted) and sand-gravel (mixed),

by dividing the length of each block (L=45 cm) with the measured effective pore water velocity *u*. We applied L=45 cm, equivalent to the length of each block in the sorted streambed, to calculate the residence time of sand-gravel habitats. This enabled us to compare Da of sand-gravel from the mixed streambeds with Da of sand and gravel sediments from the sorted streambeds standardizing it to the similar flow path length to reveal the texture specific controls on C-transformation.

3.3.6 Photic zone

The penetration of photosynthetically active radiation in the streambed was determined in the laboratory following a standard procedure described by de Winder et al. 1999. The depth of the photic zone (1% of incident light intensity) was estimated by covering a light meter (ULM-500; Walz, Effeltrich, Germany) with the same sand and gravel used for the experiment to increasing depth (incident light PAR=300mE m^{-2} s^{-1}). Depth of the sediment that decreased incident light to 1% was considered as photic zone.

Table 5 Overview of hydrological, reactive and biological characteristics of the studied sediment habitats. Gravel and sand were either incubated in the streambed as separate blocks (sorted streambed) or homogenously mixed (mixed sand-gravel streambed). VWF: Vertical water flux. Da: Damköhler number. CR: Community respiration. NEP: Net ecosystem production. Data is given as mean of n=3 to 4 with standard deviations in brackets or as single values.

Streambed treatment	**Light**	**Habitat**	Hyporheic CR	Benthic CR	Grain Size	Photic zone	VWF	Pore water velocity	Dispersion coefficient	Roughness	Mean Residence Time	Da	Algal biomass
	PAR [µmol m^{-2}/s^{-1}]		% Ecosystem CR		D50 [mm]	mm	[$dm^3m^2h^{-1}$]	[mm s^{-1}]	[cm^2s^{-1}]	[-]	[min]	[-]	[mg Chla m^{-2}]
Sorted	**Ambient 44.2±4.4**	**Gravel**	36.7 (3.7)	63.3 (3.7)	4.75	10	>4.22 (0.92)	0.2	$5.75x10^{-4}$	0.029	37.5	0.33 (0.10)	14.03 (4.95)
		Sand			0.5	2		0.08	$2.42x10^{-5}$	0.023	93.7	2.00 (0.27)	11.10 (2.06)
Mixed		**Sand-Gravel**	31.7 (3.6)	68.3 (3.6)	2.63	5	3.73 (0.83)	0.1	$1.59x10^{-4}$	0.026	75.0	1.63 (0.47)	13.73 (4.49)
Sorted	**Shaded 26.2±1.0**	**Gravel**	47.5 (3.9)	52.5 (3.9)	4.75	10	>4.22 (0.92)	0.2	$5.75x10^{-4}$	0.029	37.5	0.42 (0.12)	2.54 (1.26)
		Sand			0.5	2		0.08	$2.42x10^{-5}$	0.023	93.7	1.57 (0.18)	1.38 (0.58)
Mixed		**Sand-Gravel**	49.9 (3.6)	50.1 (3.6)	2.63	5	3.73 (0.83)	0.1	$1.59x10^{-4}$	0.026	75.0	1.20 (0.16)	4.01 (1.19)

Roughness: Manning n according Arcement and Schneider (1989).

Dispersion coefficient: Experimental findings on dispersion of fluids flowing with mean axial velocity u in packed beds. (O. Levenspiel, 1999).

*The pore water of the gravel habitats in the sorted streams was already in large quantity exchanged within 15 min due to the magnitude higher hydraulic conductivity (0.77 cm/s), pore water velocity (2mm/s) and dispersion (5.75×10-4 cm^2/s) in these habitat compared to sand habitat. Therefore in streams with sorted sediment structure the VWF measured was mainly VWF in the sand habitat underestimating the overall VWF.

3.3.7 Absorbance and fluorescence indices of DOC

Optical characteristics of DOC were used to track treatment-induced variations in DOC composition (e.g., Coble 2007; Fellman et al. 2010; Gabor et al. 2014). Absorbance spectra and fluorescence matrices were simultaneously measured from water filtrates (cellulose acetate, 0.45 µm, Sartorius Stedim Biotech GmbH, Goettingen, Germany) collected once at the end of the experiment. DOM absorbance spectra (230–600 nm, every 5 nm) and fluorescence excitation–emission matrices (EEMs, excitation wavelength from 230 to 600 nm, in 5 nm increments and emission range of 210–620 nm in 1.77 nm increments) were measured simultaneously on a spectrophotometer equipped with a CCD detector (Aqualog®; Horiba Ltd, Kyoto) using a 1 cm quartz cuvette. MilliQ water was used as optical blank. Excitation and absorbance wavelengths were scanned from low to high energy (red to UV), reducing UV exposure of the sample to limit the effects of photo-bleaching during analysis. Spectral correction procedures using the Aqualog software included instrument correction, baseline correction, normalization to the daily water Raman peak area (Murphy et al. 2011), removal of Rayleigh scatter, and correction for concentration-related inner filter effects. Fluorescence data are expressed in Raman-normalized intensity units (RU) (Murphy et al. 2010). Samples with absorbance at 254 nm greater than 3.0 were diluted and re-analyzed to ensure linearity in the wavelengths of interest and avoid excessive inner filter effects.

From optical properties of DOC we calculated the following indices: the fluorescence index (FI), the humification index (HIX), the freshness index (β:α) and the molecular weight ratio E2:E3, which served as proxies for DOC quality. The fluorescence index (FI) was calculated as the emission ration of 450 to 500 nm at an excitation wavelength of 369 nm (McKnight et al. 2001). The FI has been reported to distinguish DOC derived from terrestrial sources (degraded plant and soil organic matter; lower values) vs. microbial sources (extracellular release and leachate from bacteria and algae; higher values) (e.g. McKnight et al. 2001; Cory et al. 2007, 2010). The humification index (HIX) describes degree of humification with proceeding diagenetic stage of DOC; it is causally associated to the decrease in the ratio of hydrogen to carbon, which shifts the emission spectra of the fluorescing molecules towards longer wavelengths (Zsolnay et al. 1999; Ohno 2002). HIX was calculated as the area of emission within 435-480 nm divided by the peak area within 300-345 nm and 435-480 nm at an excitation wavelength 254 nm (Ohno 2002). The freshness index (β:α) reflects the ratio of fresh, diagenetically young DOC to old and humic-like DOC (Parlanti et al. 2000; Wilson and Xenopoulos 2009). β:α is calculated from the ratio of the emission intensity at 380 nm (β) to the maximal emission intensity within 420 and 435 nm (α)

at an excitation of 309 nm (Parlanti et al., 2000). The ratio E2:E3, reflects the absorption ratio at 250 to 365 nm and tracks changes in the relative size of DOC molecules (Peuravouri and Pihlaja 1997, De Haan and De Boer 1987). As molecular size increases, E2:E3 decreases because of stronger light absorption by high-molecular-weight DOC molecules at longer wave-lengths. In addition to the above indices, individual fluorescent components were modeled from the obtained EEMs with parallel factor analysis (PARAFAC) (Stedmon and Markager 2005) using the DOMFluor Toolbox (version 1.7; Stedmon and Bro, 2003) for Matlab (version 7.11.0, MathWorks, Ismaning, Germany). We followed common PARAFAC modeling guidelines including split-half validation and multiple random model initializations (Stedmon and Bro, 2003), and identified 6 PARAFAC components that are described in detail in the results section.

3.3.8 Statistical analysis

Analysis were performed with the statistical software R using packages vegan, MASS, multcomp and psych. Treatment-induced variations in DOC quality were evaluated by principal component analysis (PCA). PCA was performed on a correlation matrix generated from DOC absorbance and fluorescence indices computed for each stream. Further, we applied linear mixed effects (LME) modeling to test for a significant effect of light and streambed structure on ecosystem and habitat-specific microbial metabolism (CR, NEP). In the model, fixed effects (2x2 levels) were “light” and “streambed structure” while streams were treated as random effects to allow for different intercepts for each stream in the model. Each LME model was validated for normal distribution of residuals and homogeneity of variances. The statistical significance of each fixed effect and potential interaction between both fixed effects was tested using a likelihood-ratio (LR) test by comparing the model with and without the respective effect.

3.4 Results

3.4.1 Ecosystem and habitat specific CR and NEP

Ecosystem CR and NEP (Figure 10 and 11) significantly varied in relation to light conditions (*LME; CR: LR=2.34, P=0.008; NEP: LR=4.38, P=0.001*) but not among streambed structure (*LME; CR: LR=0.60, P=0.47; NEP: LR=0.16, P=0.70*).

In the hyporheic zone, however, $hz\,CR_{habitat}$ significantly differed among light conditions (Figure 10*, LME: LR=3.20, P=0.008*), and among within-habitat types (*LME: LR=5.95, P=0.001*). Under ambient light conditions, $hz\,CR_{habitat}$was significantly higher in sandy compared to gravel (*P<0.001*) habitat. Compared to sand-gravel habitat of the mixed streambeds, however, $hz\,CR_{habitat}$ was significantly higher in sandy (*P=0.003*) but significantly lower in gravel sediments (*P=0.01*). A significant stimulating effect of light on $hz\,CR_{habitat}$ was only observed for sandy sediments (*P=0.005*). Under ambient light, contribution of $hz\,CR$ to *Ecosystem CR* was significantly higher in streams with sorted, 36.7 ± 3.7%, compared to streams with mixed, 31.7 ± 3.6%, streambeds (*LME: LR=1.65, P=0.41*). In general, the contribution of *hz CR* to *Ecosystem CR* was significantly lower under ambient compared to shaded light conditions (*LME: LR=6.00, P<0.001*), whereby significant differences for sorted, 47.5 ± 3.9%, and mixed streambeds, 49.9 ± 3.6%, were not observed under shaded conditions (*LME: LR=0.77, P=0.9*).

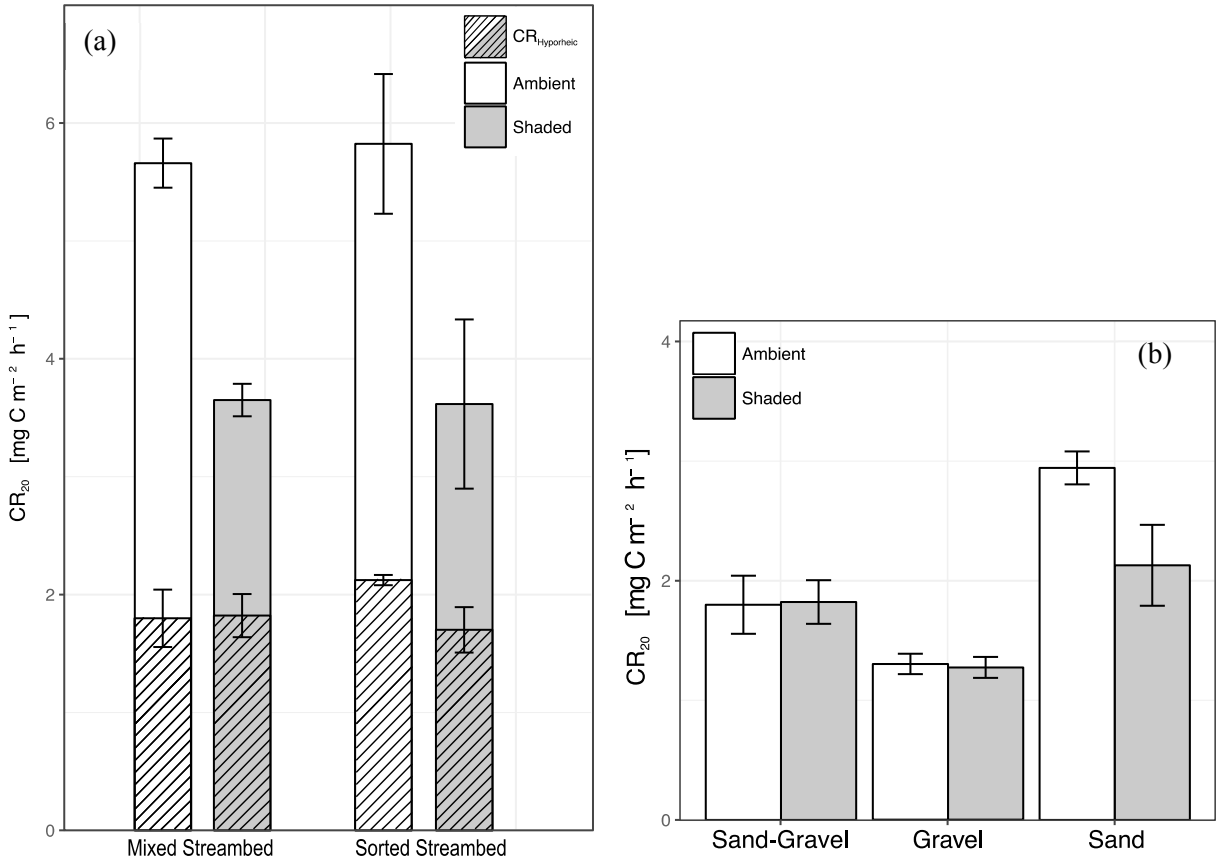

Figure 10 Bar chart of standardized (to 20 °C) community respiration (CR) rates under shaded (grey stags) and ambient light (white stags) conditions. (a) represents the estimate on the fraction of hyporheic (hatched bars; average CR for sandy and gravel habitats) on ecosystem CR; (b) represents CR rates of sandy, gravel and sand-gravel microbial habitats. n=3 to 4.

The balance approach for estimating NEP of the different habitats (Figure 11) revealed a significant influence by grain-size (*LME: LR=9.12, P<0.0001*). Thus, NEP significantly differed between sandy, gravel and sand-gravel habitats. Further, in accordance to observation on *Ecosystem NEP*, light availability significantly promoted NEP (*LME: LR=9.05, P<0.0001*) in all habitats ($P_{gravel}<0.001$, $P_{sand}=0.02$, $P_{sand\text{-}gravel}=0.001$). Under ambient light conditions, NEP was significantly higher in gravel than in sandy sediments of the sorted streambed (*LME: LR=4.05*, *P<0.001*).

