# Effects of algae on microbial carbon cycling in freshwaters

**- with focus on the utilization of leaf carbon by heterotrophic bacteria and fungi -**

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"*Algae and bacteria have coexisted ever since the early stages of evolution.*
*A coevolution, that has revolutionized life on earth in many aspects." Raman et al. 2016*

# SUMMARY

Microbial processing of organic matter (OM) in the freshwater biosphere is a key component of global biogeochemical cycles. Freshwaters receive and process valuable amounts of leaf OM from their terrestrial landscape. These terrestrial subsidies provide an essential source of energy and nutrients to the aquatic environment as a function of heterotrophic processing by fungi and bacteria. Particularly in freshwaters with low in-situ primary production from algae (microalgae, cyanobacteria), microbial turnover of leaf OM significantly contributes to the productivity and functioning of freshwater ecosystems and not least their contribution to global carbon cycling.

Based on differences in their chemical composition, it is believed that leaf OM is less bioavailable to microbial heterotrophs than OM photosynthetically produced by algae. Especially particulate leaf OM, consisting predominantly of structurally complex and aromatic polymers, is assumed highly resistant to enzymatic breakdown by microbial heterotrophs. However, recent research has demonstrated that OM produced by algae promotes the heterotrophic breakdown of leaf OM in aquatic ecosystems, with profound consequences for the metabolism of leaf carbon (C) within microbial food webs. In my thesis, I aimed at investigating the underlying mechanisms of this so called priming effect of algal OM on the use of leaf C in natural microbial communities, focusing on fungi and bacteria.

The works of my thesis underline that algal OM provides highly bioavailable compounds to the microbial community that are quickly assimilated by bacteria **(Paper II)**. The substrate composition of OM pools determines the proportion of fungi and bacteria within the microbial community **(Paper I)**. Thereby, the fraction of algae OM in the aquatic OM pool stimulates the activity and hence contribution of bacterial communities to leaf C turnover by providing an essential energy and nutrient source for the assimilation of the structural complex leaf OM substrate. On the contrary, the assimilation of algal OM remains limited for fungal communities as a function of nutrient competition between fungi and bacteria **(Paper I, II)**. In addition, results provide evidence that environmental conditions determine the strength of interactions between microalgae and heterotrophic bacteria during leaf OM decomposition **(Paper I, III)**. However, the stimulatory effect of algal photoautotrophic activities on leaf C turnover remained significant even under highly dynamic environmental conditions, highlighting their functional role for ecosystem processes **(Paper III)**.

The results of my thesis provide insights into the mechanisms by which algae affect the microbial turnover of leaf C in freshwaters. This in turn contributes to a better understanding of the function of algae in freshwater biogeochemical cycles, especially with regard to their interaction with the heterotrophic community.

# ZUSAMMENFASSUNG

Die mikrobielle Verarbeitung von organischer Biomasse in Süßwasser nimmt eine fundamentale Rolle in den globalen biogeochemischen Nährstoffkreisläufen ein. Ein Großteil der organischen Biomasse gelangt aus der terrestrischen Umgebung, insbesondere aus dem Blattlaubeintrag, in die Gewässer und stellt eine wesentliche Energie- und Nährstoffquelle für die aquatische Umwelt dar. In die aquatischen Nahrungsnetze gelangt das terrestrische Material vorwiegend durch mikrobielle Umsatzprozesse, an denen vor allem heterotrophe Bakterien und Pilze beteiligt sind. Der mikrobielle Umsatz von Blattlaub kann die biogeochemischen Prozesse aquatischer Ökosysteme signifikant beeinflussen und nicht zuletzt deren Beitrag zum globalen Kohlenstoffkreislauf. Das gilt insbesondere für Gewässer, in denen die *in-situ* Produktion organischer Biomasse durch aquatische Algen sehr gering ist.

Aufgrund ihrer unterschiedlichen chemischen Zusammensetzung wird angenommen, dass Blattbiomasse für die mikrobielle Gemeinschaft schlechter abbaubar und damit weniger bioverfügbar ist als photosynthetisch produziert Biomasse durch Algen. Das gilt insbesondere für das partikuläre Blattmaterial, welches vorwiegend aus strukturell komplexen und aromatischen Polymeren besteht. Neue Forschungsergebnisse haben jedoch gezeigt, dass Algen den enzymatischen Abbau von Blattmaterial stimulieren (*Priming Effekt*), und den Umsatz von Blattkohlenstoff innerhalb des mikrobiellen Nahrungsnetzes signifikant beeinflussen. In meiner Doktorarbeit habe ich die zugrundeliegenden Mechanismen dieses *Priming* Effekts von Algenbiomasse auf die mikrobiellen Umsatzprozesse von Blattkohlenstoff innerhalb natürlicher mikrobieller Gemeinschaften untersucht. Der Fokus lag dabei vor allem auf aquatische Pilz- und Bakteriengemeinschaften.

Die von mir erbrachten Arbeiten verifizieren, dass Algenbiomasse für die mikrobielle Gemeinschaft teilweise hoch verfügbar ist **(Studie II)**. Meine Arbeiten unterstreichen jedoch, dass Algenbiomasse vor allem von Bakterien assimiliert wird und deren Beitrag zum mikrobiellen Blattumsatz stimuliert. Die Bakteriengemeinschaft erhält über das Algenmaterial vermutlich essentielle Energie- und Nährstoffquellen, die ihnen die Assimilation des strukturell komplexen Blattkohlenstoffs erleichtert. Im Gegensatz dazu scheint die Pilzgemeinschaft das Algenmaterial nicht direkt nutzen zu können, vermutlich bedingt durch deren schwache Konkurrenz mit Bakterien um das Algensubstrat **(Studie I, II)**. Darüber hinaus liefern die Ergebnisse einer weiteren Studie Hinweise darauf, dass Umweltbedingungen die Stärke der Wechselwirkungen zwischen Algen und heterotrophen Bakterien während der Zersetzung der Blattbiomasse bestimmen **(Studie I, III)**. Die stimulierende Wirkung der photoautotrophen Algenaktivität auf den Blattkohlenstoff Umsatz blieb jedoch selbst unter hochdynamischen Umweltbedingungen signifikant, was ihre funktionelle Rolle für Ökosystemprozesse unterstreicht **(Studie III)**.

Die Ergebnisse aus den Arbeiten meiner Promotion geben Einblicke in die Mechanismen des mikrobiellen aquatischen Blattabbaus und welche funktionelle Rolle Algen hierbei haben. Das trägt zu einem besseren Verständnis der Funktion von Algen in den biogeochemischen Kreisläufen der Süßgewässer bei, insbesondere mit Hinblick auf die Interaktion der heterotrophen Gemeinschaft mit Algenbiomasse.

# Table of Contents

## List of Papers

This thesis is based on the following three papers, referred to in the text by their Roman numerals (I-III). Published papers I and III are open access.

I **Fabian, J.**, Zlatanovic, S., Mutz, M., and Premke, K. (2017) Fungal-bacterial dynamics and their contribution to terrigenous carbon turnover in relation to organic matter quality. *ISME J.* **11**: 415–425.

[The concept and the initial idea and the experimental design was elaborated by me and KP. I did the laboratory work, execution, and had the main responsibility for sample analyses (gas measurements, fatty acid, stable isotope, carbon quality) and data processing. I also performed the statistical analysis and drafted the manuscript.]

II **Fabian, J.**, Klawonn, I., Musat, N., Stryhanyuk, H., Dippoldt, M., Grossart H.-P., and Premke, K.: Algal exudates impact microbial turnover of leaf litter in freshwater systems. *Manuscript*

[ I initated the project and finalized the experimental set up together with IK and KP. I did all the measurements and analysis except of the NanoSIMS which was conducted by the help of NM, HS, and MD. I performed the statistical analysis and drafted the manuscript.]

III **Fabian, J.**, Zlatanović, S., Mutz, M., Grossart, H.-P., van Geldern, R., Ulrich, A., Gleixner, G. and Premke, K.(2018) Environmental Control on Microbial Turnover of Leaf Carbon in Streams – Ecological Function of Phototrophic-Heterotrophic Interactions. *Front. Microbiol.* **9**: 1044. DOI: 10.3389/fmicb.2018.01044

[I designed the experimental set up together with SZ, MM and KP. I conducted the gas and microbial fatty acid measurements, including stable isotopes analysis. I had the main responsibulity for the field work and laboratory analysis together with SZ. I did the statistical analysis and drafted the manuscript.]

**In addition to the papers in this thesis, I have co-authored the following papers:**

Zlatanović, S., **Fabian, J.**, Mendoza-Lera, C., Woodward, K.B., Premke, K., and Mutz, M. (2017) Periodic sediment shift in migrating ripples influences benthic microbial activity. *Water Resour. Res*. 53, DOI:10.1002/2017WR020656.

Zlatanović, S., **Fabian, J.**, Premke, K., and Mutz, M. (2017) Shading and sediment structure effects on stream metabolism resistance and resilience to infrequent droughts. *Sci. Total Environ*. DOI.org/10.1016/j.scitotenv.2017.10.105

**Fabian, J.**, Zlatanović, S., Singer, G., Premke, K., Mutz, M. Streambed structure defines the hydraulic coupling of benthic and hyporheic microbial processes". Submitted manuscript to *ES&T*

Bodmer, P., **Fabian, J**., Kamjunke, N., Lechtenfeld, O., Zak, D., and Premke K. Dissolved organic matter quality influences microbial metabolism. Manuscript will be submitted in August to *Ecology*.

Taube, R., **Fabian, J**., van der Wyngaert, S., Agha, R., Kagami, M., Gerphagnon, M., Premke. K., Phospholipid derived fatty acids as a tool to detect and quantify saprotrophic and parasitic aquatic fungi. Manuscript will be submitted in December to Methods in Ecology and Evolution.