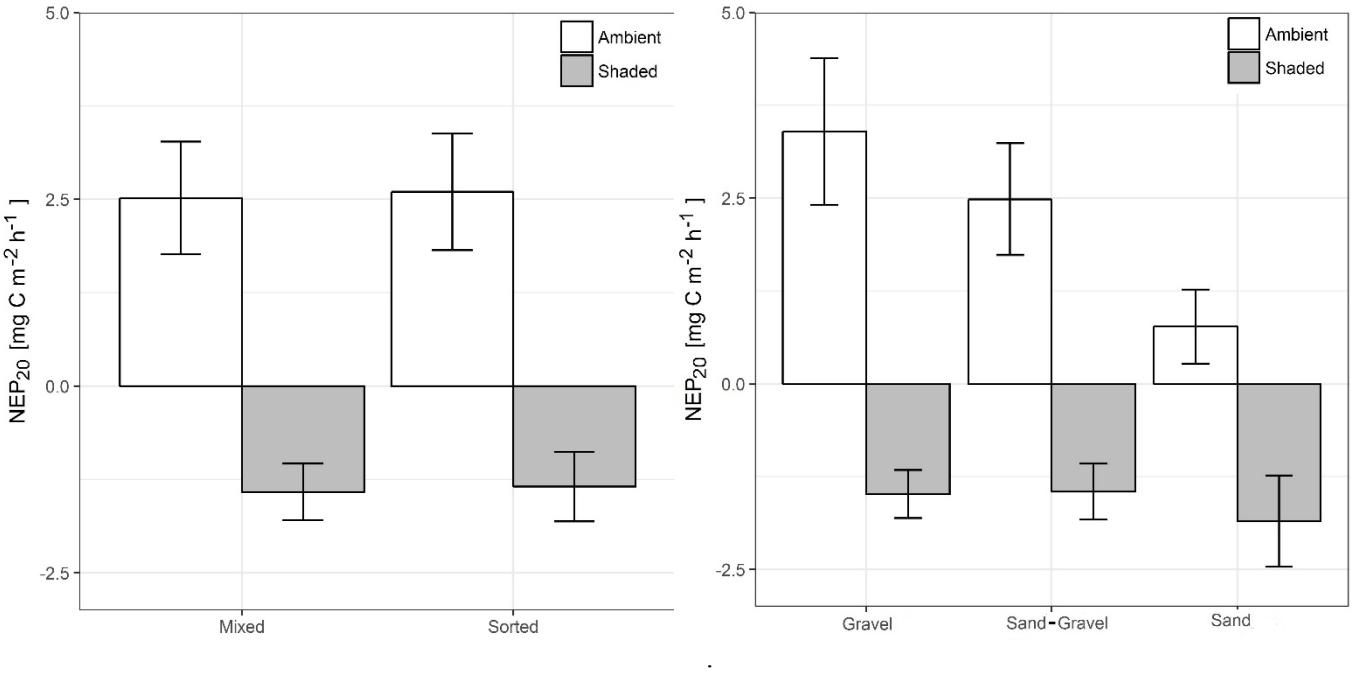

Figure 11 Bar chart of standardized (to 20 °C) ecosystem (a) and habitat specific (b) rates of net ecosystem production (NEP). Experimental streams were incubated under ambient (white) or shaded conditions (grey). n=3 to 4.

3.4.2 Controls on hyporheic C transformation

Da number significantly related to the light (*LME: LR=11.46, P=0.0032*) and sediment (*LME: LR=11.69, P=0.0085, Table 1*). *Da* characterize sorted gravel as less reactive than sorted sands and mixed sediments under the ambient light (LME: LR=4.46, P=0.008) and shaded light (*LME: LR=5.42, P=0.003*).

3.4.3 Treatment related variations in DOM quality

We identified 6 PARAFAC components (Figure 14 and 15) from which 5 could be characterized from their excitation (λ_{ex}) and emission (λ_{em}) maxima based on comparison with the literature (reviewed by Fellmann et al. 2010). Thereby, we identified two humic-like components: C 1 ($\lambda_{ex}/\lambda_{em}$: 330/410) stems from low-molecular-weight OM while C2 ($\lambda_{ex}/\lambda_{em}$: 370/440) is indicative of high-molecular-weight OM. The identified C6 ($\lambda_{ex}/\lambda_{em}$: 470/510) fits to previously identified fluorophores associated with terrestrial humic acids and lignin derivates that are associated with more complex, aromatic OM (Tadini et al. 2015). In addition, we identified two protein-like PARAFAC components: C5 ($\lambda_{ex}/\lambda_{em}$: 280/330nm) is known as tryptophane-like, C4 ($\lambda_{ex}/\lambda_{em}$: 300(250)/350nm) was found as similar to

fluorophores associated with more general low-molecular-weight, protein-like compounds. PARAFAC C3 has not been reported in the literature before and could thus not be characterized in more detail.

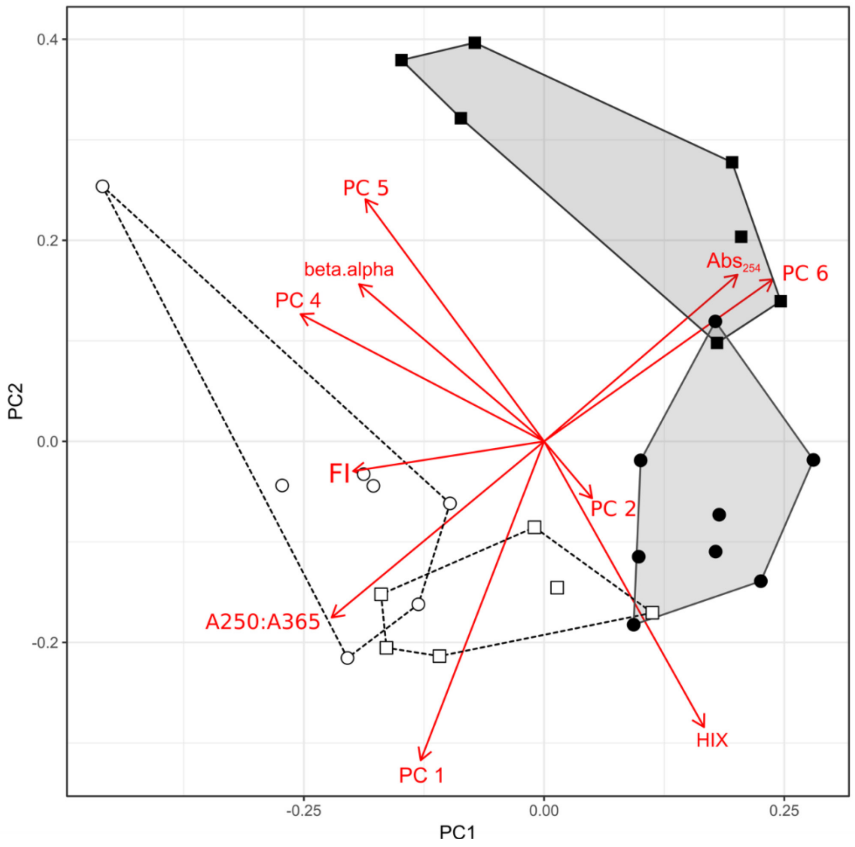

Figure 12 Principal component analysis based on absorbance coefficients, fluorescence indices and the normalized PARAFAC components suggests significant variations in DOM composition according light conditions and streambed treatment. PC1 separates streams according to light conditions, whereby streams with sorted streambeds were additionally separated along PC2. The proportion of fresh (beta/alpha), protein like (C1) DOM of recent origin was higher under ambient light conditions, whereby the proportion of aromatic (SUVA254) DOM of predominantly terrestrial origin was higher under shaded light conditions. For streams with sorted streambed, optical properties of DOM further indicate DOM to processed (HIX) under ambient light conditions

The humification index (HIX) ranged from 4.18 to 7.60, the fluorescence index (FI) ranged from 1.26 to 1.40, the molecular weight ratio (250nm/365nm) ranged from 5.77 to 6.35 and the freshness index (β:α) ranged from 0.65 to 0.76. DOC quality significantly differed between light conditions (*PERMANOVA, F=19.09, P=0.001*) and two streambed structure treatments (*PERMANOVA, F=4.67, P=0.015*), but we also observed a significant interaction between light and streambed (*PERMANOVA, light x streambed, F=5.15, P=0.01*). Principal component analysis (PCA) of optical indices reveals two prominent gradients by which sorted and mixed streambeds separate in their response to light availability (Figure 12).

Along DOM-PC1, which explained 44 % of the variance, the PCA separates both streambed treatments according to light conditions. Under ambient light conditions, composition of stream DOM was characterized by protein like (C1) DOM of high 'freshness' (beta/alpha) suggesting DOM of authochonous and recent origin. Under low light conditions, composition of stream DOM was characterized by more aromatic ($SUVA_{254}$) DOM of predominantly terrestrial origin (C6). For sorted streambeds, PCA additionally separates both light treatments along DOM-PC2, which explained 32 % of the variance, whereby DOM is indicated to be more protein, tryptophan like (C5) under shaded conditions and more humified (HIX) DOM of low molecular weight (C1) under ambient light conditions.

3.5 Discussion

The purpose of this study was to unravel how changes in streambed structure affects microbial OC transformation focusing on associated changes in the streambed hydraulics. Our results imply that a sorted arrangement of sand and gravel habitats facilitates higher VWF and a better longitudinal transport of benthic solutes through well-conducting gravel into poor-conducting sandy habitats. We further observed a positive interacting effect of our applied factors, light availability and streambed structure, on microbial activities. In this respect our findings provide evidence that a streambed with increased structural complexity promotes the hydraulic coupling of benthic and hyporheic streambed zones and hence facilitates a greater availability of phototrophic solutes for hyporheic sediment communities. Variation in hyporheic microbial activities in respect to streambed structure were associated with variations in the composition of DOC. Accordingly, combined findings suggest that streambed structure has a significant influence on microbial activity in the hyporheic zone, with profound consequences for underlying processes of OC turnover in streams.

3.5.1 Effect of grain-size arrangement on streambed hydraulics

DO concentrations in the hyporheic pore space of the streambed vary according to microbial respiration, and supply of O_2-rich surface water (Odum et al., 1956). The spatial distribution of DO in the pore water of gravel and sand habitats (Figure 9) agrees well with previous reports showing that the solute supply from the surface into hyporheic zones improves with increasing grain size (De Beer et al., 1996; Mendoza-Lera et al., 2017). When comparing sand and sand-gravel habitats, however, we expected a smaller decrease of DO with depth in sand-gravel habitats, as the on average a larger grain size creates higher surface roughness. Since the stream bed had even topography we expected that the surface roughness

promotes a stronger vertical mixing of surface water and solutes into the interstitial space of the streambed (Brayshaw et al., 1983; Grant et al., 2012) and hence enables a better re-aerations of deeper sediment (Tonina and Buffington, 2009). Yet, although we measured significantly higher CR in sandy than in sand-gravel habitats (Figure 10b), sandy habitats remained more saturated with O_2. Hence DO concentration profiles and VWF indicate a better advective supply of sandy habitats when arranged in blocks with gravel than when homogenously mixed with gravel (Table 5). During the water down-welling and upwelling in the gravel habitat at the interface with the sand, the appreciable part of water intrudes in the sand habitat elevating the DO to the full saturation in these first and last 10 cm of sand habitats (Figure 9). In this regard, our findings underline that expectations about the supply of a sediment based on surface roughness and grain size are modified by the arrangement of grain sizes in the bed (Fleckenstein et al. 2006). Thereby, a sorted arrangement of different grain-sizes in the streambed likely improves the hydraulic connectivity in poor-conducting sandy habitats through a horizontal supply component that increases the supply of solutes from well-conducting gravel habitats. Hence, simplification of streambed structure with respect to the arrangement of grain sizes results in less coupled benthic and hyporheic zones compared to structurally complex streambeds.

3.5.2 Effect of grain-size arrangement on streambed activity

In the sediments, microbial activities relate to biomass and community composition specific to grain-size surface area available for colonization (Freixa et al.,2016). In hyporheic zone however, the metabolic activity of microbial communities is modified by the supply with nutrients and redox partners like O_2 (Battin et al, 1999; Hall et al., 2012; Nogaro et al., 2013, Mendoza-Lera et al. 2017). In our study, hyporheic microbial activities significantly differed between habitats;, hyporheic CR rates in sorted gravel were significantly lower than in sorted sand and sand-gravel habitats (Figure 10b). Observed hyporheic CR rates are complemented by distinct Damköhler numbers (Da) that were well below 1 in gravel indicating lower reaction rate, probably due to lower biomass caused by intrinsic sedimentological properties (e.g. colonization area) (Higashino et al., 2009), despite a higher conductivity. Unfortunately, we lack estimations on microbial biomass in hyporheic sediments due to constraints by the experimental set up (sampling deeper sediments would have involved significant disturbance for hydraulic measurements).

As previously discussed, varying patterns of well- and poorly-conducting habitats in the streambed favors the overall supply of hyporheic microbial communities with essential solutes from the surface water and the benthic zone. Accordingly, we hypothesized that a complex streambed structure promotes a deeper penetration of not only nutrients and redox partners, but also of phototrophic OC (Rauch-Williams et al., 2006). Besides hydraulic connectivity, sediment grain size also defines the depth of the photic zone and thus the spatial distribution of photosynthetic activity and associated highly bioavailable DOC (Findlay et al., 1993; Battin et al., 2003). As reflected in a greater habitat specific NEP, gravel sediments had a deeper phototrophic zone (Table 5) and associated photosynthetic activity (Figure 11). A deeper benthic zone in well-conducting gravel could also promote lateral supply of poorly-conducting sandy sediments with not only redox partners and nutrients but also with highly bioavailable, benthic DOC. This notion is further supported by the effect of light on hyporheic CR, which differed between sand, gravel and sand-gravel habitats (Figure 10b). Microbial activities are positively related to DOC quantity and promoted by DOC of phototrophic origin (Kaplan and Bott, 1982). For gravel habitats, a stimulation of hyporheic CR by phototrophic CR was likely limited by the available colonization (Mendoza-Lera et al., 2017) as discussed above. Yet, the significant stimulation of hyporheic CR by higher light in sand but not sand-gravel habitats underlines a stronger metabolic coupling of a streambed's benthic and hyporheic zones by streambed sorting.

3.5.3 Hydraulic coupling of benthic and hyporheic zones influences microbial processing of stream DOC

Microbial activities alter the qualitative composition of the DOC pool; thus, variations in DOC quality indicate functional changes in microbial OC turnover (Miller 2009, Mladenov 2010). Principal component analysis of DOC quality indices indicates a significant shift in DOC quality from shaded to ambient light conditions (Figure 12). An increase in the extinction ratio (A250:A365) and the relative proportion of PARAFAC component 1 to DOC fluorescence with higher light availability indicates that light-induced changes in DOC quality correspond to a general decrease in aromaticity and hence increasing proportion of microbial DOC (Strome and Miller, 1978, Fellmann et a. 2010). Accordingly, decreasing DOC aromaticity with higher light availability correlated with a strong stimulation of ecosystem microbial activities (Figure 10), yet was not reflected in a common stimulation of sediment-specific microbial activity in the hyporheic zone. Microbial activities in the benthic zone are dominated by the activity of microbial primary producers (Figure 11, see also Zlatanović et al., 2017). Hence, light induced shifts in DOC aromaticity correspond primarily to an

accompanied stimulation in the production of phototrophic OC that is rich in proteins and composed of simple biomolecules (Cole et al., 1982). Even though phototrophic activities also promoted the activity of microbial heterotrophs, a heterotrophic production of low complex DOC is unlikely and thus not necessarily reflected in DOC aromaticity as heterotrophs selectively allocate phototrophic C to respiration (Guillemette et al., 2016).

PCA further indicates that shifts in DOC quality from shaded to ambient light conditions differ between sorted and mixed streambeds as they relate to distinct absorbance and fluorescence indices. For mixed streambeds, light related shifts in DOC quality relate primarily to a higher fluorescence index that is associated to the origin of DOC with respect to terrestrial versus microbial sources (McKnight et al. 2001). However, for sorted streambeds, light-related shifts in DOC quality additionally relate to a greater humification index that indicates an increase in microbial processing of OC. Accordingly, optical properties of DOC underline that the previously evaluated better vertical connectivity of benthic and hyporheic zones in geomorphological complex streambeds led to a greater overall microbial processing of OC. Further, in a related study (Fabian et al., data not published) we show that streambed structure influences the decomposition of leaf POC that resulted in a higher fraction of leaf C on ecosystem DOC for sorted versus mixed streambeds. Hence, combined findings support that underlying shifts in microbial processing of stream OC. relate to interactive effects of streambed structure and light availability on the activity of hyporheic microbial communities. Further, observed correlative variations between DOC quality and hyporheic but not ecosystem microbial activities underline that subsurface processes significantly shape the quality of stream DOC.

3.5.4 Implications for future consequences on potential streambed simplification for microbial C cycling in streams

In summary, our findings highlight that streambed structure and light availability influence microbial activities in the hyporheic streambed zone through associated changes in the hydraulic connectivity of the hyporheic zone with the benthic zone and surface water as a function of vertical exchange. We show that these changes in hydraulic connectivity alter the qualitative composition of ecosystem DOC, but have no influence on overall quantitative turnover processes, hence ecosystem CR and NEP. Ecosystem-scale measurements spatially integrate all processes, with benthic activities usually dominating metabolic rates at ecosystem scale especially in the shallow ecosystems. However, ecosystem-scale measurements do not provide information on spatial variability in metabolic processes, which

in turn underestimates the role of small-scale, habitat specific processes for ecosystem function. On the other hand, measurements on a habitat scale only resolve a part of the flow paths, as it provides little information about water returning to the stream and thus allow a limited estimate of the total ecosystem processes (Knapp et al., 2016) Owing to their complementary information about hyporheic transport, the illustrated fundamental discrepancies of the two approaches, may therefore in combination give the entire snapshot of the underlying quantitative and qualitative metabolic processes in streams. In this sense, our study confirms that the qualitative transformations of OC in subsurface are closely coupled with its hydraulic connectivity that drives transport of solutes and particles into, through and out of the subsurface sediment (van Rees et al. 1996). Yet, light availability strongly drives the magnitude of the ecosystem rates and their trophic balance (Figure 11) masking the relevance of the qualitative changes occurring in the subsurface. The disparate findings on ecosystem and habitat scale in present study further suggest that constraints for the microbial activity in the hyporheic zone driven by different factors (microbial rate in gravel $Da < 1$ and transport of reactants in sand $Da > 1$) were compensated within the sorted streambed causing ultimate similar ecosystem activity.

Our findings highlight the possible consequences of anthropogenic actions for microbial turnover of OC in streams and corroborate that streambed sorting capacity mediates the functional contribution of microbial processes in the hyporheic zones to stream ecosystem biogeochemistry, in particular regarding the decomposition of OC in streams (Battin et al., 2003). Furthermore, we highlight that streambed structure interacts with light as controlling factor for microbial C cycling and therefore forms a central link between stream metabolism and expected OC turnover in streams. Given the complexity of the processes that synergistically influence C-turnover in streams at various scales, combining reach and sub-reach scale observations has the potential to vastly improve understanding about the controlling processes and cumulative effects of hyporheic-zone reactions (Gomez-Velez et al., 2015), which will be needed to forecast how changing land use will affect river water quality and to prioritize effective management (Hester and Gooseff, 2010; Mortensen et al., 2016). Consequently, changes in microbial OC transformation through streambed hydraulics provide a hitherto underappreciated key player in contemporary landscapes and must therefore be included for a complete mechanistic understanding of ecosystem processes in streams.

Supplemental information

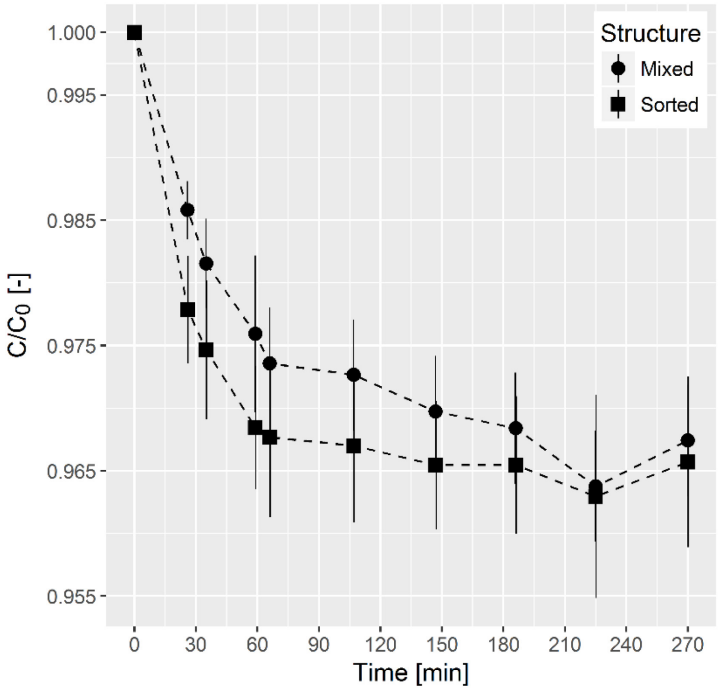

Figure 13 Uranine tracer concentrations in surface water at time t (C) relative to uranine tracer concentration when uniformly distributed in surface water (C0) during the experiment in sorted-bed (filled squares) and mixed-bed (filled circles). Symbols are measured values (mean ±SD, n=8).

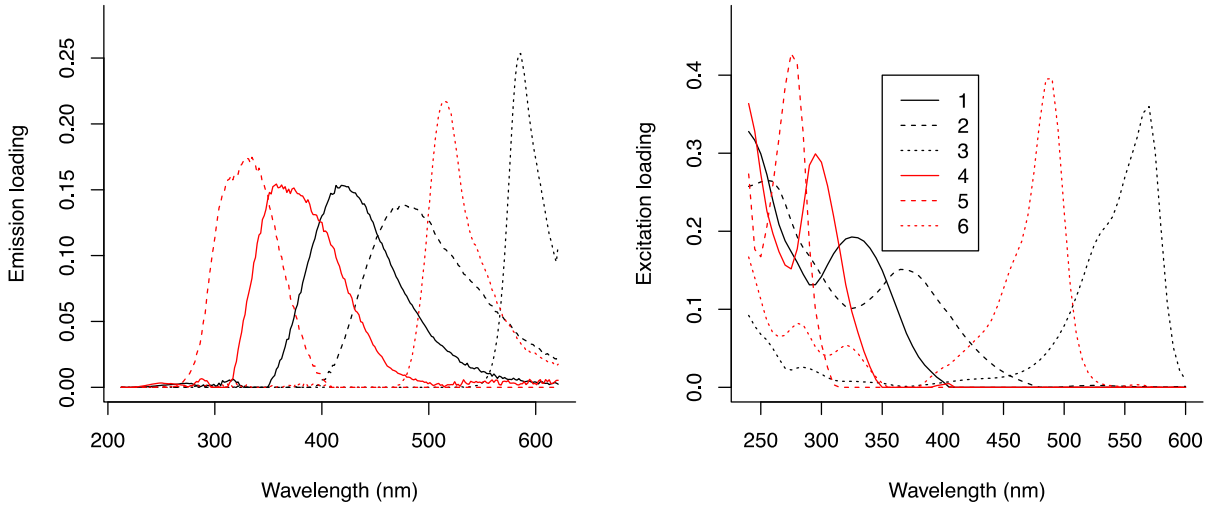

Figure 14 Emission (left) and excitation (right) loadings of six identified PARAFAC components

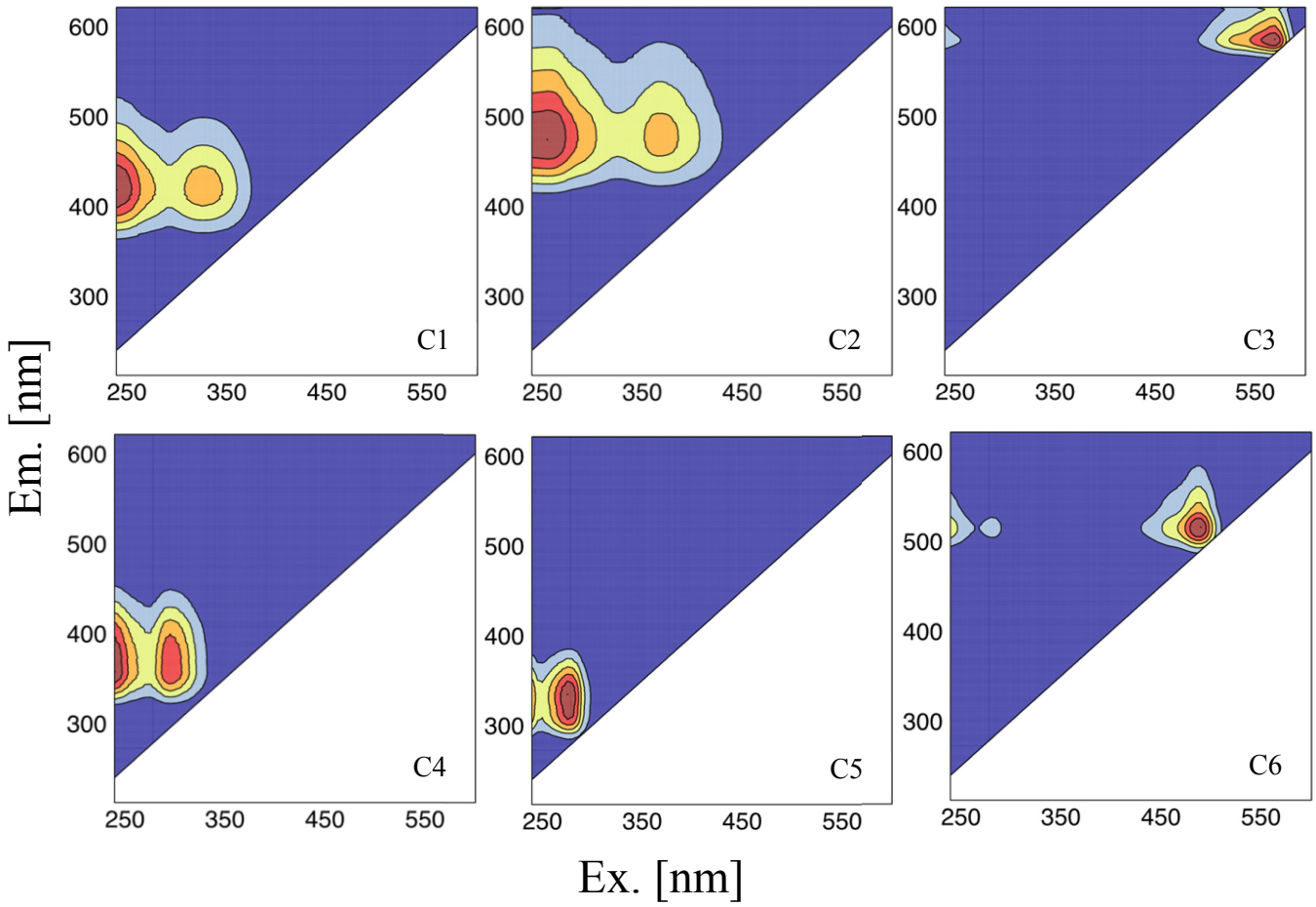

Figure 15 Excitation-emission plots of six identified PARAFAC components; C =component, Em = emission, Ex = excitation.

Acknowledgments

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4. Shading and sediment structure effects on stream metabolism resistance and resilience to infrequent droughts

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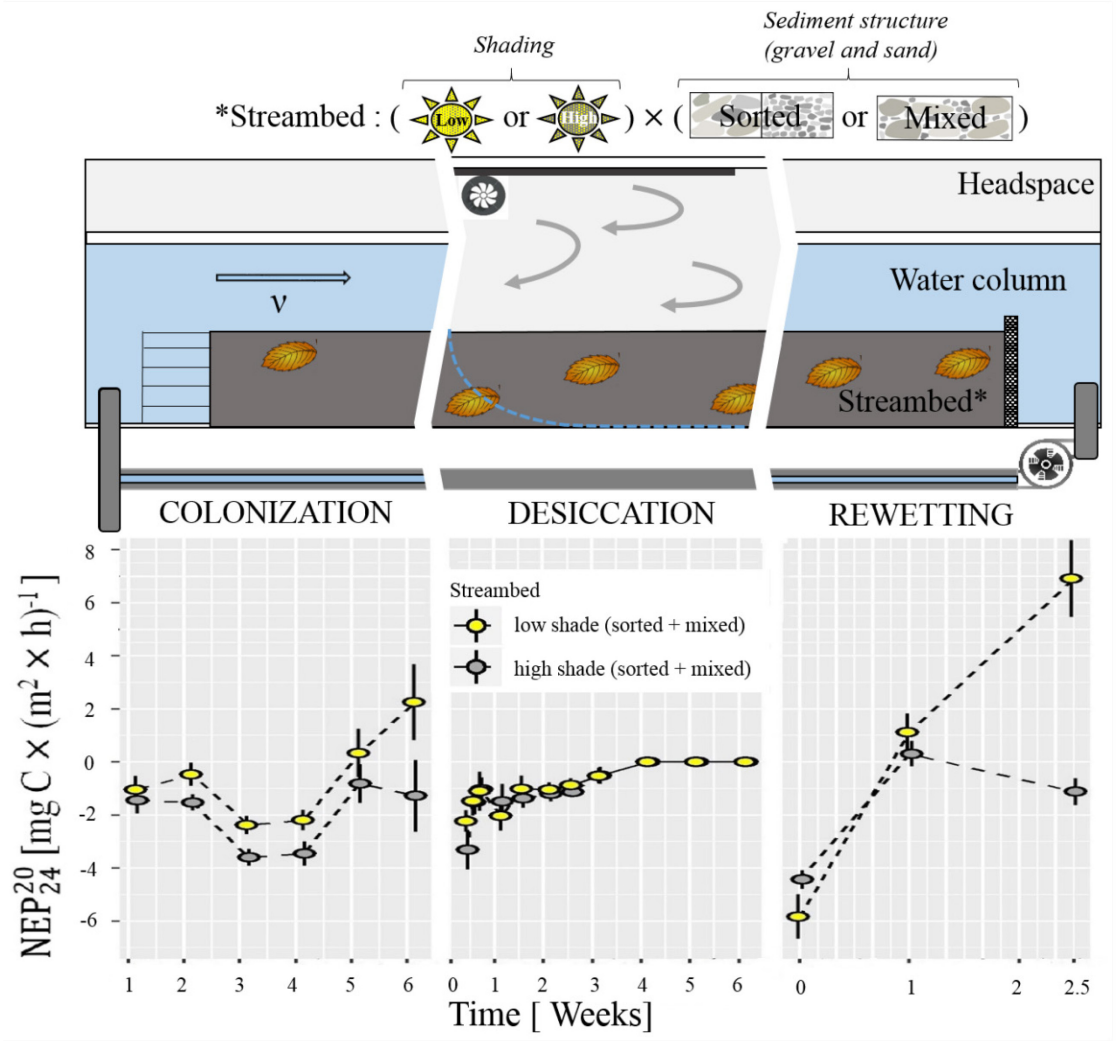
Highlights:

- Shading affects stream metabolism (NEP, CR) resistance and resilience to infrequent droughts.
- Transition to microbial community during drying is shaped by sediment structures.
- Periphyton has capacity to adapt to drying only when light exceeds given threshold.
- The microbial community shifts during drying and rewetting result in decoupled NEP and CR.
- Stream management is key to mitigate future infrequent intermittency under changing climate and land use.