## Abbreviations

| | |
|---|---|
| µm | micrometer |
| β:α | Freshness Index |
| C | Carbon |
| $CO_2$ | Carbon Dioxide |
| $CH_4$ | Methane |
| DIC | Dissolved Inorganic Carbon |
| DIN | Dissolved Inorganic Nitrogen |
| DOM | Dissolved Organic Matter |
| DON | Dissolved Organic Nitrogen |
| E2:E3 | Absorbance ratio |
| EA-IRMS | Elemental Analyser-Isotope Ratio Mass Spectrometry |
| $^{13}F$ | Fractional isotope abundance of $^{13}C$ |
| FAME | Fatty Acid Methyl Ester |
| FI | Fluorescence Index |
| GC | Gas chromatography |
| GC-c-IRMS | Gas Chromatography-combustion-Isotope Ratio Mass Spectrometry |
| HIX | Humification Index |
| IOSC | Integrated Cavity Output Spectroscopy |
| IRMS | Isotope ratio mass spectrometry |
| MS | mass spectrometry |
| N | Nitrogen |
| OC | Organic Carbon |
| OM | Organic Matter |
| P | Phosphate |
| PDB/VPDB | PeeDeeBee/ViennaPDB |
| POM | Particulate Organic Matter |
| PLFA | Phospholipid derived fatty acids |
| SIMS | Secondary Ion Mass Spectrometry |
| *spec.* | *Species* |
| SRP | Soluble reactive phosphorus |

## Introduction and Objectives

Freshwater systems cover about 1.8 % of the global surface area. However, their collective contribution to global carbon cycling is substantial (Downing *et al.*, 2006; Battin *et al.*, 2009). Microbial organic matter (OM) turnover in sediments is an active component of the carbon (C) cycle, making freshwaters "hotspots" for carbon dioxide ($CO_2$) and methane ($CH_4$) and, therefore, an essential factor in global climate. A significant portion of the processed organic material comes from the terrestrial catchment (Battin *et al.*, 2009). For many aquatic systems, these terrestrial subsidies are an essential energy and nutrient source in ecosystem processes, with their microbial processing not least depending on environmental conditions (Cole *et al.*, 2007; Tranvik *et al.*, 2009). Understanding the processing and fate of terrestrial C has thus been a high priority in aquatic ecology. Nevertheless, comprehensive knowledge of how environmental factors affect the microbial processing of terrestrial OM in freshwater sediments is still limited, but fundamental to fully integrating this process into global carbon budgets.

### Origin & Fate of Organic Matter in Freshwaters

*– Focusing on carbon cycling in microbial food webs –*

OM is mainly derived from the remains of organisms and their waste products in the environment. Another characteristic is the significant amount of C. In literature, organic matter and organic carbon (OC) are thus often used interchangeable. Organic compounds are conceptually categorized into particulate (POM) and dissolved organic matter (DOM) as fractions that either retain or pass through a membrane with a pore size of 0.2 to 0.45 µm, respectively. DOM frequently includes a significant fraction of colloids in addition to small organic molecules, some of which are complexed with trace metals and other inorganic compounds (Wetzel, 1995).

Sources and composition of both organic C pools are diverse and vary in time and space depending on proximity to sources and exposure to degradation processes (Cole *et al.*, 2007; Battin *et al.*, 2009). Figure 1 provides a simplified overview on the origin and major C processes associated with the transformation and turnover of OM within the aquatic microbial food web, i.e. the microbial loop. On the one side of the microbial loop, chemical energy and nutrients (e.g. phosphate (P) and nitrogen (N)) are stored in organic matter during photochemical fixation of dissolved inorganic carbon (DIC: $CO_{2\,(aq)}$, $H_2CO_3$, $HCO_3^{-1}$, $CO_3^{-2}$) and nutrients by autotrophic organisms including pro- and eukaryotic photoautotrophs (cyanobacteria, microalgae) and chemoautotrophs (e.g. metanotrophic bacteria) (Bertilsson and Jones, 2003). A fraction of the initially fixed inorganic compounds is either assimilated during biomass production adding to the POM

pool or extracellular released as dissolved metabolites adding to the DOM pool (Haack and McFeters, 1982, Kirchman 2002). Particulate and dissolved organic compounds are utilized for microbial energy (catabolism), by microbial heterotrophs. The carbon is either used for growth and assimilated into cell compartments, making it available to other trophic levels within the aquatic nutrient cycle (ciliates, invertebrates), or recycled to $CO_2$ via heterotrophic mineralization (Langenheder and Jürgens, 2001; Sherr and Sherr, 2002). $CO_2$ reacts with water to form DIC and may enter the organic pool again though photoautotrophic fixation.

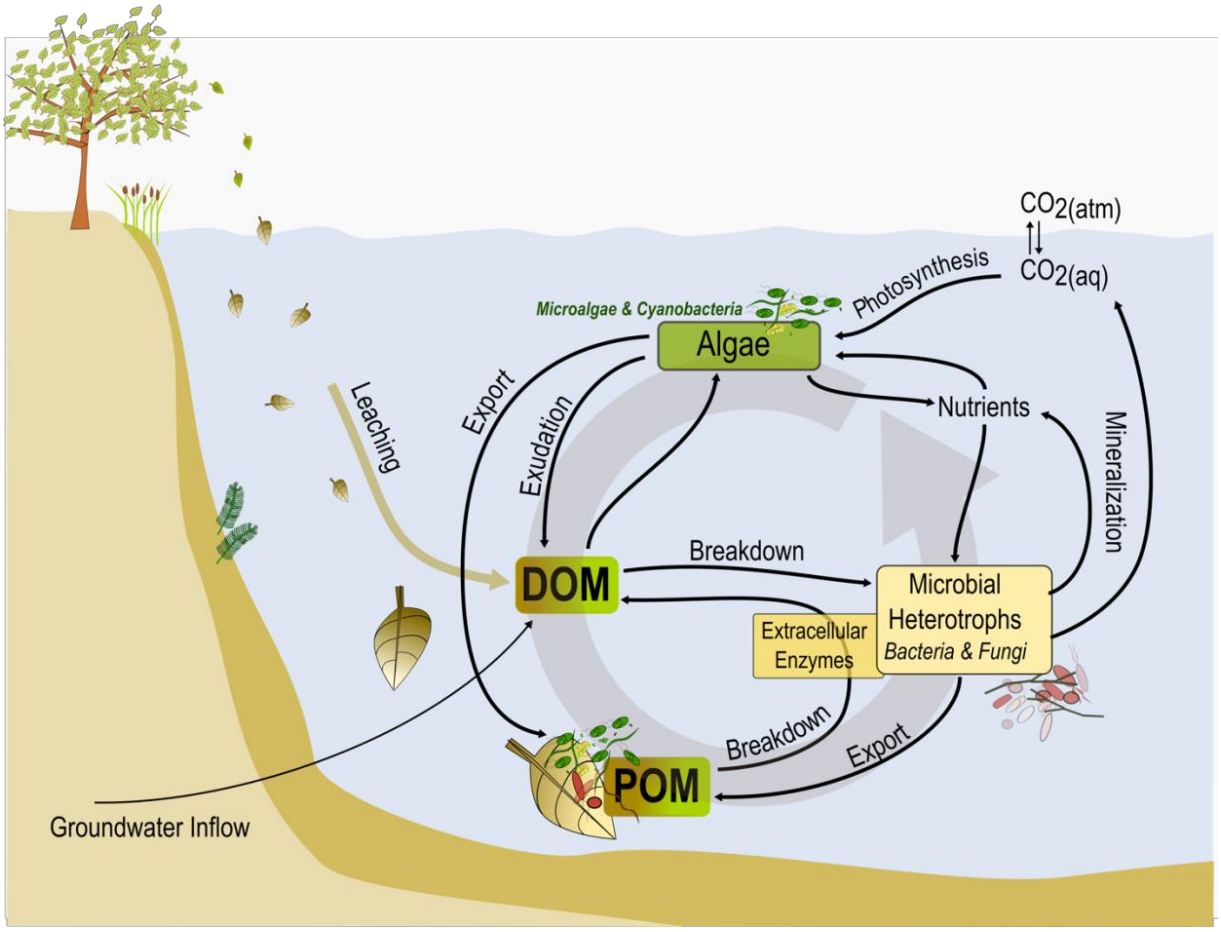


**Figure 1** Origin and processing of organic matter in the aquatic microbial loop. Carbon of terrestrial and aquatic origin is constantly transferred between photo- and heterotrophs during production, transformation and mineralization of dissolved (DOM) and particulate (POM) organic matter. Chemical energy from primary production and nutrients are stored in algae biomass and can be transported within and across systems. Heterotrophic microbes use extracellular enzymes deployed on cell surfaces to transform or mineralize organic carbon in microbial respiration. In most freshwaters, microbial breakdown of particulate organic matter is restricted to upper sediment layers.

Next to internal primary production (autochthonous C), freshwaters receive valuable amounts of organic and inorganic C-subsidies from the surrounding terrestrial landscape (allochthonous C) (Cole *et al.*, 2007; Battin *et al.*, 2009; Tranvik *et al.*, 2009). This is especially true for headwater streams and littoral lake areas, where terrestrial subsidies can account for up to 90 % of the total organic material (Cole *et al.*, 2007; Brett *et al.*, 2017). Particulate forms of terrestrial OM mainly enter the aquatic environment through falling leaves, soil abrasion and wood derives (Fisher and Likens, 1973; Aitkenhead-Peterson *et al.*, 2003; Richardson *et al.*, 2010). In addition, there is a constant inflow of dissolved organic and inorganic forms through groundwater and lateral inflow across the hyporheic zone (Boulton *et al.*, 2014). Hence, terrestrial subsidies enter the aquatic microbial loop, either by photoautotrophic fixation of inorganic compounds or via catabolism of organic compounds. In light mediated zones, photochemical decay of organic compounds provides an additional valuable pathway for the breakdown of dissolved terrestrial OM (Amon and Benner, 1996; Spencer Robert G *et al.*, 2009).

## Microbial Turnover of Leaf Litter

*– a fundamental process in freshwater biogeochemistry –*

Amongst other terrestrial sources, the annual entry of leaf litter is one of the primary sources by which terrestrial OM enters aquatic ecosystems (Fisher and Likens, 1973; Boyero *et al.*, 2016). The degradation of the subsidized leaf material results from physical (leaching, abrasion, fragmentation) and biological transformation processes, the latter being mediated by macroinvertebrates, ciliates, and to a large extent by microorganisms (Bärlocher, 1985; Webster and Benfield, 1986; Gessner *et al.*, 1999).

Leaf tissue is mainly composed of lignocellulose, that is composed of structurally complex and aromatic carbohydrate polymers (Pettit *et al.*, 2012). The most abundant are celluloses, that are heterogeneous intertwined polysaccharide chains with varying degrees of crystallinity (Yadav and Malanson, 2007). Cellulosic compounds are generally embedded in a matrix with other structural biopolymers such as pectin as well as predominantly hemicelluloses and lignin, the latter being a complex, crosslinked phenolic polymer containing aromatic hydrocarbons (Lynd *et al.*, 2002). Structurally simple biochemical compounds such as simple carbohydrates, lipids and proteins account for only a small proportion of the plant tissue (Martínez *et al.*, 2005).

Once submerged in the water, microbial species quickly colonize the leaf litter, grow in the leaf tissue, and form species-rich communities upon it (Figure 2, Das *et al.*, 2007; Gessner *et al.*, 2010). A significant portion of the leaf-forming compounds are immediately leached from the tissue into the surrounding water as soon as the leaf material is submerged (Gessner *et al.*, 1999). Leached constituents include inorganic elements such as Calcium, Kalium, Magnesium and mostly structurally simple

biochemical compounds (Bernhard-Reversat *et al.*, 2003; Ibrahima *et al.*, 2008) that are highly bioavailable to the microbial community and are therefore rapidly metabolized within the pelagic microbial food web (Wantzen *et al.*, 2008; Attermeyer *et al.*, 2013, 2014).

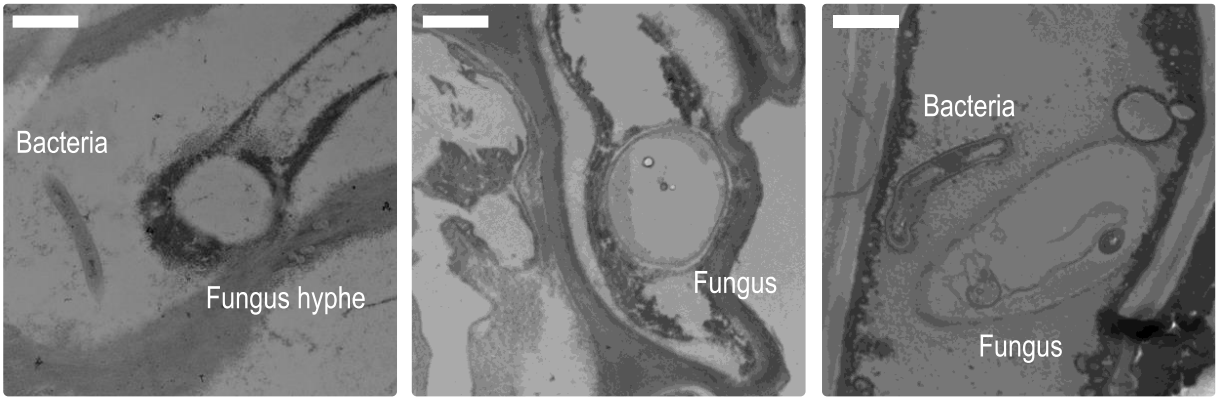


**Figure 2** Photomicrographs of fungal and bacterial cells growing in leaf tissue. The scale bar is 2 µm. Source: The images were taken with trans-electron microscopy and kindly provided by Gerd Hause.

The remaining particulate fraction, on the other hand, consisting predominantly of lignocellulose that is highly resistant to enzymatic breakdown by microorganisms (Wetzel, 1995). Structural leaf compounds must be degraded into simpler intermediate compounds before they can be taken up by microbial cells (Chróst, 1992; Romaní *et al.*, 2006). Their processing is energy demanding and requires specific enzymes that can only be synthesized by certain species within the microbiome (Das *et al.*, 2007). Accordingly, particulate leaf litter is largely degraded in the sediment and at a significantly slower rate than the leaf leachate (Webster and Benfield, 1986; Gessner *et al.*, 1999; Hieber and Gessner, 2002). To break down lignocellulosic compounds, microorganisms produce enzymes that are released into the surrounding water. These so-called extracellular enzymes split the structure-forming polymers cellulose, lignin and hemicellulose into smaller molecules, which can ultimately be absorbed by the microbial cells (Romaní *et al.*, 2006; Lundell *et al.*, 2010). In addition, high-molecular-weight intermediates, so-called humic substances, often remain longer in the DOM pool and are only slowly degraded by either microbial or photochemical processes (McKnight and Aiken, 1998; Spencer *et al.*, 2009).

The complete degradation of leaf litter results from a complex network of ecological interactions between different microbial species (Slater and Lovatt, 1984; Romaní *et al.*, 2006). Microorganisms encompass a wide range of metabolic potentials and requirements (Arndt *et al.*, 2013). Many are biochemical specialists equipped with specific enzymes that target particular molecular bonds (Brett *et al.*, 2017), are highly specialized to the use of particular substrates, whereas other species are very versatile

using many different ones (Gómez-Consarnau *et al.*, 2012). Leaf degrading microbial communities include members of all three domains, that is, eukaryotic fungi, bacteria and archaea. Amongst all, least is known about the physiological capabilities and specific biochemical roles of archaea in leaf turnover. The ongoing discovery of unknown archaea strains, in particular in subsurface sediments, indicates potential functions of archaea in anaerobic leaf decay (Biddle *et al.*, 2006; Lazar *et al.*, 2017). Upon today, however, only a few archaea are known to degrade aromatic substrates as those composing leaf litter (Fuchs *et al.*, 2011). Hence, fungi and bacteria are commonly considered to contribute most significantly to leaf decomposition (Abelho, 2001; Frossard *et al.*, 2012), even though existing gaps in environmental microbiology may underestimate archaeal functions.

Aquatic hyphomycetes, an ecological group of anamorphic species from the fungal phyla *Ascomycota* and *Basidiomycota* (Ingold, 1975; Bärlocher *et al.*, 2008), has traditionally been considered to dominate the degradation of lignocellulose (Baldy *et al.*, 1995). Yet, methodological developments increasingly highlight the so far underestimated diversity of bacterial and fungal species involved in leaf decay, including their varying contribution throughout decomposition stage (Das *et al.*, 2007; Schneider *et al.*, 2012). Species from the fungal phyla *Mucoromycotina* belong to the first colonizers of the submerged leaf litter, whereas *Basidiomycota* and in particular *Ascomycota* become more prominent during later decomposition stage (Bärlocher and Kendrick, 1974; Torres *et al.*, 2005; Osono and Takeda, 2006). The phyla *Mucoromycotina* and *Ascomycota* have the ability to degrades cellulose or sugar whereby *Basidiomycota* can degrade the aromatic counterparts of lignin in leaf OM (Osono, 2007; Lundell *et al.*, 2010). In addition, other studies have documented many diverse members of the phylum *Chytridiomycota* in leaf-associated communities suggesting a so far uncovered functional role of this fungal phylum in leaf degradation (Nikolcheva and Bärlocher, 2005). Moreover, the proportion of yeasts in leaf-associated communities was shown to increase under low-oxygen conditions (Baldy *et al.*, 2002). With respect to the bacterial domain, *Actinobacteria* dominate bacterial communities inhabiting leaf OM (Bärlocher and Kendrick, 1974; Das *et al.*, 2007) and are known for their ability to degrade lignin derived compounds (Kirby, 2005). Moreover, species of the phyla *Bacteroidetes* and *Proteobacteria* are commonly observed, the latter mainly in leaf associated biofilms. *Proteobacteria* degrade humic substances, hence primarily contribute to the decomposition of dissolved leaf OM (McNamara and Leff, 2004; Battin *et al.*, 2016). Members of the phylum *Bacteroidetes* actively inhabit the leaf tissue, in particular during later decomposition stage, owning to their capability to degrade various polysaccharides including cellulose (Kirchman, 2002; Reichenbach and Dworkin, 2006; Das *et al.*, 2012).

Due to their physiological differences, bacteria and fungi are generally conceded with different functions and different contributions to the degradation of leaf OM (Hieber and Gessner, 2002). Fungi are assumed to dominate early stages in the decomposition of the particulate leaf material and initiate the

degradation of lignocellulosic compounds due to their enzymatic capabilities (Gessner *et al.*, 1999; Soares *et al.*, 2017). Fungal biomass normally exceeds that of bacteria on particulate leaf OM, typically accounting for over 90% of the total microbial biomass (Baldy *et al.*, 1995; Komínková *et al.*, 2000). This refers especially to filamentous fungus species which, in addition, mechanically pave the way for bacteria into the particulate leaf tissue via their mycelial growth in the leaf tissue (Gulis and Keller Suberkropp, 2003). Bacterial cells often occur closely associated with fungal hyphae on particulate leaf OM and utilize the lignocellulosic detritus or intermediate leaf compounds produced by fungal activity (Benner *et al.*, 1984; Ruttimann *et al.*, 1991; Romaní *et al.*, 2006). Above all, bacteria dominate the mineralization of simple leaf compounds and hence the degradation of the nutrient-rich leaf leachate (Attermeyer *et al.*, 2013). In addition, bacterial probably complement fungi in advanced stages of decomposition as the size of the particles gets smaller (Abelho, 2001). It was also noted that bacterial contribution to the degradation of particulate leaf OM might be significantly greater due to their higher turnover rates than assumed from their biomass fraction (Benner *et al.*, 1984). However, detailed studies on the specific functions of the various microbial groups in the leaf OM turnover are lacking (Schneider *et al.*, 2012). It is known, though, that the contribution of the different fungal and bacterial species to degradation of leaf OM varies depending on the stage of degradation, leaf type and environmental conditions (Hieber and Gessner, 2002; Güsewell and Gessner, 2009). In addition, interactions among species of the microbial food web have a fundamental influence as well (Møller, 1999; Mille-Lindblom and Tranvik, 2003; Das *et al.*, 2012).

## Photosynthetic Stimulation of Heterotrophic Metabolism

*– Influence on how fungi and bacteria utilize carbon from leaf litter –*

There is both theoretical and empirical evidence that the dynamics of consumption of specific OM pools should differ based on their molecular size, chemical composition and elemental stoichiometry (Chróst, 1992; Amon and Benner, 1996). Nevertheless, it is becoming increasingly clear that the conversion of organic compounds is more environmentally driven than solely by their molecular nature. The investigation of the abiotic and biotic factors that determine microbial turnover of leaf litter, including underlying mechanisms, are among the most studied topics in freshwater science today (reviewed in Gessner *et al.*, 2010; Boyero *et al.*, 2016).