4.1 Graphical abstract

Perennial, temperate, low-order streams are predicted to become intermittent as a result of irregular droughts caused by global warming and increased water demand. We hypothesize that stream metabolism changes caused by irregular droughts are linked to the shading and bed sediment structure of temperate streams. We set up 16 outdoor experimental streams with low or high shade conditions and streambeds either with alternating sorted patches of gravel and sand or homogeneous gravel-sand mix sediment structures. We assessed community respiration (CR), net ecosystem production (NEP) and periphyton biomass and structure (diatoms, green algae, cyanobacteria) in the course of 6 weeks colonization, 6 weeks desiccation, and 2.5 weeks after rewetting. The heterotroph to autotroph (H:A) and fungi to bacteria (F:B) ratios in the microbial biofilm community were assessed at the end of the colonization and rewetting phases. Streams with different bed sediment structure were functionally similar; their metabolism under desiccation was controlled solely by light availability. During flow recession, all streams showed net heterotrophy. As desiccation progressed, NEP and CR decreased to zero. Desiccation altered the periphyton composition from predominantly diatoms to green algae and cyanobacteria, particularly in streams with low shade and mixed sediments. Rapid post-drought resilience of NEP was accompanied by high cyanobacteria and green algae growth in low shade, but poor total periphyton growth in high shade streams. Variable periphyton recovery was followed by increased H:A in relation to shading, and decreased F:B in relation to sediments structure. These shifts resulted in poor CR recovery compared to the colonization phase, suggesting a link between CR resilience and microbial composition changes. The links between drought effects, post-drought recovery, shading level, and streambed structure reveal the importance of low-order stream management under a changing climate and land use to mitigate the future impact of unpredictable infrequent droughts on stream metabolism in temperate ecosystems.



4.2 Introduction

Intermittent streams and rivers, watercourses that cease to flow at some points in time and space, represent 69% of global first-order streams below 60° latitude (Raymond et al. 2013). Most predictions agree that the combined effects of irregular climate patterns (Botter et al. 2013; Reynolds et al. 2015) and increasing water abstraction (Barceló and Sabater 2010; Larned et al. 2010) will cause a shift in many temperate low-order streams from permanent to intermittent flow regimes, with infrequent and brief zero-flow periods and stream bed desiccation (Datry et al. 2017; Krysanova et al. 2010). Hence, unpredictable desiccation will challenge microbial communities and ecosystem functions in temperate streams (Bogan et al. 2015; Lake 2003).

Desiccation and rewetting are increasingly recognized as major stressors of streams ecosystems (Sabater et al. 2016). Species richness and diversity decline significantly in most biofilm microbial communities during prolonged dry periods (Acuna et al. 2015; Pohlon et al. 2013; Rothrock and Garcia-Pichel 2005). Flow resumption in previously dried streambeds causes rewetting that adds additional stress to the sediment community (Romaní and Sabater 1997). Studies of laboratory microcosms with disturbed hyporheic sediments indicate that more intense drying results in more distinct changes and reduction in the microbial community (Marxsen et al. 2010). In streams impacted by desiccation, algal communities react quickly after flow cessation, while heterotrophs take longer to react (Acuna et al. 2015). Given the disparate reactions of communities to drying, autotrophic and heterotrophic dominated ecosystems controlled by the light availability may respond differently to drying stress affecting stream metabolism and its recovery after flow resumption (Timoner et al. 2012).

Impact of drying intensity in a natural streambed is related to sediment structure (Shokri et al. 2010). Natural streambeds are composed of textural patches (grain-size facies) (Buffington and Montgomery 1999a; Buffington and Montgomery 1999b) of variable sediment grain size and hydraulic conductivity (Malard et al. 2002). Such heterogeneous sediment structures support solute supply to sediment associated biofilms (Salehin et al. 2004), and provide metabolic functions (Battin et al. 2003). However, during desiccation, coarser sediment patches become sites of higher initial evaporation rates (Shokri et al. 2010), potentially increasing the impact of drying on benthic communities in sorted coarse bed sediments. In contrast, bed hydraulic conductivity is limited in hydromorphologically degraded streams. The uniformly mixed bed sediments impacted by sand from catchment erosion (Meyer et al. 2008; Wagner et al. 2015), restrict solute supply and, during drought, limit evaporation rates from deeper sediments. Opposing roles of sediment structure on microbial communities and their functions during flow and non-flow periods may have a critical impact on intermittent stream metabolism.

Although it is assumed that drought stress interacts with light availability and bed sediment structure, the response of microbial communities and functions to drought in the complex environment of canopied temperate streams with variable bed sediment structure and shading remains unclear. We examined the functional dynamics of autotrophic and heterotrophic ecosystem metabolism during drying and subsequent rewetting in streams. We focused on the interactions of light availability (shading level) and sediment structure with

stream metabolism resistance and resilience to desiccation and rewetting. While definitions vary (Allison and Martiny 2008; Pimm 1984; Shade et al. 2012), we define resistance as the ability of communities to withstand drying, and resilience as the capacity of communities exposed to drying and rewetting to recover rapidly, and revert to pre-drying ecosystem functions (Acuna et al. 2015; Todman et al. 2016). The hypothesis of this study was that shifts of stream ecosystem functions caused by infrequent and unpredictable droughts interact with the shading and sediment structure of temperate streams. We tested this hypothesis by analyzing 16 experimental streams, varying the light availability and bed sediment structure, and assessing microbial ratios (H:A, F:B), periphyton biomass and structure (diatoms, green algae, cyanobacteria), community respiration (CR), and net ecosystem production (NEP).

4.3 Material and Methods

4.3.1 Experimental set-up

We designed a 2 × 2 factorial experiment comprising 16 outdoor experimental streams, using flumes (400 × 12 × 8 cm) which were filled with a 3 cm layer of sediment (Figure 16). Two streambed structures (both with 30% porosity) were generated through different arrangements of gravel (2–10 mm grain size, d50 = 4.75 mm) and sand (0.2–2 mm grain size, d50 = 0.57 mm), rinsed with 10% hydrochloric acid and washed with groundwater prior to use. A natural streambed (sorted structure) was created by arranging eight alternating patches of gravel and sand, each 45 cm in length, in eight streams. A hydromorphologically degraded streambed (mixed structure) was created by homogenously mixing sand and gravel (50:50 vol.%), which was evenly distributed in the other eight streams. In all streams, 31.25 g C m^{-2} of beech litter was homogeneously distributed in the bed sediments as a source of organic carbon to simulate the leaves deposited in natural streams during spring and summer (Romaní et al. 2013). Additionally, two shade conditions were generated. Low shade was achieved by placing a white, water-resistant polyethylene tent (180 g m^{-2}) above the streams, which reduced ambient light by 25%. High shade was achieved by also placing black net (mesh size 1.2 mm) 10 cm above half of the streams, resulting in a 55% further reduction in light. The combination of both factors, streambed structure and shading, resulted in four different experimental treatments, each in replicates of four: 1) High shade sorted, 2) Low shade sorted, 3) High shade mixed, and 4) Low shade mixed.

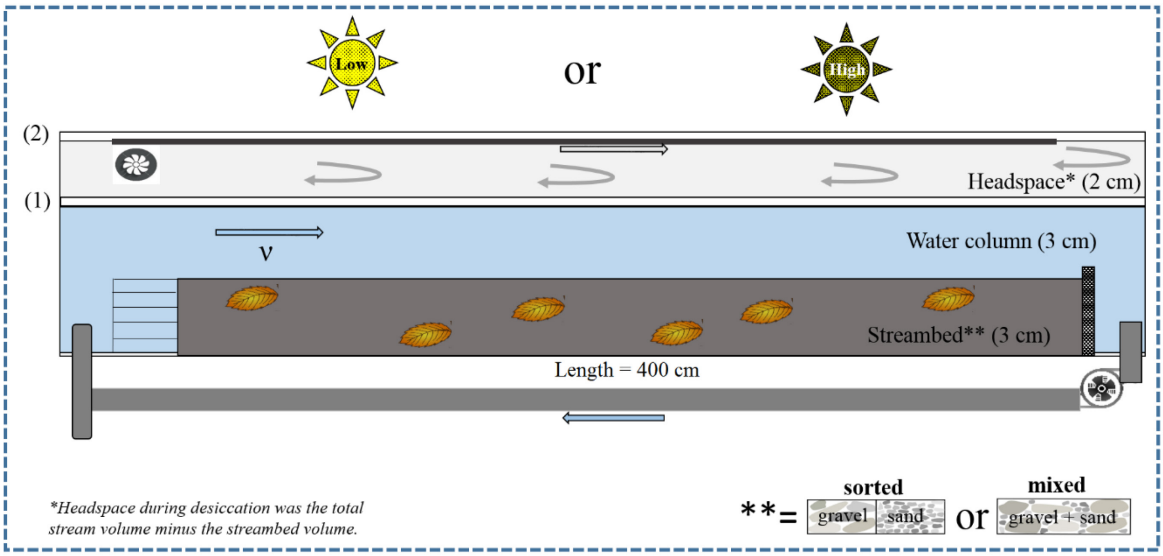


Figure 16 Longitudinal section of the experimental stream (n=16). Eight streams were filled with a 1:1 mix of gravel and sand (mixed) and eight streams were filled with alternating 45-cm long patches of gravel and sand (sorted). Leaves were homogeneously mixed in the sediments. Water was recirculated by pumps and pipes outside of the streams. Four streams of each substrate treatment were exposed to low shade and high shade. Streams were open to the atmosphere except during metabolism measurements when streams were covered by airtight acrylic glass lids that were lowered over the water surface without headspace during colonization and rewetting (1), or fixed to the top of experimental streams allowing headspace above the bed during desiccation (2). For measurements during desiccation, a fan attached to the lid ensured complete mixing of the headspace prior to gas sampling.

The experiment involved 6 weeks of initial colonization, 6 weeks of desiccation, and 2.5 weeks of rewetting. 20 L of water from a local groundwater well (+1.0 mg L^{-1} $N\text{-}NO_3^-$, + 0.2 mg L^{-1} $P\text{-}PO_4^{3-}$, + 0.3ml L^{-1} SL10, Lehman 1980) were added to each stream. Streams were operated as individual recirculation systems (pumps by EHEIM GmbH & Co.KG, Germany) with a constant mean water velocity of 2.54 ±0.03 cm s^{-1} and a water depth of 2.8 ±0.2 cm. Water evaporation during colonization and rewetting under ambient temperature was compensated for by adding deionized water whenever the water level decreased ≥0.5 cm below the initial level. Dissolved nitrogen ($NaNO_3$) and phosphorus ($K_2HPO_4 \cdot 3H_2O$) were added to the water column to maintain nutrient concentrations at a molar ratio of 20:1 whenever depletion was observed due to microbial growth. Concentrations of total organic carbon (TOC), dissolved inorganic nitrogen (DIN), and soluble reactive phosphorus (SRP) were monitored weekly during the colonization and rewetting phase.

Streams were inoculated with a microbial community isolated from the groundwater-fed Waldbach stream, Germany (52°16'N and 14°03'E), a temperate, perennial, first-order stream (average annual precipitation: 576 mm), which last experienced drought in 2003 (Breda et al. 2006). The stream runs through deciduous forest and is fully canopied by beech (*Fagus sylvatica* L.) and alder (*Alnus glutinosa* L.). Its streambed is composed mostly of coarse sand and fine to medium gravel, and is occasionally scattered with rocks. The inoculum was generated from 16 randomly collected 500 ml sediment samples, which included litter and woody particles. Sediment samples were homogeneously mixed and incubated in 8 L of well aerated stream water for 48 h under ambient conditions. Each stream received 500 ml of inoculum suspension, previously screened through a 125 µm mesh to exclude macroinvertebrates and larger organic particles. Colonization of the streams lasted 6 weeks, followed by desiccation and rewetting (Figure 17). The streams were open to the atmosphere and exposed to ambient temperature and atmospheric humidity between May and September, with the exception of incubation periods for O_2 and CO_2 dynamic measurement. Light intensity and water temperature were measured every 10 min by sensors deployed in each stream (ONSET HOBO Pendant® Temperature/Light 64 K Data Logger).

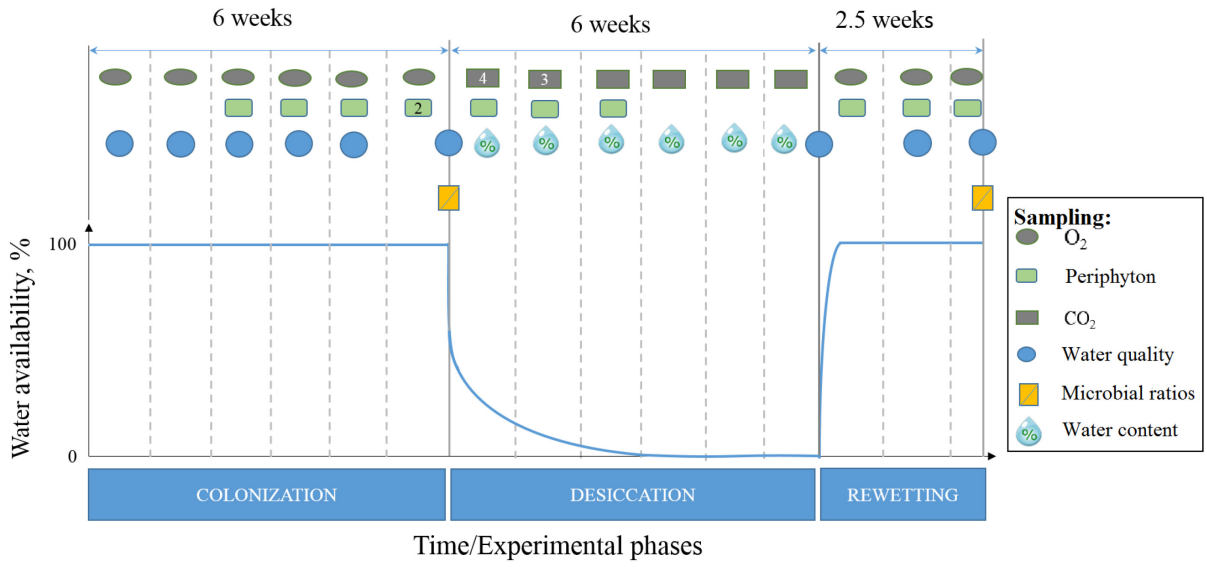


Figure 17 Timeline of the experiment. Blue line represents the water availability in the streams during the three experimental phases. Vertical dotted lines indicate weeks. Numbers within symbols represent the number of samplings each week.

4.3.2 Stream metabolism measurements

We assessed stream metabolism on a weekly basis using day and night dissolved O_2 concentrations during the colonization and rewetting phases. Dissolved O_2 was measured in the water column with multichannel fiber oxygen optodes (Oxy 10 mini; Presens, Regensburg, Germany) at 30 min intervals over a 24 h period. During measurement, we inhibited gas exchange with the atmosphere by sealing each stream from the atmosphere with acrylic glass lids. During the desiccation phase, we assessed stream metabolism by monitoring headspace CO_2 dynamics with a gas analyzer (CCIA, Los Gatos Research, CA, USA). Streams were sealed against the atmosphere with acrylic lids on Neoprene foam (Zellpren) along the edges of the experimental stream and further with silane-terminated polymer adhesive (Terosan, Henkel, Germany) on all connections, allowing ≤4% of air intrusion during the 24 h period. A small tube (0.7 mm diameter), open to the atmosphere and situated on the opposite side of the headspace sampling, prevented pressure reductions while sampling.