One of the key themes is whether access to highly bioavailable OM improves the microbial degradation of structurally complex OM, such as terrestrial substrates, in aquatic ecosystems, also known as *priming* effect (Blagodatskaya and Kuzyakov, 2008; Guenet *et al.*, 2010; Bengtsson *et al.*, 2018). In sediment areas that are exposed to light, algae (microalgae, cyanobacteria) co-occur with heterotrophic bacteria and fungi in microbial communities associated with the leaf tissue. Hence, phototrophic and

heterotrophic processes are closely linked by an immediate mass transfer (Kirchman, 2012). Photosynthetic OM consists predominantly of simple carbohydrates and amino acids, and thus represents a readily available source of energy, but also a source of nutrients to the heterotrophic community (Myklestad, 1995; Espeland et al., 2001; Kragh and Søndergaard, 2004). In particular, dissolved algal compounds are rapidly metabolized by fungi and bacteria and promote their activities (Cole *et al.*, 1982; Baines and Pace, 1991; Kuehn *et al.*, 2014). In this regard, it has been speculated that algal exudation of photosynthetic metabolites stimulate the degradation of terrestrial OM (Romani and Sabater, 1999; Espeland *et al.*, 2001). Further research promotes evidence that the utilization of algal exudates enhances the overall breakdown of leaf matter by bacteria and fungi (Rier *et al.*, 2007; Danger *et al.*, 2013; Kuehn *et al.*, 2014). Thus, algae activities could significantly determine microbial leaf degradation in fresh waters and, consequently, leaf C turnover in biogeochemical cycles of freshwaters.

Yet, the results of previous studies do not provide a clear picture. Both positive, negative, and no effects on microbial leaf degradation, and hence carbon turnover, were observed (Bengtsson *et al.*, 2014; Wagner *et al.*, 2017), indicating an additional impact of other biotic or abiotic factors. Nor is it clear that algae OM promotes the actual microbial degradation of leaf OM or merely alters the use of leaf-derived carbon and nutrients within the microbial food web. It is believed that the high-energy content of the algal material provides additional sources for the energy demanding, enzymatic breakdown of the structural complex lignocellulose. (Espeland *et al.*, 2001; Danger *et al.*, 2013; Ward *et al.*, 2016). This assumption is supported by other authors that denote a stimulated enzyme production through algae activities (Rier *et al.*, 2014). Yet, additional research has documented associated shifts in the biomass fraction of fungi and bacteria in heterotrophic communities, as well as in bacterial activity (Danger *et al.*, 2013). Hence, effects of algal OM on microbial leaf turnover may be related to associated functional shifts in microbial communities. The results so far, however, do not allow unambiguous conclusions as to whether algal OM affects the functional role of bacteria and fungi equally in the degradation of leaves or possibly leads to functional shifts within both domains (Kuehn *et al.*, 2014; Soares *et al.*, 2017).

Hence, a complete understanding of the underlying mechanisms by which algae influence the uptake and metabolic use of leaf compounds by fungi and bacteria remains open, as well as how this relationship is shaped by environmental conditions.

## Photo-Heterotrophic Interactions in a Multivariate Space

*– Factors that modify the influence of algae activities on microbial leaf turnover –*

Several environmental factors are known that influence the functioning and composition of phototrophs and heterotrophs and in turn potentially shape their interaction.

Respectively, the availability of light energy is the ultimate limiting factor for the occurrence of algal metabolites and thus their availability to the heterotrophic community (Wetzel, 2001). Despite, recent findings confirm intensified metabolic interactions of bacteria and fungi with algal DOM exudates under low nutrient availability and consequently stronger stimulating effects on leaf turnover (Danger *et al.*, 2013; Rier *et al.*, 2014). In addition, it has been shown that heterotrophic species interact to different extent with algae OM and prefer different compound classes (Sarmento and Gasol, 2012; Cooper and Smith, 2015). Hence, stimulating effect of algal OM on leaf decay likely depend on the nature and compound composition of the algae OM pool, but also on the species composition of the microbial community. Respectively, the studies so far also give no indication as to whether particulate, dead algal material is likely to have the same positive effect on leaf degradation as documented for dissolved algal material. Yet, both algae OM pools differ in their compound composition, as well as nutrient stoichiometry. In addition, the energy and nutrient content in the algae OM source potentially determines the intensity in the effect on leaf degradation, and whether the catabolized leaf compounds are used for microbial activity or biomass growth (Elser and Kuang, 2002; Guillemette *et al.*, 2016).

In addition, local barriers determine the coupling of phototrophic and heterotrophic processes and, consequently, the functional contribution of algal biomass to the microbial turnover of leaf C. This is especially true in sediments of headwater streams, where ecosystem processes are largely defined by physical processes (Battin, 2000). The sediment structure considerably drives underlying processes in microbial leaf turnover, and the resulting changes in its contribution to freshwater biogeochemistry (Battin *et al.*, 2003; Santmire and Leff, 2007; Mendoza-Lera *et al.*, 2017). The structure of the streambed is characterized by the distribution of different grain sizes as well as their arrangement in the streambed (Figure 3). The latter defines the light penetration depth and, in turn, the spatial distribution of phototrophic communities in the streambed, as well as their structural composition and functionality (Santmire and Leff, 2007; Lowell *et al.*, 2009). In addition, the grain size distribution defines the area available for colonization, which in turn determines the biomass of the microbial community and thus the quantity of algal OM produced in the streambed. The sediment structure further influences the hyporheic flow, that is the velocity as well as the pathways, by which the surface water flows through the streambed. The hyporheic flow results from the hydraulic pressure gradient in the river bed that is formed by the spatial distribution of the different grain sizes, which are different permeable to water, and by existing bedding elements such as ripple and OM fragments (Mutz *et al.*, 2007), as well as turbulence near the bed (Higashino *et al.*, 2009). The hyporheic flow also transports nutrients, organic substrates, and redox partners dissolved in the water through the streambed (Mendoza-Lera *et al.*, 2017). Photosynthetic production of algal metabolites is restricted to the light mediated, surface area of the streambed, the benthic zone, whereas heterotrophic activities occur throughout the entire streambed. Hence variations in streambed structure will likely not

only affect the composition and function of streambed communities, but also the spatial availability of algal OM in the streambed and consequently influence the potential influence of algae OM on microbial leaf turnover at ecosystem scales.

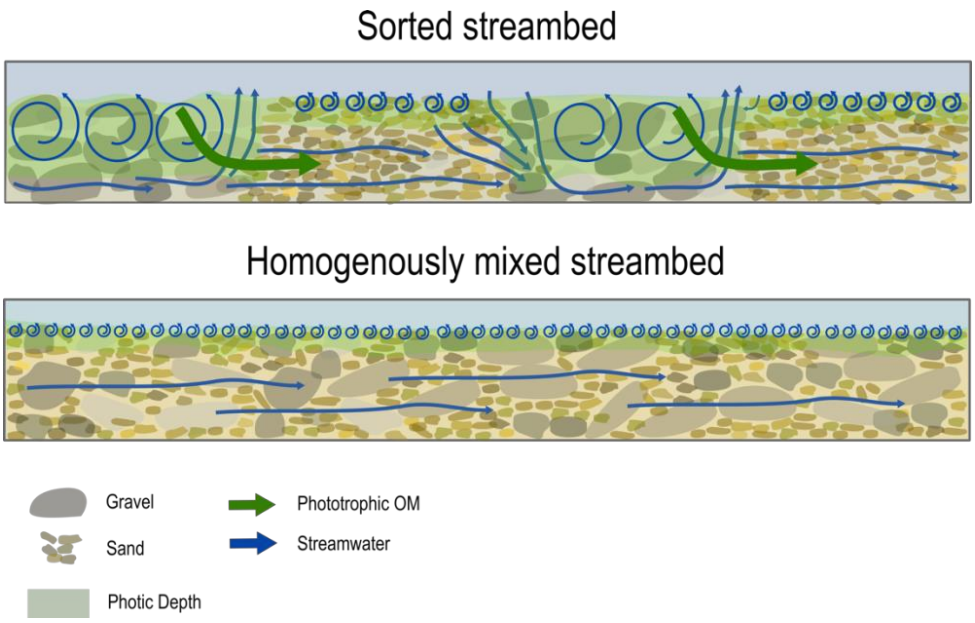


**Figure 3** Schematic overview of how variations in the arrangement and distribution of different sediment grain sizes change the hyporheic flow through the streambed. The different streambeds shown here correspond to the treatments used in the study of Paper III: a sorted versus a homogenous mixed arrangement of gravel and sand grains. A sorted arrangement enhances the hyporheic exchange of stream water across the sediment surface and favors areas of deeper photic zones. Both promote a spatial distribution of algal metabolites in the riverbed beyond its production in the photic zones.

## Objectives

Understanding the mechanisms, by which algae affect the utilization of particulate leaf OM by fungi and bacteria, and how these are modulated by environmental conditions, is the general focus of my thesis. I addressed that in three different experimental set-ups by focusing on two processes: First, the quantitative and relative use of C originating from particulate leaf litter for respiratory and assimilatory processes in microbial communities. Second, the contribution of bacterial and fungal organisms to community metabolism of leaf C, that is, their functional role in microbial processing of leaf OM.

In two laboratory studies, I investigated the mechanisms by which algal metabolites alter the turnover of leaf C within the microbial food web. In **paper I**, I investigated if the availability of dead algal biomass changes the microbial utilization of leaf C and whether observed relationships are associated with the C: P stoichiometry of the algal material. Furthermore, I looked at whether variations in microbial metabolism relate to shifts in the composition and function of the microbial community with respect to fungal-to-bacterial organisms. In this respect, I investigated whether the use of OM sources differs between bacteria and fungi. In **paper II** I focused more on understanding possible mechanisms by which algal exudates (DOM) affect microbial leaf turnover. I investigated whether algal DOM either generally stimulates bacterial cell metabolism or instead, relates to functional shifts within bacterial communities. Respectively, my focus was particularly on finding out how the use of algae and leaf material differs between the different bacterial groups.

In an outdoor study, which was conducted in flow channels, I addressed the question of how environmental conditions modify the occurrence of phototrophic- heterotrophic interactions during leaf turnover. In **paper III**, I examined how the structure of streambeds alters the influence of phototrophic processes on the metabolism of leaf C in the microbial food web, and what consequences this has for the fate of leaf C in ecosystem processes. This work is closely related to two additional papers (Zlatanović *et al.*, 2017, Fabian & Zlatanovic et al., submitted). The latter focused on the understanding of how the structure of the streambed affects the hyporheic flow and thus the availability of algal OM in the streambed.