Gas from the headspace above the sediment was pumped (650 ml min^{-1}) through a 5 m gas-tight tube for 3 min and automatically measured (10-universal multiport selectors, Vici Valco, Houston, TX, USA) every 3 h over 24 h in each experimental stream. Previous tests revealed that gas diffusion from one side of the stream to the other occurs within 50 min. Therefore, a mini fan (ACT-RX Technology Corporation, Taiwan, air flow capacity 116 L min^{-1}) was installed on the inside of the acryl lid that recirculated the headspace to ensure good mixing prior to gas sampling. The selector-analyzer network was flushed with outside air between each measurement. Before each measurement campaign, we ran a reference gas (0.15% CO_2 in 70% N_2+30 % O_2, Airliquide, Germany) to correct for instrument concentration drifting, yielding a precision of 1 ppm for CO_2 concentration.

Changes in O_2 and CO_2 were converted to metabolic rates according to the following equations:

$$\boldsymbol{CR_{24}} = \left(\frac{\boldsymbol{dC_{treatment}}}{\boldsymbol{dt}}\right)_{night} \times \left(\frac{\boldsymbol{V_{water}}}{\boldsymbol{A_{sed}}}\right) \quad (1)$$

$$\boldsymbol{NEP_{24}} = \left(\frac{\boldsymbol{dC_{treatment}}}{\boldsymbol{dt}}\right)_{day} \times \left(\frac{\boldsymbol{V_{water}}}{\boldsymbol{A_{sed}}}\right) - \boldsymbol{CR_{24}} \quad (2)$$

where $C_{treatment}$ is oxygen (mg L^{-1}) or carbon dioxide concentration (ppm), t is time (h), *night* and *day* represent the dissolved O_2 and CO_2 concentration data set used during nighttime

(between 10 pm and 4 am, sunset and sunrise, respectively) and daytime (between 4 am and 10 pm) incubation, V_{water} is the total water volume (L) in the experimental streams, and A_{sed} is the stream bed surface (m^2). The rates were expressed as carbon equivalents using a respiratory quotient of 0.85 (Del Giorgio and Peters 1994) and standardized to 20ºC using a temperature coefficient Q_{10} of 2. Both CR_{24}^{20} and NEP_{24}^{20} were expressed as daily values.

4.3.3 Periphyton biomass and structure

We monitored periphyton biomass and structure at eight sites in each experimental stream each week throughout the experiment using an *in vivo* fluorometer (Bentho Torch, bbe-Moldaenke, Germany). The Bentho Torch quantified total algal biomass via chlorophyll a content, and differentiated between cyanobacteria, green algae, and diatoms based on pigment fluorescence. The method allowed repeat measurements and avoided extensive sediment disturbance that would have altered drying by creating large voids. The relative proportion of cyanobacteria, green algae, and diatoms in the total algal biomass was further validated by microbial biomarkers (PLFA) specific to the three algal typesfrom sediment samples collected at the end of colonization and rewetting phases. To avoid interference of ambient light with the instrument light beam and prevent disruption of the upper sediments, the Bentho Torch was positioned 1–2 mm above the sediment by a carrier that allowed sliding of the instrument along the stream channel. Near-bed hydraulic disturbance of the sediment by the Bentho Torch was avoided by stopping the flow during measurement (15 min).

4.3.4 Microbial ratios in the benthic community

After the 6 week colonization phase, and at the end of the experiment, the uppermost benthic sediments were sampled randomly using a laboratory spoon, and immediately stored at -20 °C until further processing. Microbial ratios of the sediment microbial community (heterotroph to autotroph, H:A, and fungi to bacteria, F:B) were obtained by analyzing phospholipid fatty acids (PLFAs) (Frostegard et al. 1991; Steger et al. 2011) extracted from 60 to 70 g of lyophilized sediment. PLFAs are present in the membranes of all living cells and rapidly degrade to neutral lipids upon cell death (Willers et al. 2015); hence, the method allows for sensitive and reproducible measurements characterizing viable microbial communities (Boschker and Middelburg 2002; Weise et al. 2016). After extraction, PLFAs were separated from other lipids on silicic acid columns (BondElut®LRC-Si, Altmann Analytic, Germany) using solid-phase extraction, and methylated using mild alkaline methanolysis. The resulting fatty acid methyl esters were quantified on a GC-IRMS system (Agilent 6890, Agilent 5973, Germany). The Bayesian mixed model (FASTAR) (Galloway et

al. 2014; Strandberg et al. 2015) was used to calculate the relative occurrence of autotrophs, fungi, and heterotrophic bacteria from biofilm communities obtained from the PLFA sample profiles. H:A and F:B ratios were calculated by relating the occurrence of heterotrophs to autotrophs and fungi to bacteria, respectively, in the total community biomass.

4.3.5 Water quality parameters

Weekly sampled water was analyzed for soluble reactive phosphorus (SRP), dissolved inorganic nitrogen (DIN: NO_3^-+ NO_2^- +NH_4^+), and total organic carbon (TOC). Water samples for SRP and DIN were pre-filtered (Cellulose Acetate, 0.45 µm, Whatman, Germany) to remove all particles. SRP was analyzed photometrically with a UV/VIS-Photometer CARY 1E (VARIAN, Darmstadt, Germany) following a standard method (DIN EN ISO 11732) (Murphy and Riley 1962). DIN was measured with a CFA-Photometer Skalar SAN (Skalar Analytical B.V., Breda, The Netherlands) according to DIN EN 1189. TOC was analyzed with a TOC autosampler (TOC-L CPN, Shimadzu, Japan).

4.3.6 Sediment hydraulic characteristics, drying, and rewetting

We measured vertical water flux (VWF) between the water column and the sediment at the beginning of the colonization phase to determine the hydraulic properties of the streambed. We added uranine to the water column of each experimental stream (3 µg L^{-1} final concentration). Surface water was uniformly colored with uranine after 15 min (based on previous tests). A field fluorometer (LLF-M Fiber-Optic fluorometer; Gotschy Optotechnik, Adnet, Austria) measured the decrease in uranine concentrations by measuring the exchange of colored surface water with uncolored water from the sediment pore space, allowing calculation of the vertical water exchange (Mutz et al. 2007). Before desiccation, surface and free sediment pore water were drained from the experimental streams by elevating one side of the stream by 5 cm for 30 min, after which the streams were returned to their horizontal position. The remaining water was evaporated from the moist sediment under ambient temperature and humidity in July and August.

We measured the pore water loss during desiccation in two additional streams, one for each sediment structure, that were weighed daily, and then calculated the gravimetrical water content. When no difference in weight was observed by weighing entire streams, transects of moist sediments were sampled instead, dried at 105 ºC for 48 h, and the gravimetric water content was calculated. Streams were rewetted by simulating a heavy rain shower (23 mm) on the dry streambed. Low nutrient groundwater was sprayed from watering cans for 20 min on

the streambed, taking care not to destroy the sediment surface. After pores were filled and the bed was covered by a thin film of water, we stopped rewetting to simulate a short period (3 h) without strong surface flow, as observed in streams rewetted by rain in nature (Datry et al. 2014). Then, the water column was filled to a defined depth, and flow was restored.

4.3.7 Statistical analysis

We applied a linear mixed-effects (LME) model to relate the observed differences in stream metabolic function (CR, NEP) and periphyton between experimental phases (colonization, desiccation, and rewetting) to levels of shading and sediment structure. The fixed-effects influence in the model was set as the interaction related to each treatment (four levels) and sampling time (the number of levels depended on parameter and phase: six, eleven and three levels for CR and NEP during colonization, desiccation, and rewetting, respectively; and five and three levels for periphyton during colonization and during desiccation and rewetting, respectively). For the random-effects influence in the response, we allowed different intercepts for each stream in the model. We compared CR, NEP and periphyton of different treatments between experimental phases, running an additional LME without sampling time in a fixed structure of the model. H:A ratio, F:B ratio, and water quality parameters were compared only between the colonization and rewetting phase. Each LME was followed by model validation to check residuals for normal distribution and homogeneity of variances. The statistical significance of relationships was tested using a likelihood-ratio test by comparing the model with and without the interactions between factors. The LMEs were followed by a conservative Tukey's post-hoc test to examine significant differences between treatments. All statistical analyses were performed in the statistical software R, using the packages lme4, MASS, and lsmeans, at a significance level of $P \leq 0.05$.

4.4 Results

4.4.1 Community respiration (CR) and net ecosystem production (NEP)

CR and NEP showed a significant relationship to shading during all phases (Figure 18, CR: χ^2_{col} = 12. 08, p<0.001, χ^2_{des} = 5. 27, p = 0.02, χ^2_{rew} = 4. 92, p=0.03; NEP: χ^2_{col} = 4. 78, p = 0.03; χ^2_{des} = 103. 82, p < 0.001; χ^2_{rew} = 25.53, p < 0.001), and were strongly affected by drying (pairwise t-test, p < 0.001) and rewetting (p < 0.001). CR decreased more (60%) in high shade streams than in low shade streams (40%) after flow cessation (p < 0.001). NEP and CR decreased to near-zero after 20 days' drying. After flow resumption, CR peaked within a few hours and declined thereafter to lower than before drying (p < 0.0001). NEP

recovered slowly, starting from similar low values in all experimental streams ($p = 0.2$), but reaching higher levels in high shade and similar levels in low shade streams than before desiccation ($p < 0.0001$).

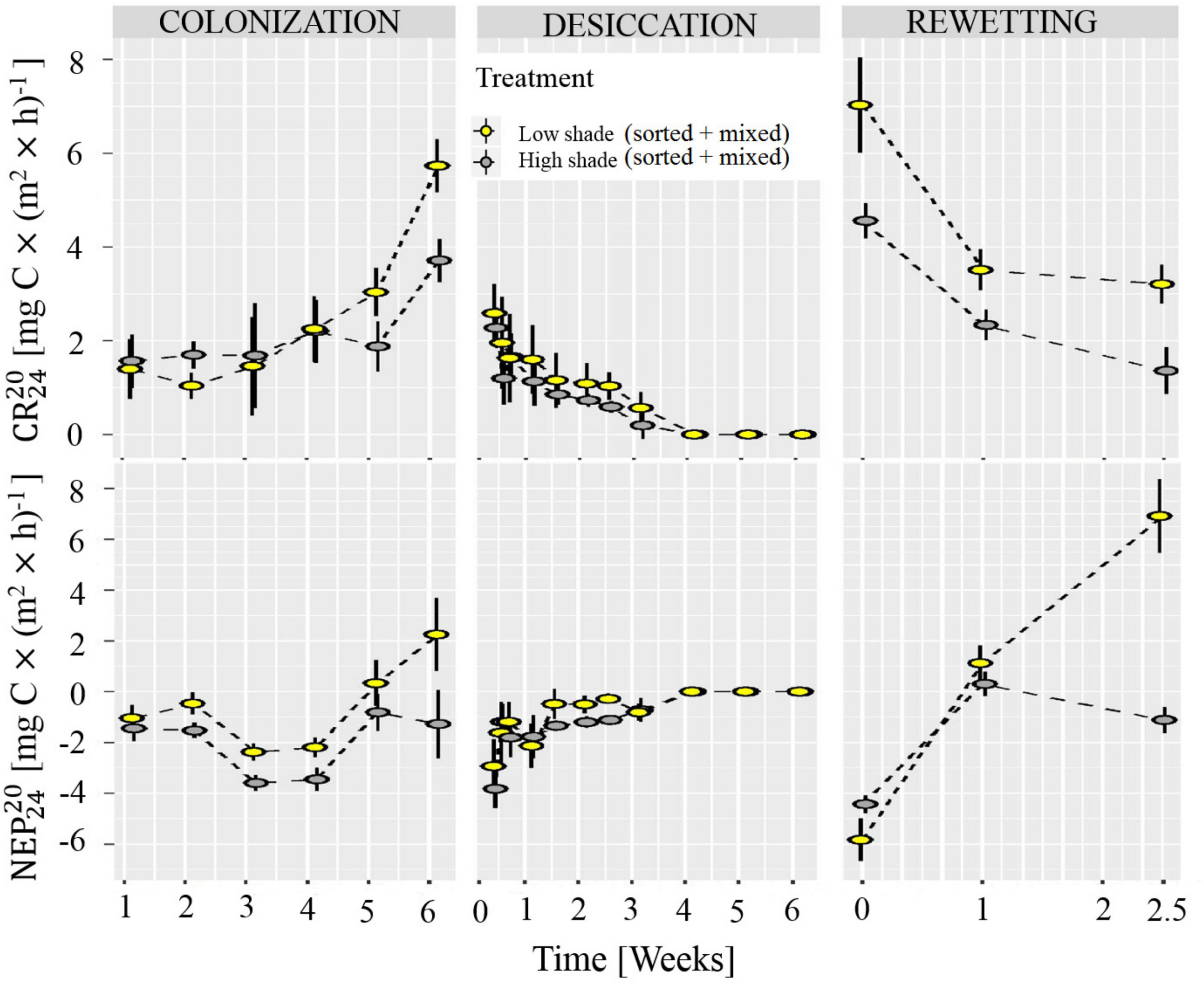


Figure 18 Dynamics of daily community respiration (CR_{24}^{20}) and daily net ecosystem production rates (NEP_{24}^{20}) for low shade (yellow filled markers) and high shade streams (grey filled markers). Values are means (± SD, n = 8) within each experimental phase (colonization, desiccation, and rewetting).

4.4.2 Periphyton biomass and structure

Periphyton biomass was significantly related to shading in all phases (Figure 19, $\chi^2_{col} = 32.82$, $p < 0.0001$; $\chi^2_{des} = 23.24$, $p < 0.0001$; $\chi^2_{rew} = 37.11$, $p < 0.0001$). During colonization, algal biomass was doubly high ($p < 0.0001$) in the low shade stream. During the first week of desiccation, total biomass in all streams changed little, but decreased in the subsequent two weeks (high shade: $p = 0.006$, low shade $p = 0.03$). After rewetting, the total periphyton biomass in low shade streams was similar to pre-desiccation levels ($p = 0.61$), but lower in streams with high shade ($p < 0.0001$).