*Research hypotheses*

1 Bacteria and filamentous fungi comprise different functions in microbial leaf turnover (Baldy *et al.*, 1995). The activity and production of degrading enzymes of both, fungal and bacterial cells is promoted through the uptake of algal OM (Rier *et al.*, 2014). However, bacteria have a significantly higher energy and nutrient requirement than fungi, and their ability to grow on structural complex OM is therefore more limited by additional energy and nutrient source (Romaní *et al.*, 2006).

Therefore, I assumed that the contribution of bacteria to leaf turnover is more stimulated by algal material compared to fungi.

2 The question remains as to how algae material controls leaf C utilization in bacterial communities. Accordingly, the high energy and nutrient content in algae material could stimulate microbial activity and, at the same time, enzyme production (Rier *et al.*, 2014). Yet, changes in the use of C sources from leaf OM could also be associated with both functional changes in the bacterial community, as well as shifts in their composition (Gómez-Consarnau *et al.*, 2012; Wagner *et al.*, 2015). Bacteria show an incredible diversity in their metabolic abilities and life strategies from strongly substrate-specific *("specialist")*, to substrate-unspecific, ("*generalist")*. Accordingly, bacterial groups differ in the strength with which they interact with algal material which is why photo-heterotrophic interactions are commonly assumed species-specific (Sarmento and Gasol, 2012; Horňák *et al.*, 2017). I therefore assumed, that algae OM promotes the assimilation of leaf OM for some bacterial groups, while the reverse is true for others, underlining associated functional shifts in the community.

3 Heterotrophic microorganisms homeostatically regulate their elemental composition in a relatively narrow range of characteristic biomass ratios C: P and N: P and therefore require nutrients in a certain ratio to the carbon source for assimilation (Elser and Kuang, 2002; Danger *et al.*, 2016). The nutrient content in leaf material is significantly lower than the microbial requirement (Keiblinger *et al.*, 2012; Rinkes *et al.*, 2014). Therefore, I assumed that the availability of additional nutrients, either bound in the algae material or dissolved in the surrounding water, determines whether the catabolized leaf C is primarily respired or assimilated. Equally, I assumed that the composition of the algae OM pool defines the fate of leaf C in the microbial cells metabolism.

4 Environmental factors shape the level of interaction between microbial photo- and heterotrophs. In headwater steams, benthic biogeochemistry is largely driven by biophysical processes in the streambed that in turn relate to the structural composition of the streambed (Battin *et al.*, 2003; Singer *et al.*, 2010; Perujo *et al.*, 2017). Microorganisms are tightly linked with biogeochemical cycles and ecosystem functions. I therefore assumed that variations in the streambed structure will modify the interaction of the heterotrophic community with algae OM and consequently, define the functional role of photo-heterotrophic in microbial leaf turnover at the ecosystem scale.

## Experimental Approach and Methods

I applied algae and leaf substrates, labelled with $^{13}C$ stable isotopes, to closely follow how the uptake of algal biomass is associated with variations in the quantitative and relative use of leaf C for respiratory ($CO_2$ production) and assimilatory (phospholipid derived fatty acids (PLFA), NanoSIMS) processes in microbial communities. Stable isotope analysis of individual PLFA biomarker allowed me to investigate variations in the source use of individual community members. In addition, I investigated variations in community PLFA profiles as proxy for variations in the structure, hence composition of microbial communities.

### Stable Isotopes Tracers

*– Methodical tool to follow microbial C assimilation and respiration –*

Stable isotope tracers are widely used to quantify the microbial transformation of a substrate and to link the identity of microorganisms with their functions (Dumont and Murrell, 2005). $^{13}C$-labeled organic C-compounds were the main approach to study the metabolism of algae and leaf OM by natural microbial communities from freshwater sediments.

Isotopes are atoms of a given element that vary in the number of neutrons in their nucleus, and hence in their atomic mass. The isotopes of a particular element occupy the same (greek *iso*) position (greek *topos*) in the periodic table. Both, stable and radioactive forms of isotopes exist for each element on earth, with the latter having half-times of minutes to seconds, though.

In the natural sciences, the stable isotopes of carbon (C), $^{12}C$ (mass 12) and $^{13}C$ (mass 13), are commonly applied to track processes during element cycling (Figure 4). Both stable isotopes have skewed distribution on earth, reflecting largely details of their synthesis long ago in stars (Fry, 2006). Accordingly, natural C pools constitute to 99.8944 % of the lighter element $^{12}C$ and to 1.1056 % of the heavier element $^{13}C$. Due to the low abundance of $^{13}C$ but nearly identical chemical behavior and stability of stable isotopes, variations in the abundance of $^{13}C$ in C-compounds are commonly used to study OM cycling (McAlpine*, 1971; Lehmann *et al.*, 2002). This includes, in particular, understanding the involvement of microorganism in underlying turnover processes.

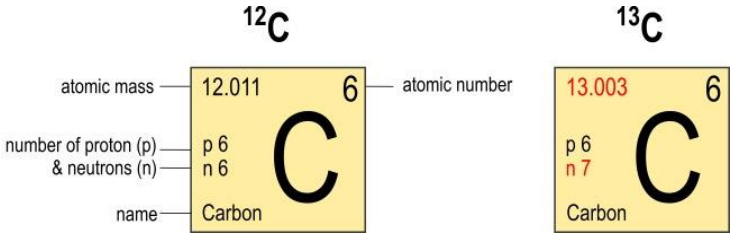


**Figure 4** Stable Isotopes of carbon applied in this thesis. Carbon-13 and carbon-12 differ in their number of neutron and thus in their atomic mass (highlighted in red).

The absolute abundance of an isotope is commonly reported either as its *fractional abundance F* in or in terms of *atom percent %* in the target element pool (Hayes, 2004).

$$^{13}F = \frac{^{13}C}{^{13}C + ^{12}C}\,;\; ^{13}F \times 100 = atom\ \% \qquad \textbf{(1)}$$

Both *fractional abundance* and *atom %* may be applied for accurate mass balance calculations, while it has become conventional to express Isotope abundances using a differential notation, the *delta notation* $\delta$.
As an exception, the $\delta$ notation is commonly applied when dealing with tracer concentrations of either natural abundance or below 1 atom %. The $\delta$ values expresses the abundance of a particular isotope in a sample element pool, relative to the abundance of the same isotope in a reference element pool, an international standard. For carbon the international standards are PeeDee Belemite (PDB) and Vienna-PDB (VPDB). $\delta$ is calculated as follows:

$$\delta = \left[\left(\frac{R_{SAMPLE}}{R_{STANDARD}} - 1\right)\right];\; R = \frac{^{13}F}{^{12}F} \qquad \textbf{(2)}$$

$R$ is the ratio of the abundance ratio of the heavy $(^{13}F)$ to light $(^{12}F)$ isotope in the sample $(R_{SAMPLE})$ and standard $(R_{STANDARD})$ element pool. Isotopic variations are expressed either as ratio without unit or in parts per thousand and assigned the unit ‰ (permil, from the Latin *per mille*) by multiplying equation 2 with 1000 as follows:

$$\delta[‰] = \left[\left(\frac{R_{SAMPLE}}{R_{STANDARD}} - 1\right) \times 1000\right];\; R = \frac{^{13}F}{^{12}F} \qquad \textbf{(3)}$$

As elements circulate through the biosphere, the combination of isotope mixing, and process-specific fractionation of enzymes for the heavier or lighter isotopes, changes the natural isotopic composition of the process-related element pools. This in turn leads to characteristic $^{13}C$ isotope patterns across the various C

pools on earth that provide information about origin and history and with regard to microbial processes about their metabolic linkage and the organisms involved (Peterson and Fry, 1987). Yet, we often face an overlap in the isotope composition of different C containing compounds that complicate estimation on microbial function based on natural stable isotope abundances. In addition, changes in isotope composition by process related fractionation is often too small to elucidate changes in specific pathways and to identify the microorganisms in environmental samples that use a particular growth substrate (Peterson and Fry, 1987). Stable isotope labeling, or tracing, is a technique that is widely used to overcome problems with natural isotope abundances when for example specifically identifying the microorganisms in environmental samples that use a particular growth substrate. In the case of carbon, the method is based on the microbial transformation of a substrate added to the natural OM pool, which is highly enriched in the $^{13}$C-stable isotope (Dumont and Murrell, 2005). Subsequent analysis of the $^{13}$C-carbon tracer in product pools can then provide information about nutrient pathways mediated by the microorganism group of interest. In addition, the selective recovery and analysis of the $^{13}$C-carbon tracer in cellular compounds facilitates the identification and differentiation of metabolically active microorganisms within a community (Neufeld *et al.*, 2007; Dijkman *et al.*, 2009). In this thesis, microbial turnover of $^{13}$C-labeled algal OM and leaf OM was evaluated under varying aspects.

## Stable Isotope Analysis

Traditionally, the abundance of different stable isotopes in a particular element pool was quantified by mass spectrometry (Muccio and Jackson, 2009). Briefly, the C pool of interest is first converted to gaseous $CO_2$ molecules, either by combustion, gas chromatographic feeds, or chemical trapping in an elemental analyzer coupled to the mass spectrometer via an interface (Brenna *et al.*, 1998; Meier-Augenstein, 1999). Upon entrance to the mass spectrometer, $CO_2$ molecules are ionized and subsequently separated and deflected in a magnetic field according to their masses to a detector. In this thesis, I applied different types of elemental analyzer and mass spectrometers to analyses various C-compounds in the dissolved or particulate phase (Table 1). Today, *Elemental Analysis - Isotope Ratio Mass Spectrometry (EA-IRMS)* and *Gas Chromatography (GC) - IRMS* are common facilities for stable isotope analysis due to their automated systems and lower analysis cost (Werner Roland and Brand Willi). Yet, particular in aquatic microbiology, the application of *Secondary Ion Mass Spectrometry (SIMS)* has greatly increased. This technique makes it possible to analyze stable isotope abundances of individual microbial cells and thus to investigate metabolic activities at the cellular level in natural microbial assemblages (Ploug *et al.*, 2010; Adam *et al.*, 2011).