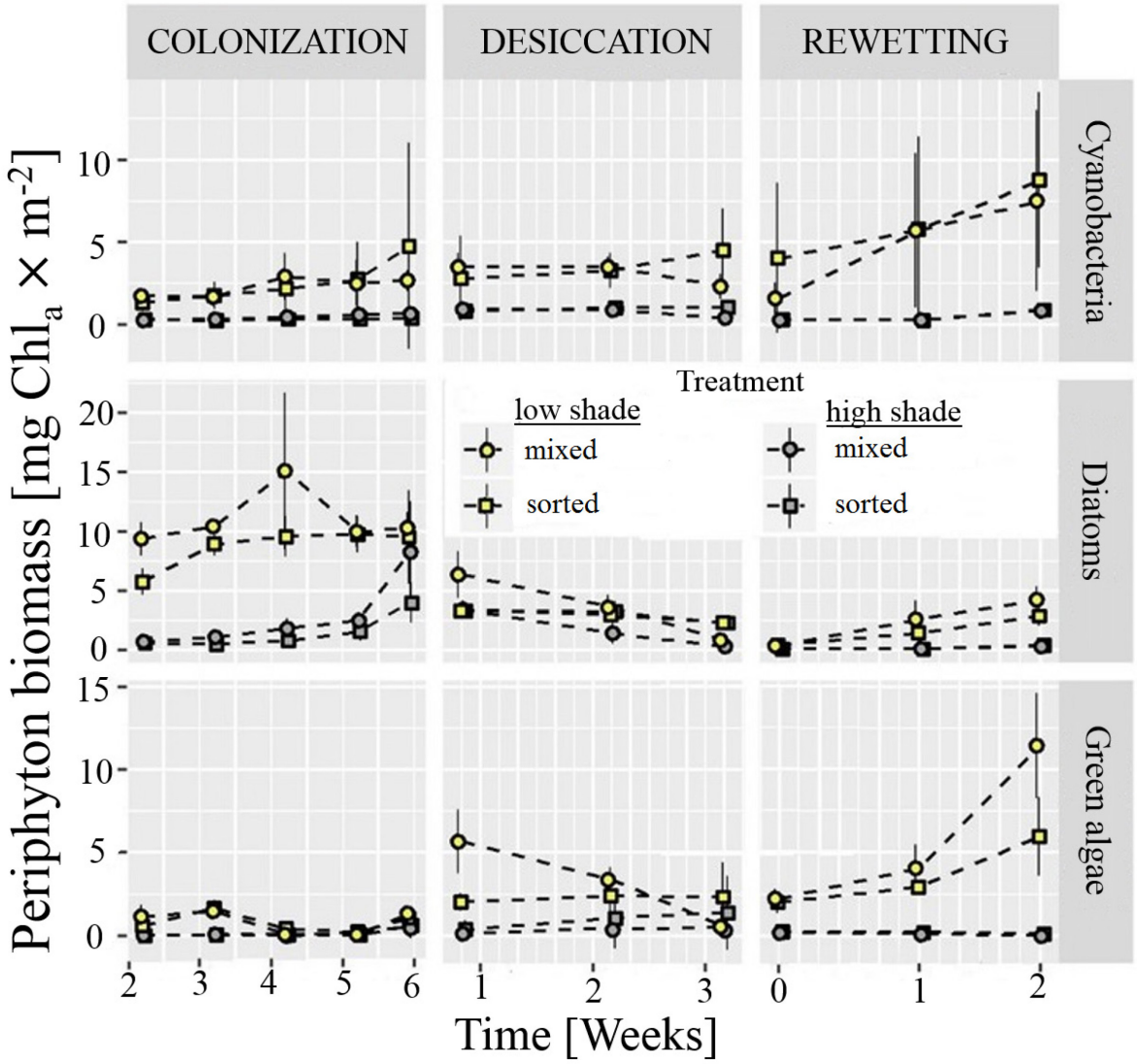


Figure 19 Dynamics of cyanobacteria, diatom, and green algae biomass for low shade (yellow filled markers) and high shade streams (grey filled markers). Squares and circles represent periphyton biomass in streams with sorted and mixed bed sediment, respectively. Values are means (± SD, n = 4) within each experimental phase (colonization, desiccation, and rewetting).

Shading affected all periphyton groups during all experimental phases ($p < 0.0001$) (Figure 19). Biomass increased within each group ($\chi^2_{cyano} = 32.82$, $p < 0.0001$; $\chi^2_{diatom} = 27.89$, $p < 0.0001$; $\chi^2_{green} = 7.63$, $p = 0.006$) in low shade streams compared to high shade streams (Figure 19). In streams with more light, desiccation caused a shift among periphyton groups related to sediment structure. During the first days of desiccation, diatoms decreased significantly ($p < 0.0001$) and green algae increased ($p = 0.003$), recovering to stable levels similar to pre-desiccation. However, after the first week of drying, cyanobacteria increased ($p = 0.02$) and green algae decreased ($p = 0.001$), irrespective of sediment structure.

Diatoms were affected by interactions between sediment structure and shading ($\chi^2_{des} = 9.29$, $p = 0.0023$). In mixed sediment, their biomass continued to decrease ($p < 0.0001$) regardless of shading, while in sorted sediments, biomass remained constant during the entire desiccation period. Shading was also a dominant factor after flow resumption. Under low shade conditions, the biomass of all groups steadily increased, and in the case of cyanobacteria and green algae, rose to higher levels than before drying ($p < 0.0001$). However, growth of cyanobacteria and green algae was faster than that of diatoms, which did not return to pre-desiccation biomass levels ($p < 0.0001$), leading to a shift in biomass dominance during recovery towards green algae. Under low light, growth of all groups was considerably reduced, and biomass was much lower than before desiccation. Consequently, the shift among groups was clear and mainly caused by the substantial decrease of diatoms during desiccation.

4.4.3 Microbial ratios in the benthic community

The heterotroph to algae ratio (H:A) was significantly related to shading (Figure 20, $\chi^2 = 24.53$, $p < 0.0001$) at the end of the colonization and rewetting phases, and was higher in high shade streams ($p < 0.0001$), regardless of sediment structure. After rewetting, the H:A ratio for both shading treatments was higher than at the end of colonization ($p < 0.001$). The fungi to bacteria ratio was significantly related to sediment structure (Figure 20, $\chi^2 = 10.82$, $p < 0.01$) at the end of the colonization and rewetting phases, and was lower in sorted streams ($p = 0.01$), irrespective of shading level. After rewetting, the F:B ratio was lower in all streams ($p = 0.002$) than at the end of colonization.

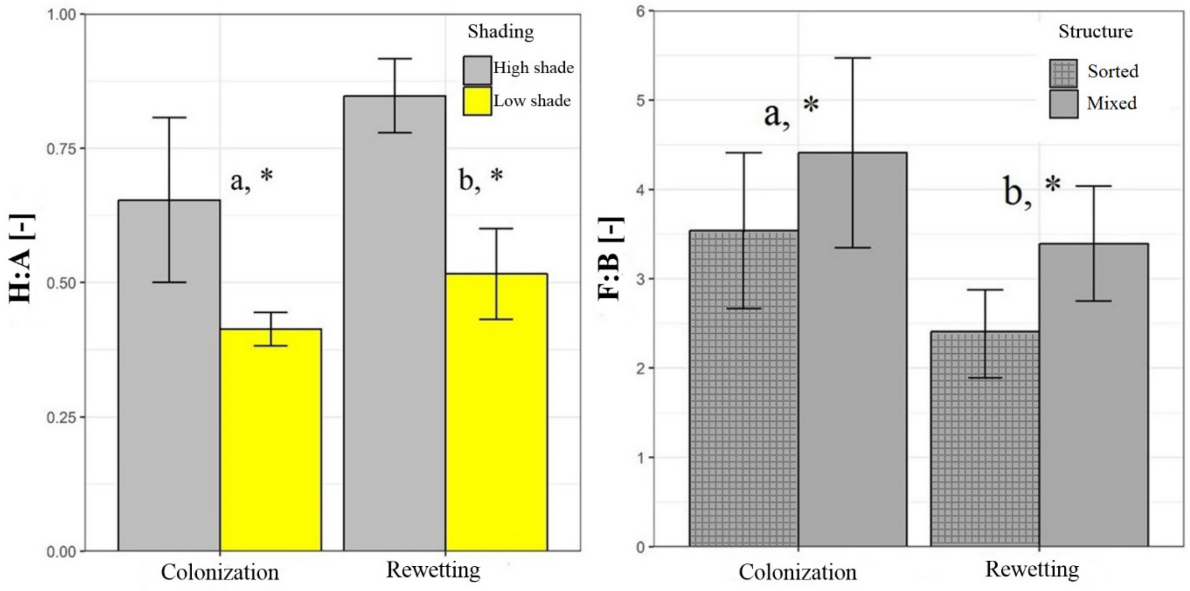


Figure 20 Ratio of the heterotroph to autotroph biomass (left) for high shade (grey) and low shade streams (yellow), and the fungal to bacterial biomass (right) for sorted (mesh pattern) and mixed bed sediments (grey). Values are means (± SD, n = 8) at the end of the colonization and rewetting phases.

4.4.4 Water quality parameters

Table 1 shows the differences in the main water parameters between treatments. TOC was significantly related to experimental phase ($\chi^2_{phase} = 10.8$, $p < 0.01$), and was higher upon flow resumption than during the colonization and rewetting phases ($p<0.001$), that were similar. SRP was significantly related to shading ($\chi^2 = 10.8$, $p < 0.01$); high shade streams had higher SRP than low shade streams during colonization and rewetting phases ($p_{col} < 0.001$, $p_{rew} < 0.0001$). SRP upon flow resumption was higher than during the colonization phase in all streams ($p < 0.0001$). DIN was significantly related to shading ($\chi^2 = 36.6$, $p < 0.0001$) and sediment structure ($\chi^2 = 7.1$, $p = 0.03$). Low shade had lower DIN than high shade streams during colonization and rewetting phases ($p_{col} = 0.03$, $p_{rew} < 0.0001$). DIN upon flow resumption was lower in mixed streams than in sorted streams for both shading levels ($p_{low} < 0.001$ and $p_{high} = 0.04$).

Table 6 Environmental and water quality parameters of experimental treatments. T_{avg} is the mean daily temperature, PAR is photosynthetically active radiation and represents a mean daily value. TOC, DIN, and SRP are total organic carbon, dissolved inorganic nitrogen, and soluble reactive phosphorus in surface water, respectively. VWF is the vertical water flux at the beginning of the colonization phase. Water quality parameters are averages for the colonization and rewetting phase. Flow resumption water quality parameters are for the day of flow resumption. All values are means (± SD, n = 4/n = 8).

Experimental phase	Parameters	Low shade		High shade	
		Sorted	**Mixed**	**Sorted**	**Mixed**
Colonization	**Tavg [°C]**	16.5±0.1		16.3±0.1	
	PAR [µmol/m²/s]	44.2±4.4		26.2±1.0	
	VWF[a] [dm³/m²/h]	4.22±0.92	3.73±0.83	4.22±0.92	3.73±0.83
	TOC [mgC/L]	7.31±0.10	7.41±0.41	8.20±0.21	7.71±0.25
	DIN [mgN/L]	0.20±0.10	0.27±0.04	0.46±0.13	0.48±0.07
	SRP [µgP/L]	9.0±4.8	11.5±4.1	88.8±35.5	87.9±34.5
Desiccation/Flow resumption[b]	**Tavg [°C]**	21.7±0.2		21.4±0.2	
	PAR [µmol/m²/s]	63.0±14.7		34.9±5.1	
	TOC [mgC/L][b]	12.79±2.00	13.71±4.00	9.82±0.46	10.2±1.10
	DIN [mgN/L][b]	0.12±0.03	0.09±0.02	0.25±0.11	0.13±0.03
	SRP [µgP/L][b]	109.9±30.8	125.93±30.49	161.8±42.9	207.95±23.01
Rewetting	**Tavg [°C]**	16.5±0.0		16.6±0.1	
	PAR [µmol/m²/s]	26.1±5.6		17.0±2.0	
	TOC [mgC/L]	7.58±0.86	7.55±0.32	7.72±0.76	7.84±0.56
	DIN [mgN/L]	0.10±0.04	0.06±0.02	0.68±0.16	0.53±0.17
	SRP [µgP/L]	5.6±2.5	4.2±0.3	136.5±31.6	113.6±12.4

[a] The pore water of the gravel patches in the sorted bed sediments was exchanged within 15 min due to an order of magnitude higher hydraulic conductivity (0.77 cm/s), pore water velocity (2 mm/s) and dispersion (5.75×10^{-4} cm²/s) in these patches compared to sand patches. Therefore, in streams with a sorted sediment structure the measured pore water exchange was VWF only in the sand patches.

[b] Values are representative for the flow resumption situation.

4.4.5 Vertical water flux (VWF) and sediment water content

VWF was higher in streams with sorted sediments, regardless of shading and experimental phase ($p < 0.0001$, Table 1). Water drainage prior to natural desiccation reduced the sediment water content to 72% and 58% of sediment saturation (226.9 mg H_2O g^{-1} DM_{sed}) in mixed and sorted sediments, respectively (Figure 21). Both sediment structures lost the largest amount of water during the first week (93 and 60 mg H_2O g^{-1} DM_{sed} in mixed and sorted bed sediment, respectively). The differences in water loss between treatments were smaller in the second week (64 and 58 mg H_2O g^{-1} DM_{sed} in mixed and sorted bed sediment, respectively), while differences during the third and following weeks were ≤3%.

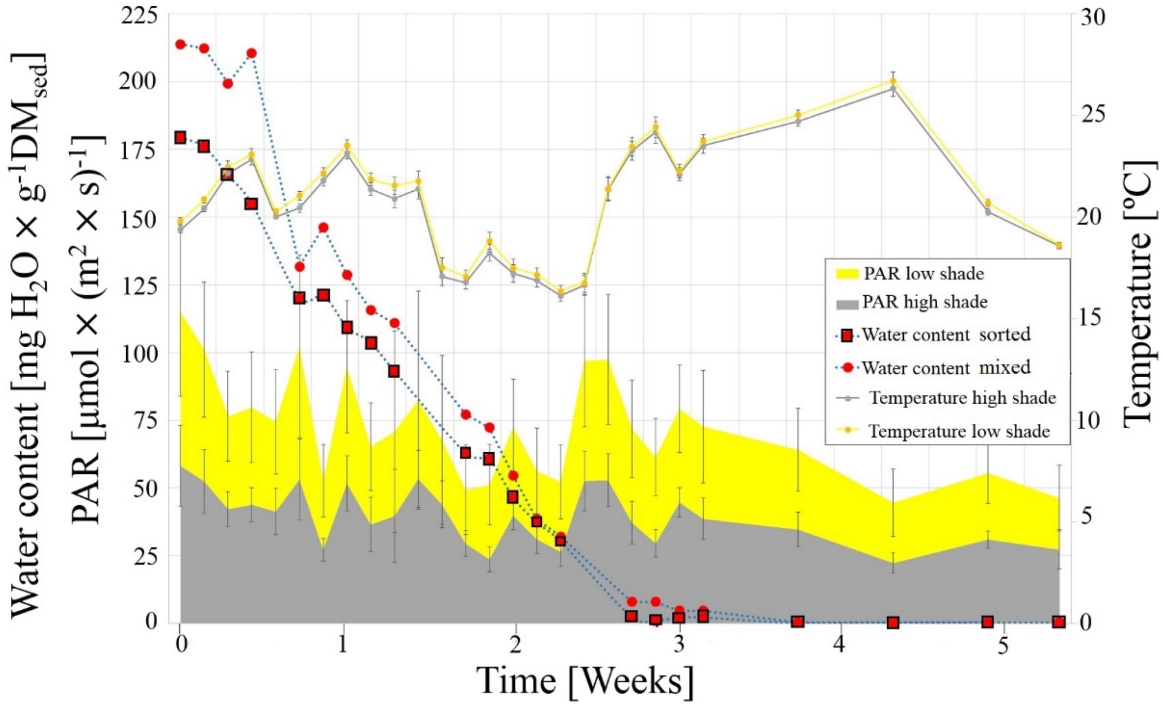


Figure 21 Water content, sediment temperature, and photosynthetically active radiation (PAR) in the experimental streams during desiccation.