**Table 1** Overview of the applied measuring instruments and methods for the analysis of stable isotopes from different sample matrices. Both different types of mass spectrometers (MS) were used, as well as laser absorption spectroscopy. Typical MS measurement precisions for $\delta^{13}C$ in $CO_2$ are 0.02–0.1‰ depending on the methods used, and for laser absorptions spectroscopy between 0.2-5 ‰.

| Method | | Sample | C-Species | Target process | Preservation | Paper |
|---|---|---|---|---|---|---|
| Elemental Analysis-Isotope Ratio Mass Spectrometry | EA-IRMS | solid | $^{13}C$-POC<br>$^{15}N$-PON | Concentration of $^{13}C$ in particulate organic matter | Freeze-dried | **I, II** |
| Gas Chromatography Isotope Ratio Mass Spectrometry (or Trace gas- IRMS) | GC-IRMS | liquid | $^{13}C$-DOC<br>$^{13}C$-DIC | Concentration of $^{13}C$ in respired (DIC) and decomposed OM | 0.2 µm filtrated, stored cool | **I - III** |
| Gas Chromatography-combustion Isotope Ratio Mass Spectrometry (Compound specific isotope analysis) | GC-c-IRMS | liquid | $^{13}C$-FAME | $^{13}C$ incorporation into phospholipid derived fatty acids | Freeze-dried | **I - III** |
| Nanoscale Secondary Ion Mass Spectrometry | Nano-SIMS | solid | $^{13}C$-OM | $^{13}C$ and $^{15}N$ incorporation into single cells | Resin embedded samples | **II** |
| Off-Axis Integrated Cavity Output Spectroscopy | Off-Axis ICOS | gas | $^{13}C$-$CO_2$ | $^{13}C$ in gaseous $CO_2$ | None (Automatic headspace sampling) | **I, III** |

EA-IRMS is a standard technique for the isotopic composition of particulate and dissolved organic C (Gandhi *et al.*, 2004; Kaplan *et al.*, 2008). GC-IRMS is widely used for C isotope analysis of $CO_2$ in gaseous samples and of its dissolved compounds ($CO_2$, $H_2CO_3$, $HCO_3^-$, $CO_3^{2-}$) in water samples. Gas chromatography-combustion isotope ratio mass spectrometry (GC-c-IRMS) is increasingly applied for analyzing the stable isotopic ratios of individual compounds, as in this thesis, for the analysis of phospholipid derived fatty acid (PLFA) biomarkers. Besides mass spectrometry, laser absorption spectrometry is gaining in popularity in the natural sciences as method for stable isotope analysis due to the online applications in field surveys at minimal loss of accuracy, as well as the ease of use of the equipment and low maintenance costs.

Compound specific isotope analysis and Off-Axis Integrated-Cavity Output Spectroscopy (Off-Axis ICOS) have been intensively applied to study catabolic and anabolic processes, respectively. Both techniques are therefore presented in more detail in the next section.

## Phospholipid-Linked Fatty Acid Biomarkers

*- Chemotaxonomic markers for natural microbial communities -*

Phospholipid-derived fatty acids (PLFAs) are widely used in microbial ecology for total biomass estimation and as chemotaxonomic markers for observing broad changes in the composition of the living microbiota (White *et al.*, 1979; Willers *et al.*, 2015). In addition, stable isotope analysis of individual fatty acids enable the identification of species involved in specific processes during nutrient cycling, as applied in this thesis (Boschker *et al.*, 1998; Boschker and Middelburg, 2002). The basic premise is, that PLFAs decompose within weeks of cell death, thus providing information on viable or recently viable communities. In addition, the composition and occurrence of particular PLFAs varies between individual microbial groups, which in turn provides information about the composition of the microbial community (Boschker and Middelburg, 2002).

Phospholipids are an essential structural component of all microbial cell membranes (Figure 5 A). They belong to the class of lipids and have amphiphilic properties, hence they have both hydrophilic (water-loving, polar) and lipophilic (fat-loving) properties. The structure of the phospholipid molecule generally consists of two hydrophobic fatty acid "tails" and a hydrophilic "head" consisting of a phosphate group. The two components are linked together by a glycerol molecule. PLFAs differ in their length, that is, in the number of carbon atoms, in their branches and in the chemical bonds between the carbon atoms. These characteristics emerge from the biochemical nomenclature of the respective fatty acid:

$$A{:}\,B\omega D \tag{4}$$

where $A$ represents the length of the carbon chain, and $B$ refers to the number of double bonds. Unsaturated fatty acids have one or more C = C double bonds, with its position indicated by number $D$. The prefixed symbol $\omega$ (omega-nomenclature) or $\Delta$ (delta-nomenclature) indicates whether the position of the first double bond was determined from the aliphatic end or from the carboxyl end, respectively. Using the ω notation, iso-branched fatty acids have methyl group at positon n-1, while anteiso- branched fatty acids

have a methyl group at n-2. It is common for polyunsaturated fatty acids to indicate only the position of the first double bond. Furthermore, the prefix "cyclo" symbolizes a cyclic chain formation.

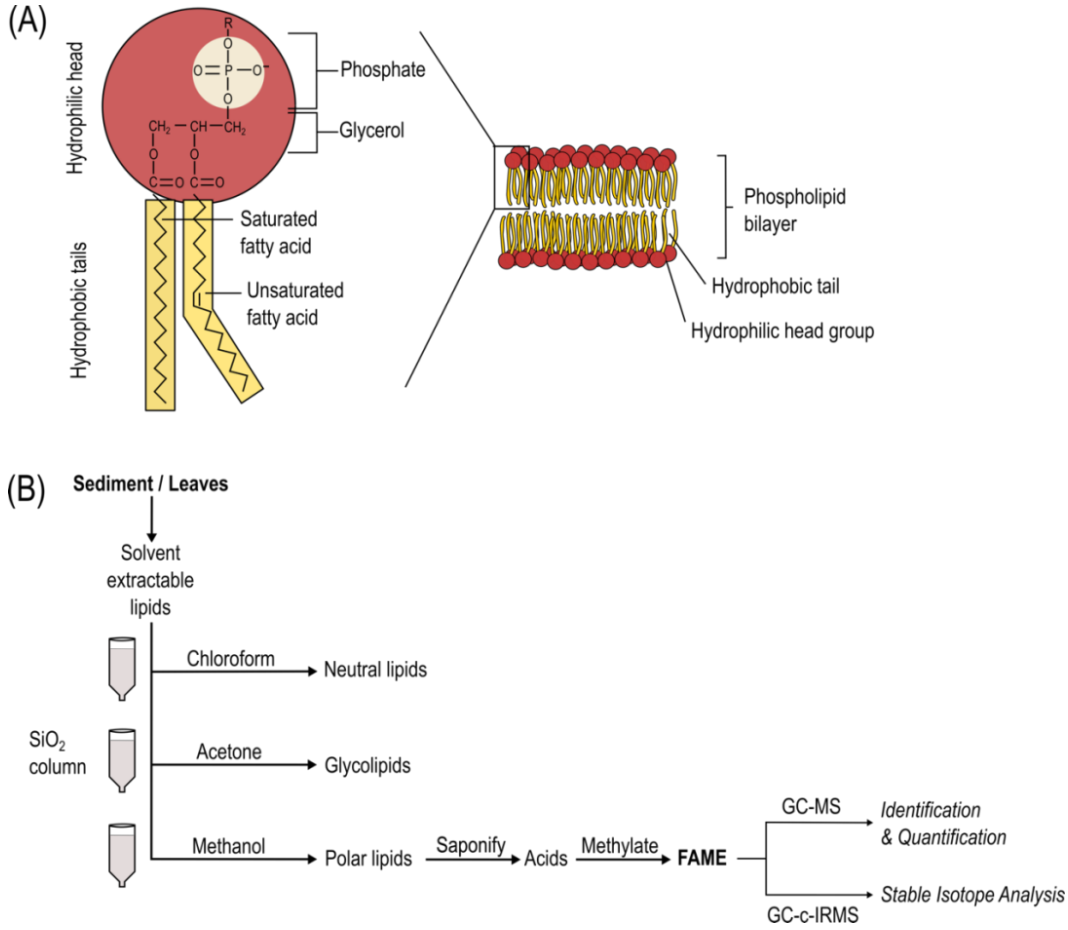


**Figure 5** (A) Overview of the structural composition of a phospholipid that forms the phospholipid bilayer of cell membranes. Modified after *Marc T. Facciotti (https://bio.libretexts.org/4.2:Lipids).* A phospholipid is a molecule with a modified phosphate group and two fatty acids bound to a glycerol backbone. The phosphate can be modified with charged or polar chemical groups (R). (B) Schematic overview of the extraction method of phospholipid fatty acids from the sediments and leaves as applied in this work.

Certain fatty acid side chains are synthesized only by selected microbial groups (Boschker *et al.*, 2002). Methyl-branched fatty acids are common constitutes of bacterial phospholipids and identified by the prefixes "iso" and "anteiso", depending on whether the branch point lies on the penultimate (ω-1) or on the ante-penultimate (two of the end or (ω-2)) carbon atom. Other commonly applied bacterial biomarkers are cyclopropane fatty acids (e.g. cyclo 17:0), as also almost exclusively found in bacterial organisms. On the other hand, polyunsaturated fatty acids (e.g.18:4ω3, 16:3ω4, 18:3ω6, 18:2ω6) are characteristic for algae

and cyanobacteria. Yet most of the algal fatty acids are also found in a variety of fungal groups. Particularly, 18:2ω6 is a commonly used biomarker for filamentous fungi (*Ascomycota* & *Basidiomycota*) in terrestrial soils. In aquatic systems, however, the application of fungal biomarkers in combination with stable isotope analysis is so far only possible in absence of viable algal and cyanobacterial cells due to their overlapping PLFA profiles.

For PLFA analysis (Figure 5 B), all solvent-extractable lipids are first extracted from dried samples using a single-phase organic extractant (Bianchi and Canuel, 2011). Depending on the sample matrix, different extraction methods are common. For aquatic sediments, a 2-4 hour extraction using a chloroform / methanol / buffer mixture is typically applied (Bligh and Dyer, 1959; Steger *et al.*, 2011). The extracted lipids are first separated from other compounds like proteins, carbohydrates via a two-phase formation of water and organic phase. Subsequently, the solvent-extractable lipids are fractionated on a solid phase extraction column and the phospholipid fraction is separated from neutral lipids, glycolipids, as well as free fatty acids and other materials. The fatty acid chains are then separated from their glycerol skeleton by a saponification step and methylated to fatty acid methyl esters (FAMEs) for subsequent analysis by GC-MS and GC-c-IRMS. For this thesis, mild alkaline transesterification was applied.

Prior to stable isotope analysis, FAMEs are identified via analysis on an GC-MS system, which is a gas chromatograph coupled to a mass spectrometer. FAMEs are usually analyzed by gas chromatography flame ionization using a polar column (Boëchat *et al.*, 2014). In the gas chromatograph, the FAMEs pass into the gas phase via flame ionization and pass through a polar capillary column by means of a carrier gas, typically helium. During the passage of the column, the ionized FAME molecules are retained by the column matrix to different extents and thus elute from the column at different times (called the retention time). In the downstream mass spectrometer, the ionized molecules are captured separately and broken down into fragments by ionization. The fragments are then accelerated and deflected in a magnetic field according to their mass-to-charge ratio and finally detected. FAMEs are identified by their mass spectra and retention times, which are validated with standard FAME or by comparing their equivalent chain lengths with literature reports for the column used (Eder, 1995; Santercole *et al.*, 2012). Fatty acid standards are used for system calibration.

Stable isotope analysis of FAMEs is undertaken on an GC-c-IRMS system. A GC is coupled to an IRMS via a miniature oxidation reactor in which FAMEs are oxidized to $CO_2$ after passaging through the GC column. Water is removed on-line and the purified $CO_2$ is passed into the Isotope Ratio Mass Spectrometer (IRMS). Finally, Isotope data is corrected for the carbon atoms added during derivatization.

## Off-Axis Integrated-Cavity Output Spectroscopy

*– A novel technique in stable isotope analysis –*

As with conventional laser absorption spectroscopy, the Off-Axis ICOS technique is based on directing a laser beam through a material. The light energy absorbed by the material represents the attenuation of the radiation intensity following Beer's law.

In the Off-Axis ICOS technique, a diode laser is pulsed through a cell containing sample gas such as air under controlled temperature and pressure conditions. The diode laser is tuned to a certain narrow emission band which coincides with a single absorption line of a particular molecule of interest. The line width of the laser emission is much smaller than the absorption line width of the molecule. This in turn allows the acquisition of high-resolution absorption spectra and the selective distinction between the individual components of a sample mixture without interference from other molecules. This is especially true for low molecular concentrations where the absorption lines are narrower. After passing through the cell, the laser beam strikes a detector which measures the energy losses (Bowling *et al.*, 2003).

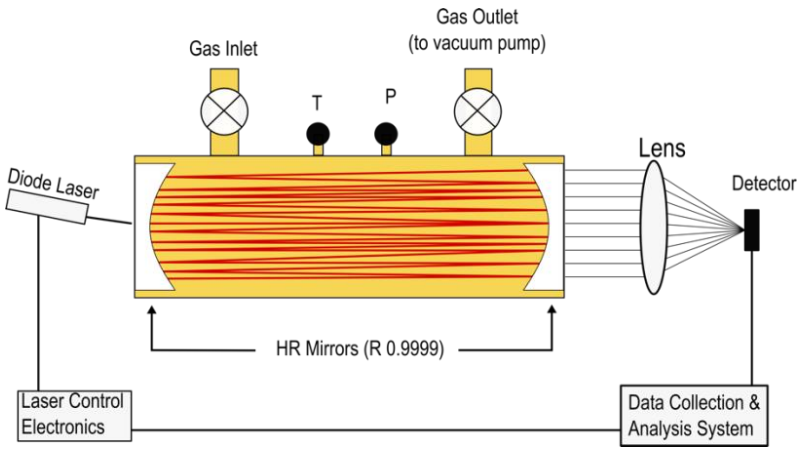


Figure 6 Schematic diagram of off-axis ICOS instrument. The optical path length of the laser is largely extended by highly reflective mirrors.

The sensitivity of laser absorption spectroscopy depends on the absorption strength of the chosen conduit and on the length of the absorption pathway. Based on the low abundance of $^{13}C$-$CO_2$ isotopes in natural samples, normal laser absorption spectroscopy does not allow accurate down-measurement. However, by using highly reflective resonator mirrors with 99.99% reflectivity in the measuring cell, the optical path length through the measuring cell is extremely prolonged (Figure 6). This in turn dramatically increases the sensitivity of measurable absorption after passing through the cavity and thus allows for

ultrasensitive trace gas measurements. The subsequent integration of the measured spectra together with measured values of the gas temperature, pressure, path length and the line thickness of the investigated transition make it possible to determine the mixing ratio of the gas.

I determined by laser absorption spectrometry the isotopic composition in $CO_2$ respired by microorganisms. For this purpose, incubation chambers were connected with a carbon dioxide isotope gas analyzer (CCIA, Los Gatos Research, Mountain View, California, USA) via Valco selectors (VICI AG, Schenkon, Switzerland). The headspace of the incubation chamber was sampled automatically at different time intervals. The incubation chambers opened and closed automatically before and after measurements by a pressure valve installed on the headspace lid. Variations in the concentration and isotopic composition of atmospheric $CO_2$ in the headspace during incubation were then further processed by Keeling-Plot analysis to determine the isotopic composition of $CO_2$ produced via microbial respiration (Pataki *et al.*, 2003). Respectively, the concentration of a substance measured in the ecosystem ($c_a$) constitutes the combination of the background substance concentration ($c_b$) and the additional concentration generated by the source ($c_s$), which increases the substance concentration above the background.

$$c_a = c_b + c_s \tag{5}$$

These arguments and application equally apply to molecule concentrations and isotope ratios. Given conversation of mass,

$$^{13}\delta_a[CO_{2\,(a)}] = {}^{13}\delta_b[CO_{2\,(b)}] + {}^{13}\delta_s[CO_{2\,(s)}], \tag{6}$$

where $^{13}\delta$ represents the C isotope ratio of each $CO_2$ component. Combining equation 5 and 6,

$$^{13}\delta_a = [CO_{2\,(b)}]({}^{13}\delta_b - {}^{13}\delta_s)(1/[CO_{2\,(a)}]) + {}^{13}\delta_s, \tag{7}$$

where $^{13}\delta_s$ is the integrated values of the $CO_2$ source in the ecosystem, as illustrated graphically in Figure 7. In Paper II and III, I additionally applied the keeling plot approach to variations in the concentration and isotopic ratio of dissolved inorganic carbon (DIC: $H_2CO_3$, $HCO^-$, $CO_3^{2-}$) in the water column of the ecosystem (Karlsson *et al.*, 2007).

(B)

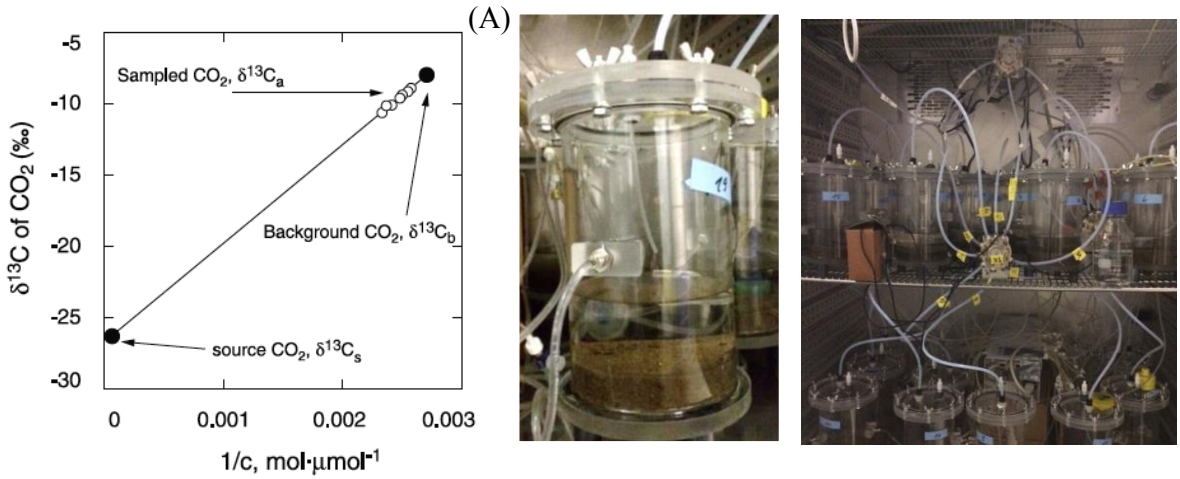


**Figure 7** (A) Keeling plot approach: The inverse of the increase in $CO_2$ over time due to respiration is plotted against the related change in the stable isotopic signature of $CO_2$ and the isotopic signature of the source $CO_2$ evaluated from the intercept. Source: (Pataki *et al.*, 2003) (B) Overview of the automatic sampling system implemented for this thesis. Incubation chambers were connected with a carbon dioxide isotope gas analyzer (CCIA, Los Gatos Research, Mountain View, California, USA) via Valco selectors (VICI AG, Schenkon, Switzerland). The headspace of the incubation chamber was sampled automatically at different time intervals.

## Central Results & Discussion

Photoautotrophs can considerably influence the incorporation of terrestrial C into aquatic pools, not only on ecosystem scales but particularly at the microbial level (Figure 8). Respectively, results confirm that photosynthetic metabolites influence heterotrophic turnover of leaf C in microbial communities. At the same time, however the outcomes of my work emphasize that the actual extent of this interaction is determined by several additional factors, which are both of abiotic and biotic nature.