4.5 Discussion

4.5.1 Effect of light availability on stream metabolism resistance and resilience

We traced stream metabolism changes from periods of low flow to desiccation, subsequent rewetting, and flow resumption, and tested the relationships between the observed dynamics sediment structure, and shading. As expected streams with low shade became net autotrophic during colonization, while those with high shade remained net heterotrophic. Shade-affected streams were not influenced further by bed sediment structure (Figure 18),

confirming the strong control of light over both NEP and CR prior to disturbance (Wagner et al. 2015). Desiccation can induce changes in the relationships between autotrophic and heterotrophic metabolic processes, causing trophic cascades, and shifting carbon sources and major pathways of energy flow (Power et al. 2008). Just as photosynthetic activity decreases substantially shortly after desiccation (Wyatt et al. 2014), NEP rapidly and sharply declined in our study, shifting stream metabolism to net heterotrophy. The rapid NEP and CR decrease during the first days of desiccation is due to the mortality of benthic communities (Acuna et al. 2004; Castenholz and Garcia-Pichel 2000), dominated mainly by algal biomass (54% in high shade and 58% in low shade streams). Most drying streams support desiccation-adapted algal communities, like those that remain dormant on dry streambeds until flow resumes, enabling quick reactivation. However, unexpected and rapid drying due to summertime water diversion for agriculture (Barceló et al. 2010) may prevent resistance structures forming (e.g., spores, thickened cells) (Stanley et al. 2004); hence random resistance processes then become important, causing fast, harmful changes to stream metabolism (Holling 1973; Peterson 1987).

Progressed desiccation and consequent low moisture content (from 72% and 58% in mixed and sorted streambeds, respectively, after flow recession to <5% after 20 days in all streams) diminishes the ability of microbial communities to use water as a transport and reaction medium. Consequently, nutrients required to sustain microbial metabolism are limited (Schimel et al. 2007), resulting in low and eventually decreasing metabolic rates, observed in both NEP and CR during the desiccation phase (Febria et al. 2012). In parallel, this situation promotes structural and metabolic adaptations to maintain basic ecological functions (Moyano et al. 2013), which are further controlled by environmental factors (light, temperature, water content). We observed that higher NEP and CR rates in desiccated low shade streams were generated by periphyton activity, which shifts to types with lower nutrient requirements only in streams with higher light availability (Figure 20). Consequently, our study reveals that the sensitivity of periphyton communities and hence metabolic functions to drought is modified by light availability, implying that it is a strong mediator of metabolic resistance to drought stress in canopied streams.

Following flow resumption, CR peaked within a few hours in all experimental streams (Figure 18). Extracellular enzymes have been reported to resist sediment desiccation (Marxsen et al. 2010; Pohlon et al. 2013), and activity pulses that alleviate microbial processes from moisture limitations after initial rehydration have previously been recognized

(Timoner et al. 2014c; Baldwin and Mitchell, 2000). Accumulation of organic matter and nutrients in a drying streambed has previously been reported and attributed to biomass senescence and low mineralization rates in the dry sediments (Amalfitano et al. 2008; Humphries and Baldwin 2003). Rapid mobilization of such readily available organic matter and nutrients from the dry bed sediments is reflected in the elevated TOC and SRP values of the re-established circulating water (Table 1). Microbial cells could rapidly shift out of dormancy (Schimel et al. 2007) using these nutrients, resulting in the initial pulse of CR. CR rates leveled out in the two weeks following rewetting (Figure 18), suggesting a link between respiration recovery and the growth of communities after the initial pulse (Meisner et al. 2015). Growth of periphyton after rewetting was again positively related to light (Figure 19), and probably also supported higher CR in lower shade streams (Figure 18). However, for the 2.5-week rewetting period, CR remained lower than pre-desiccation levels while NEP recovered fast and even exceeded pre-drying levels in low shade streams. This further implies a distinct resilience of photosynthetic and respiration activities after rewetting (Figure 18), and suggests a shift in community composition with desiccation.

4.5.2 Effects of light availability and sediment structure on shifts in microbial communities during desiccation and rewetting

Drying can reduce microbial biomass and eliminate species because few are able to resist drying. In our study, streambed desiccation reduced periphyton biomass by 70%, compared to 60–90% of active chlorophyll loss during sediment desiccation in air-dried sediments (Qiu and McComb 1995). Interactions between shading and sediment structure influenced periphyton biomass structure shifts during desiccation and after rewetting (Figure 19). In low shade streams, rapid decreases in dominant diatom biomass (Cardinale et al. 2002; Steinman et al. 1992) within the first week of desiccation were accompanied by an increase in green algae biomass, suggesting interspecies competition for nutrients in periphyton communities. In comparison to diatoms, which are more competitive for nutrients during early colonization (Stevenson 1983), late colonists such as green algae and cyanobacteria have a high affinity for limiting nutrients (Peterson and Grimm 1992). These features are advantageous when the availability of nutrients for algal cells was limited to capillary diffusion (Haghighi et al. 2013) due to the lack of nutrient transport during flow recession (Figure 19).

Progressive drying of low shade streams caused further decreases in diatoms related to the sediment structure. While the biomass of diatoms in mixed bed sediments continually

decreased, in accordance with the initially higher drying intensity found in these streams (Figure 21), it remained constant with desiccation in the sorted bed sediments. Capillary water from the shallow hyporheic zone of sand patches serves as a refuge for diatoms seeking wet areas (Minckley and Marsh 2009) after rapid drying of the gravel patches. Lower DIN and SRP concentrations after rewetting in sorted streams (Table 1) with low shade further imply longer community persistence in sorted bed sediments where autotrophic and heterotrophic communities preserve their activity by consuming these nutrients (Minckley and Marsh 2009). In drying streams diatoms are generally limited to a few species (Tornés and Ruhí 2013), whereas the most desiccation-resistant species are primarily green algae and cyanobacteria (McKew et al. 2010). All algal groups' biomass decreased during desiccation in high shade streams, and no community shift was observed (Sabater et al. 2000). The increase of cyanobacteria in low shade streams reflects their better structural adaptation to desiccation with higher light availability. The ability of green algae and cyanobacteria to persist with decreasing moisture is positively related to their ability, driven by light availability, to recover their optimum hydric conditions (Timoner et al. 2012). For instance, scytonemin occurs in the extracellular polysaccharide sheaths of cyanobacteria (Garcia-Pichel and Castenholz 1991), and has been observed in microbial mats (Fernandez-Valiente et al. 2007) and soil crust biofilms exposed to desiccation and high solar radiation (Belnap and Lange 2001). This pigment helps communities resist desiccation and recover faster after rewetting (Romaní and Sabater 1997).

The rewetting phase in our study was indeed accompanied by a further increase in cyanobacteria, fast recovery of dormant green algae, and poor recovery of diatoms in low shade streams. Conversely, periphyton biomass remained the same as that during desiccation in high shade streams. The dry phase has lasting effects on communities in subsequent hydrological phases, causing a lagged response in algal diversity and biomass (Datry et al. 2017). The length of these effects is further controlled by light availability after flow resumes. The lower F:B ratio after rewetting in sorted sediments than mixed bed sediments (Figure 20) indicates the significance of the preceding drying intensity also for heterotrophic recovery (Acuna et al. 2015; Marxsen et al. 2010), which has also been found in other studies (Amalfitano et al. 2008; Chauvet et al. 2016; Tzoraki et al. 2007). While both bacterial and fungal communities seek refuge in the hyporheic zone when surface flow ceases, conditions for fungal development in this habitat are suboptimal. For instance, dissolved oxygen concentrations are low, especially in the longer wet sandy patches of the hyporheic zone without water flow, oxygen demand (Field and Webster 1983; Medeiros et al. 2009) is high,

and hence the biomass and diversity of aquatic hyphomycetes are much lower in the hyporheic zone than in the benthic zone (Bärlocher et al. 2006; Cornut et al. 2010; Sudheep and Sridhar 2012), therefore making benthic fungi less resistant to desiccation. Consequently, drying conditions favor cyanobacteria and green algae (Ylla et al. 2010), and change the occurrence of fungi and bacteria in biofilms. Moreover, the capacity to establish this resilient transient community during desiccation is strongly related to drying conditions, light availability and sediment structure, and promotes varying resistance to desiccation among transient aquatic species (Lake 2003).

4.5.3 Decoupled resilience of stream metabolism as consequence of microbial shifts

Despite microbial shifts in the benthic zone during desiccation and rewetting, stream metabolic functions were highly resilient, and their recovery increased with increased light conditions. However, differing recovery levels of NEP (full recovery) and CR (50% recovery), imply decoupled resilience of metabolic activities after rewetting (Figure 18). Other studies also show that photosynthetic efficiencies can increase with rewetting (Timoner et al. 2012), and prolonged desiccation can induce shifts in dominance to taxa with a physiological features adapted to desiccation conditions (Minckley and Marsh 2009) but with different metabolic rates (Steinman and McIntire 1987). Hence, the dominance of green algae in the rewetting period, which are shown to have higher photosynthetic efficiency (≈ 0.83) (Büchel and Wilhelm 1993; Flameling and Kromkamp 1998; Koblizek et al. 2001), promoted photosynthesis in streams compared to the colonization phase where diatoms dominate (photosynthetic efficiency usually < 0.75). Although CR was found to recover fully within 10–14 days after desiccation and rewetting in isolated sandy sediments incubated under laboratory conditions (Pohlon et al. 2013), good recovery of periphyton biomass (Figure 19), together with the increase in H:A and decrease in F:B (Figure 20) after rewetting, was not accompanied by full CR resilience. The lower F:B ratio at the end of rewetting than during colonization may have decreased leaf mineralization rates (Malik et al. 2016), reflected in poor CR resilience two weeks after rewetting in all streams. Moreover, improved nutrient supply in sorted streams, which promotes stream metabolism (Boulton 1998; Edwards 1998) likely compensated for higher mineralization rates (Malik et al. 2016) induced by the higher F:B ratio in mixed streams. This compensation generated similar CR resilience between different sediment structures, whose rates of recovery were modulated by periphyton biomass and structure recovery mediated by shading after rewetting. This shows that respiration resilience is linked to the balance of autotrophs' and heterotrophs' biomass and structure which are controlled by light availability and sediment structure in intermittent streams.

4.5.4 Implications of infrequent droughts for low-land temperate streams

Our experiment was designed to compare the responses of moderate and well-canopied temperate streams with different sediment structure to infrequent droughst. We showed that streams with a naturally sorted bed structure, and streambeds degraded by sand input are functionally similar, and their metabolism under desiccation is strongly controlled by light availability. However, community composition changes during desiccation, which are responsible for the recovery of ecosystem functions after flow resumption, are shaped by variable sediment moisture due to differing evaporation from the two sediment structures. The after rewetting restructured periphyton biomass, increased H:A driven by light availability, decreased F:B ratio linked to sediment structure, and decreased CR, indicate that unpredictable and infrequent droughts in lowland streams may substantially alter microbial resistance and resilience, affecting organic matter mineralization. Photosynthesis recovery limits heterotrophy to the desiccation period and to the first week after flow resumption. The shifted balance of ecosystem processes between desiccation and rewetting however might imply that the biogeochemical changes induced by predicted climate change and increased water withdrawal, light availability and sediment structure of intermittent streams and rivers, underlie ecosystem function resistance and resilience. Given estimates on the increasing number of intermittent streams and rivers, this study have significant benefits for the future management of intermittent streams under a changing climate and land use, particularly for the prediction of short and long-term stream responses to infrequent droughts in temperate streams.

Acknowledgments

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5. Final discussion and conclusions

The present doctoral thesis examined microbial C-transformation influenced by the environmental stresses of sediment transport and drought, two of the land use- and climate-related factors that directly alter microbial habitat. The altered habitat stability and structure driven by sediment transport, and altered hydrological regime driven by drought interact with streambed microbial communities (Figure 22), thus in turn modulate in-stream C-transformation processes. The research questions (sections 1.2.1–1.2.3) were discussed individually in the previous chapters; this section discusses the implications of the studied influences within a broader context, including the implications of the findings for regulation of environmental impacts on freshwater ecosystems, and the possibilities for mitigating their impacts.

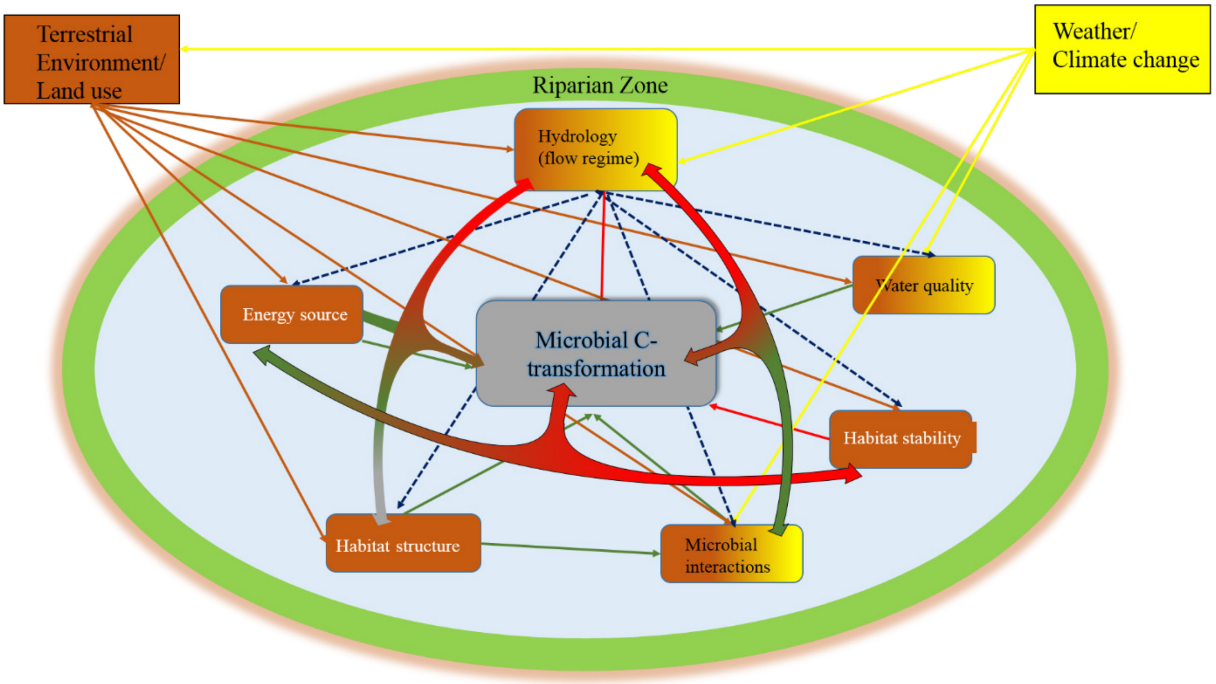

Figure 22 Significance of the studied influences (sediment transport and droughts) on in-stream C-transformation. Gradient-shaded arrows represent the contribution of each studied factor to microbial C-transformation; green and red, respectively, represent a positive and negative influence on microbial C-transformation, and gray represents a neutral influence on ultimate microbial C-transformation. Dotted-blue lines represent the direct or indirect influence of hydrology on microbial C-transformation.

5.1 Influence of sediment transport on in-stream microbial C-transformation

One of the novel findings of the present doctoral thesis is the potential for in-stream sediment transport to decrease C-transformation and modulate its rate of reduction in relation to the quality of available particulate organic matter (POM). As discussed in Chapter 2, the significance of in-stream sediment transport (i.e., periodic sediment shift) for C-transformation is a function of the number of shifting–resting cycles of sediment. This number is imposed by the wavelength of topographic feature (i.e., ripples and dunes) occurrence in the streambed and by the related frequency of disturbance. In parallel to the resulting periodic sediment shift, co-occurring perturbation of head gradients at the interface between the water surface and bed-form porous boundary facilitates higher advective solute mass transfer to the subsurface, consequently increasing the magnitude of C-transformation. The wavelength of head gradient perturbation is assumed to be equal to that of the bed-form occurrence, while the amplitude of its perturbation is related to the bed-form height (Elliott, 1990). This leads to cases where very different bed topographic configurations can produce the same amount of solute penetration (Elliott and Brooks, 1997a) but different frequencies of mechanical disturbance. Hence, in beds exposed to in-stream sediment transport in nature, the magnitude of C-transformation is balanced by the disturbance effects of periodic sediment shifts shown in this study and by advective mass transfer related to the bed-form geometry. Therefore, its variability in streams reflects the dominance of both co-occurring processes.

As discussed in Chapter 3, the significance of altered in-stream bed-load transport (Powell et al., 2005; Bridge, 2009) after river regulation for C-transformation is a function of established advective connectivity between streambed compartments of differing water conductivity. The flume experiments revealed that homogeneously mixed sand and gravel sediments within the streambed curtails the vertical connection of hyporheic with benthic microbial processes that results in decreased C-transformation. Indeed, sorting capacity increased overall processing of organic matter (OM), suggesting that interconnected pathways between streambed compartments, and a subsequent shift toward longer residence times in mixed streambeds, alters OM transformation pathways and thus the composition (i.e., quality) of the dissolved organic matter (DOM) pool at the reach-scale. The advective supply of redox partners, nutrients, and higher-quality C sources through well-conducting gravel patches maintains the downstream microbial community in sandy patches of poor conductivity also under well-supplied conditions. Thus, structural complexity of the streambed may maximize the flux of water across the streambed (Bridge, 2009; Segura et al., 2011), enabling C-

transformation in the subsurface for maximal biogeochemical cycling (Ward et al., 2011) as discussed in Chapter 3.

In streams, other factors, such as riparian vegetation that controls light availability (Steinman, 1992; Hunt et al., 2012) for in-stream-produced OM and the quantity and composition of terrestrial DOM (Fisher and Likens, 1973; Webster and Meyer, 1997), influence sediment microbial C-transformation (Wallis and Ladd, 1983; Volk et al., 1997) and may potentially alter the interrelation of advective mass transfer and sediment transport. Indeed, as reported in Chapter 2, with increasing POM quality and related trophic status of the stream, there is a higher requirement for specific and repeated moderate disturbances in order to reach the observed functional limit. Similarly, although the relationships between metabolism and grain size at the patch scale were reported in Chapter 3, the independence of stream metabolism from streambed structure at the experimental reach scale suggests that the relevance of sediment structure for stream metabolism is predominantly mediated by the strong effect of light on benthic biofilms. Hence, given the disparate influence of sediment grain size on colonization within benthic and hyporheic zones, the contributions of these zones to whole-streambed metabolism are modified by additional factors. In fact, relative contributions of these zones to reach-scale metabolism are controlled by interacting parameters: The benthic zone promotes biomass and composition in relation to mean grain size and light availability (hence nutrient consumption) regardless of sorting, as shown in Chapter 3, whereas grain size sorting controls hyporheic zone activity in relation to connectivity with the benthic zone. The results presented in Chapter 3 therefore indicate that altered vertical water flux (VWF) strongly controls the contribution of the benthic zone to overall microbial activity through grain size arrangement (sorted vs mixed) affecting the quality of DOM pool. However, under constant grain size distribution, the deeper mixing of solutes in benthic sediments of mixed streambeds leads to increased metabolism in these treatments to levels comparable to those driven by increased hyporheic metabolism in sorted streams influenced by the better connectivity in such treatments.

Previous research has further suggested that differences in streambed metabolism resulted from heterogeneity of VWF, interstitial flows, and sediment dynamics (Findlay, 1995), and that co-occurring processes may render metabolic rates similar at the reach scale (Aubeneau et al., 2014; Knapp et al., 2017). The present results highlight that the degree to which sediment disturbance frequency, stream trophic status, and the extent of advective solute transfer are connected determines the effect of sediment transport on C-transformation. The influence of periodic sediment disturbances depends firstly on the transport cycles and

compensation of their effect by the co-occurring promoted water exchange on the bed-form interface and secondly on the influence of POM on stream trophic status. The influence of the altered structural complexity is driven by the benthic process that compensates for the differences in the hyporheic zone in terms of the microbial C-transformation when the sediment size distribution is constant. Therefore, the ultimate significance of bed-load in streambeds for microbial C-transformation will depend firstly on the extent of the hyporheic and benthic zones as a function of channel geomorphology and secondly on the dominance of sediment fractions in the streambeds as a function of altered shear stress.

Hence, as the significance of the sediment transport for ecosystem function is counteracted by other stream metabolism drivers that differ between ecosystems, the protection or restoration of aquatic ecosystems in the face of growing anthropogenic pressures requires an understanding of hydrological and biogeochemical functioning across multiple spatial and temporal scales. This is illustrated by findings in Chapter 2, where the undisturbed physicochemical gradient in stable sediments yielded increased C-transformation under oligotrophic conditions. Microbial activity is known to be strictly linked to the development and maintenance of biofilm architecture, in which the production of extracellular polymeric substances enhances the solute retention and uptake of organic substrates used as a source of carbon and energy (Battin et al., 2003; Fischer et al., 2003). The inhibition of the development and maintenance of an organized biofilm architecture in the ripples modifies the existence of the favorable physicochemical gradient associated with stable sediments (Findlay, 1995).

In agricultural areas, which are often polluted and display eutrophic water conditions, an undisturbed physicochemical gradient can result in drastic metabolic limitation by the interruption of advective solute transfer between the water column and stable sediments (Nogaro et al., 2010; Navel et al., 2011). This might generate migrating dunes and ripples that are perfused by river water (Elliott and Brooks, 1997a, b; Packman and Brooks, 2001), resulting in relative hotspots within agricultural streams despite the limitation of metabolic function by mechanical stress. Furthermore, sediment structures are shown to modify the subsurface activity, and its significance for the overall C-transformation is further modified by the light-induced benthic processes under similar grain size distribution. Hence, altered grain size distribution might affect the dominance of hyporheic processes over benthic processes, ultimately suggesting that the number of patches and the proportion of well- and poorly conducting patches play significant roles in expected maximal C cycling (Ward et al., 2011).

5.2 Impact of drought-driven changes on microbial C-transformation in altered streambeds

Anthropogenic activities, such as urbanization and agriculture, commonly involve river regulation and channel clearance, which largely reduce the variability of flow and shear stress and result in ongoing degradation of streambeds from a spatially sorted to a more homogeneously mixed bed-load. Due to a reduction in percolation of surface water below ground, the promoted bed-load diminishes the catchment water storage and leads to an increase in flash runoff events. Furthermore, urbanization increases the extent of impervious surfaces and may reduce catchment water storage and produce more flash hydrographs (Walsh et al., 2005). In parallel, water extraction reduces flow volumes in streams, thereby increasing their susceptibility to the effects of drought. As a drought continues, the increased demand for water may lead to high levels of water extraction, and, as droughts usually occur in times of high temperature, evaporation may increase sharply, hastening the damaging impacts of desiccation. This scenario may apply to large-scale irrigation as well as to numerous local and small extractions to meet stock and domestic demands. As the demand for consumptive water use is high and rising, while human disturbances in catchments have reduced the natural resistance and resilience of aquatic ecosystems (e.g., loss of riparian vegetation, barriers to movement, and altered flow regime), this situation is causing marked declines in habitat and water quality (Arthington and Pusey, 2003).

As shown in Chapter 4, the spatial pattern of desiccation in streams is a function of channel morphology and hydrological connectivity between sediment compartments (benthic and hyporheic zones, and gravel and sand patches). The availability of slow-drying compartments in streams may provide refugia for biota during droughts (if they are not depleted by stock watering and/or waterhole pumping) that may persist throughout the drought after rapid desiccation of stream sections, such as riffles. Indeed, historically, the capacity of streams to recover from drought was ensured by the fact that, even during severe droughts, some refuge habitats persisted, and the biota that survived in these areas were able to breed and repopulate other areas as the drought broke. However, Chapter 4 showed that the community composition severely changes during unpredictable, infrequent desiccation and that such altered community composition affects the recovery of ecosystem functions after flow resumption. The moderately increasing light availability in temperate ecosystems promoted the establishment and maintenance of a transitional microbial community. Hence, the findings of restructured post-rewetting periphyton biomass, an increased H:A ratio with a magnitude linked to light availability, decreased F:B ratio linked to sediment structure, and

decreased CR and its decoupling from NEP, indicate that unpredictable and infrequent droughts in lowland streams may substantially alter microbial resistance and resilience, affecting primarily OM mineralization. Moreover, the induced changes in the relationships between autotrophic and heterotrophic metabolic processes during desiccation and rewetting caused trophic cascades that shifted the balance of ecosystem C-transformation processes. This suggests that the changes (in biogeochemical conditions, light availability, and sediment structure) observed in drying streams, which may be induced by land use, climate change, and increased water withdrawal, underlie the resistance and resilience of ecosystem functions in terms of C-transformation.

The results indicate that in light of the relative frequency and unpredictability of irregular droughts caused by global warming and increased water demand, there is an urgent need to develop long-term management strategies capable of contending with droughts and their impacts. This is particularly the case in regions where aquatic ecosystems have lost or greatly reduced their natural capacity (both resistance and resilience) to cope with drought as in the majority of contemporary streams with altered catchments. However, to date, biogeochemical studies of desiccated streambeds have focused mostly on describing changes in ecosystem variables over time and space, resulting in an incomplete understanding of stream ecological processes and thus limiting the effectiveness of management and restoration efforts. The findings in Chapter 4 contribute to the understanding of the resistance and resilience of temperate microbial communities to desiccation and rewetting, linking these to streambed conditions. The results thus suggest that sediment structure alterations and riparian vegetation are strong modulators of stream metabolism and should therefore be incorporated into management scenarios for temperate streams under predicted drought stress. Therefore, seeking a mechanistic understanding of (i) how ecosystem processes (such as C/nutrient cycling or spiraling and the nature of trophic interactions) change with drought and (ii) whether permanent or lasting changes occur in drying streams (addressed in Chapter 4) may have significant benefits for the future management of streams under a changing climate, particularly regarding the prediction of short- and long-term stream responses to infrequent droughts in temperate streams.

In an applied sense, future research should pursue the question of what long-term measures need to be progressively implemented to contend sustainably with drought and desiccation due to climate change (Bond et al., 2008). The results in Chapter 4 indicated sharp and rapid decreases in metabolic processes after surface water cessation, and key steps should

include rethinking the ways in which water is distributed between consumptive and environmental needs during drought and non-drought periods, together with improving the overall condition of catchments to restore their natural capacity to withstand drought events. The results in Chapter 4 support the premise that proactive measures for restoring the resilience of aquatic ecosystems may involve strengthening hydrological and biological connectivity (both longitudinally and laterally) by (i) improving channel geomorphic complexity and catchment water storage, including targeted environmental flows, (ii) protecting, if not augmenting, refugia, and (iii) managing targeted species and populations. Moreover, observed faster functional recovery with increased light availability in Chapter 4 suggests that restoration measures may include the selection of riparian vegetation with less canopy for restored ecosystems in temperate regions. This implies the design and implementation of long-term measures that protect catchments, their water resources, and the associated aquatic ecosystems in the expectation that both contemporary and prolonged droughts will become the usual state of the environment rather than the exception.

5.3 Significance of revealed effects of sediment transport and droughts revealed by micro- and mesocosms

Model systems (see section 1.4) allowed me to detect and elucidate mechanisms by which streambed mobility and structure interact with organic C quality and quantity to influence reach-scale microbial C-transformation hence whether this interaction remains significant in streams affected by sediment transport and drought. Contemporary streams are largely altered, and additional variables (e.g., periodic sediment shifts, altered streambed complexity, and droughts) have to be considered in conceptual models to obtain insights into the ultimate environmental controls on microbial C-transformation.

As various factors simultaneously influence C-transformation in streams at variable spatiotemporal scales, the significance of their resultant interaction for C-transformation might change over time and space if one factor becomes dominant over another (e.g., light, hydraulic connectivity, and OM quality) as highlighted in this thesis. The use of model systems can help to overcome the limitations imposed by spatiotemporal variability, as they provide standardized, tractable physical conditions and allow isolation of the effects of interest (e.g., periodic sediment shift, drought, and geomorphic complexity) from confounding factors. Even though model systems cannot adequately disentangle the set of possible causal interconnection because of nature's uncontrollable complexity (Cadotte et al., 2005), they

allow the revealed effects to be linked to the explanatory variable (e.g., light, OM quality, and hydraulic connectivity), revealing clearer and more predictable underlying patterns in microbial C-transformations. However, to better constrain future consequences of human activities for the functioning of fluvial ecosystems in general, improved understanding of the significance of hydrological and biogeochemical interconnections for ecosystem functioning across multiple spatial and temporal scales in streams in particular is needed, as suggested in studies with model systems. Thus, the design and employment of multifactorial studies with good replication value that allow appropriate multivariate statistical evaluation of patterns have great potential for the generalization of the revealed effects in model systems. This approach is likely to allow the successful implementation of protection and restoration measures that match the heterogeneous and dynamic nature of fluvial ecosystem functioning in terms of C-transformation.

5.4 Summary

In summary, the findings obtained using model systems (micro- and mesocosms) mimicking sandy lowland streams indicate that in-stream sediment transport and droughts in temperate regions may have profound and complex impacts on microbial communities. Progressive migration of ripples and duration of infrequent droughts have both been shown to exponentially decrease the magnitude of microbial function in C-transformation. Moreover, altered streambed structural complexity was shown to change the metabolic pathways of stream C-transformation, affecting stream water quality. Under extreme environmental conditions, sediment transport, and droughts, streambed features (ripples and sorting) affect the relevance of the physical and chemical factors of microbial habitats to C-transformation. However, other environmental variables, such as variability in in-stream C quality, light availability or hydrological connectivity, may modulate the ultimate impact of sediment transport and droughts on microbial C-transformation. Thus, these findings have implications for understanding the causal and modulating mechanisms that underlie streambed C-transformation, which is impacted by environmental extremes and can provide insight into the need for protection or restoration measures of affected critical ecosystem functions.

Given the complexity of the processes that simultaneously influence C-transformation in streams at variable spatiotemporal scales, the findings of this study suggest that a more comprehensive understanding of hydro- and biogeomorphological interactions will provide a basis for fully understanding, predicting, and mitigating general ecosystem functions. The

employment of micro- and mesocosms is of particular benefit, as it can reveal the abovementioned interactions and their mechanisms, particularly for catchments where human disturbances have increased in complexity, reducing the natural resistance and resilience of aquatic ecosystems, while the demand for consumptive water use is high and rising. Finally, the revealed interactions and their mechanisms using model systems may provide fundamental knowledge for the development of coupled hydro–biogeochemical models that could predict the responses and the resilience of stream networks to the expected increase in the extent of channels, impacted by human activities, under global change in many regions worldwide.

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STATEMENT OF ACADEMIC INTEGRITY

I hereby certify that the submitted thesis "In-stream microbial carbon transformation under opposing stresses—drought and sediment transport" is my own work and that all published or other sources of material consulted in its preparation have been indicated. I have clearly pointed out any collaboration that has taken place with other researchers and stated my own personal share in the investigations in the Thesis Outline. I confirm that this work has not been submitted to any other university or examining body for a comparable academic award.

Berlin, 14.12.2017

Sanja Zlatanović

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