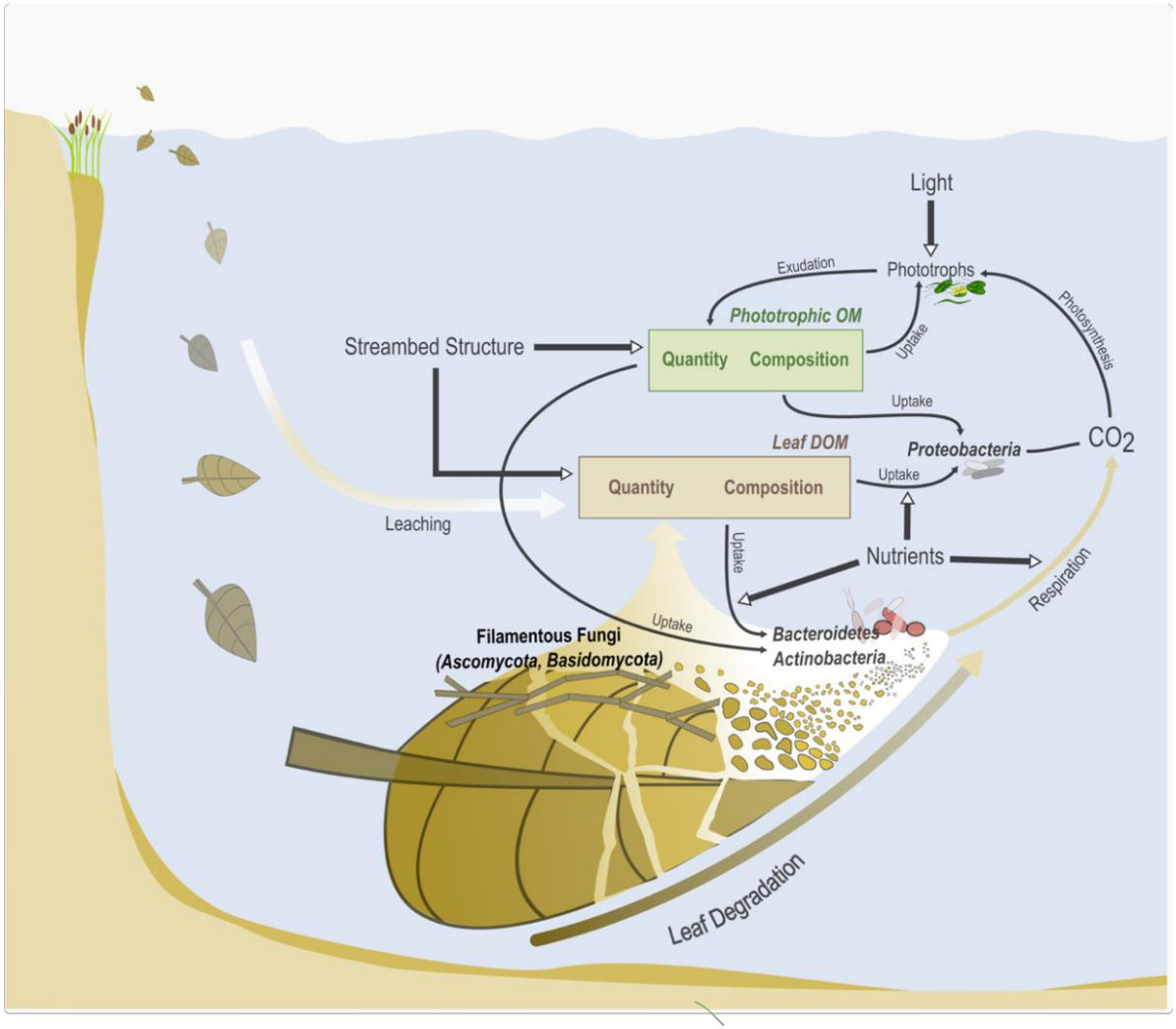


Figure 8 Schematic overview on the processes investigated in my thesis and how these contribute to the decomposition of leaf litter in aquatic environments.

## Algae Induced Shifts in the Contribution of Bacteria to Leaf Turnover

Indeed, previous work indicate that both, fungi and bacteria, assimilate algae OM (Kuehn *et al.*, 2014; Soares *et al.*, 2017). Yet, fungi and bacterial activities in leaf turnover were not equally stimulated by algal OM. Accordingly, algae OM promoted the occurrence of bacteria over fungi within degrader communities (**Paper I**). In addition, while algae-OM promoted the bacterial assimilation of leaf C, fungal assimilation of leaf C remained almost exclusively unaffected (**Paper I, II**). Rather, it seems that fungi either take up algal compounds via the metabolic exchange with bacteria, or use the degraded algal C primarily for energy production, i.e. predominantly for C respiration, and not assimilation **(Paper II)**.

The reasons for algal OM promoting primarily bacterial but not fungal use of leaf C are manifold. One possible explanation may be the generally distinct physiology between bacterial and fungal species. In general, fungi have longer generation times and process matter at slower rates than bacteria (Moore *et al.*, 2005). Fast growing microorganisms need more nutrients and energy than slow growing microorganisms (Makino *et al.*, 2003), wherefore I assumed that bacteria respond generally more sensitive to the availability of additional energy sources than fungi. Another possible explanation may be the occurrence of antagonistic behavior of bacteria against fungi, which was shown to be pronounced when highly bioavailable OM becomes accessible resulting in the inhibition of fungal activity (Mille-Lindblom and Tranvik, 2003; Wargo and Hogan, 2006). This assumption is supported by the associated increase in the occurrence of bacteria over fungi within degrader communities **(Paper I, II)**. Yet, variations in fungal-bacterial ratios were primarily driven by an increase in bacterial biomass while I observed only a marginal decrease in fungal biomass when algae OM became accessible **(Paper I)**. Furthermore, measurements of antagonistic interactions between fungi and bacteria are limited to laboratory studies and it is not clear whether production of antagonistic substances truly occurs under natural conditions (Gulis and K. Suberkropp, 2003). Both argue rather against antagonistic interactions between bacteria and fungi. Instead, resource competition between bacteria and fungi seems more likely to suppress the availability of algae OM for fungi. Respectively, bacteria successfully compete with fungi for the acquisition of highly bioavailable substrates, most likely due to their higher metabolic rates (Fontaine *et al.*, 2003; Mille-Lindblom *et al.*, 2006).

## Functional and Structural Variations in Bacterial Communities

A vast variety of existing research proved evidence for a promoted utilization of structurally complex organic compounds by readily bioavailable ones (Blagodatskaya and Kuzyakov, 2008; Guenet *et al.*, 2010). Nevertheless, the underlying mechanisms that drive this so called priming effect in aquatic ecosystems

remain little understood (Bengtsson *et al.*, 2018). The same applies to the observed influence of algae matter on the utilization of leaf C by microbial heterotrophs. The results of my work indicate that the stimulatory effect of algae OM on the turnover of leaf C in the microbial loop is associated with functional shifts towards a greater contribution of bacteria to leaf turnover processes. Yet, the question arises whether

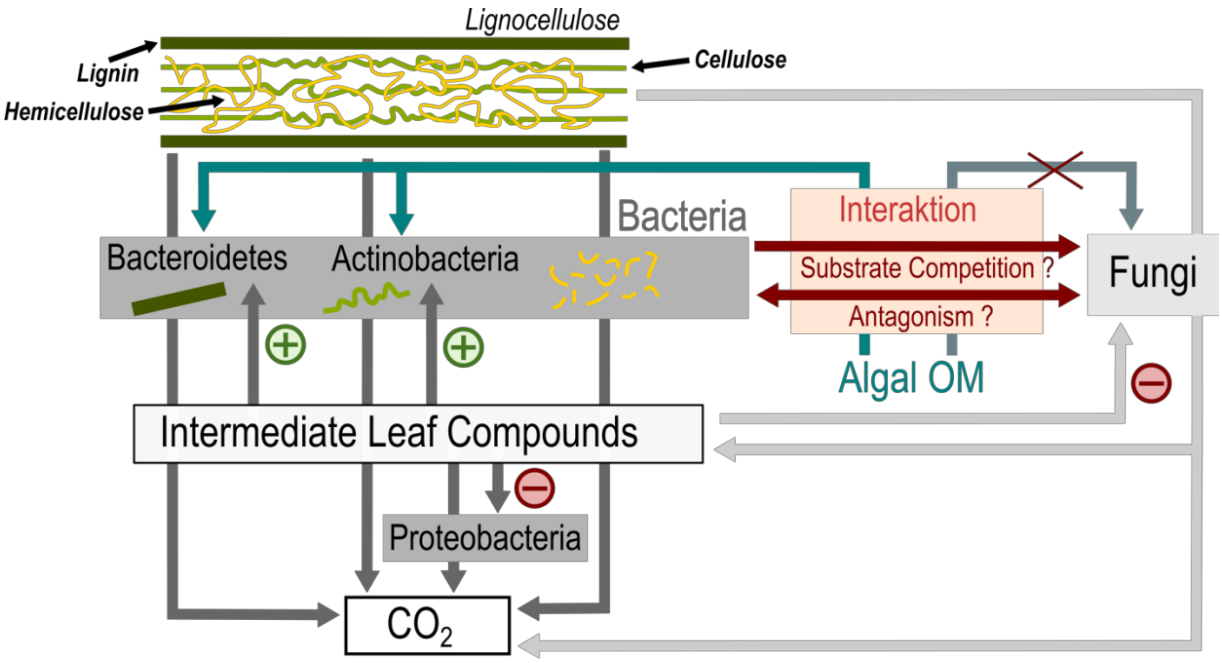


**Figure 9** Algal induced functional shifts in bacterial communities. + and – indicate algal induced effects on the uptake of leaf C into bacterial biomass. The results of this work imply that fungi do not assimilate algal carbon. One possible reason could be interactions between fungi and bacteria.

promoting effects are related to an overall increase in the assimilation of leaf C into bacterial cells, or instead are associated with functional and structural changes of bacterial communities.

In general, this entire bacterial community appears to take up the algal material **(Paper I)**. However, neither the uptake of the algae material nor the associated effects on the uptake of leaf C were equally pronounced among the different bacterial groups **(Paper I, II)**. Only *Bacteroidetes* and *Actinobacteria* increased their intake of leaf C in presence of algal OM, while the substrate use of *Proteobacteria* shifts from leaf to algal C **(**Figure 9, **Paper II)**. These findings are consistent with similar work (Wagner *et al.*, 2015), and emphasize that bacteria growing on leaf OM react differently to the availability of phototrophic metabolites. While *Bacteroidetes* and *Actinobacteria* contribute to the decomposition of cellulose and lignin into smaller intermediate leaf compounds, *Proteobacteria* grow on these pre-degraded leaf compounds (McNamara and Leff, 2004; Battin *et al.*, 2016). Respectively, observed differences among bacterial groups

likely relate to their distinct contribution to the decomposition of leaf OM, hence depend on their catabolic capability.

It is often unclear whether resource-driven functional changes in microbial communities are due to variations in their composition or, instead, to an adaptation of the metabolic performance of microbial cells (Findlay *et al.*, 2003; Langenheder *et al.*, 2005). Variations in the PLFA profiles indicate shifts in community composition with respect to the presence of algae OM in the substrate pool, as well as the nutrient composition of the algal substrate itself **(Paper I, III)**. These findings agree well with earlier work (Wagner *et al.*, 2014, 2015), and hence emphasize that resource-dependent changes in the functioning of microbial communities are associated to changes in their composition.

## Environmental Effects on Photo-Heterotrophic Interactions

A growing body of studies investigated the influence of algal activities on heterotrophic C utilization from structurally complex OM; yet, with varying results (Danger *et al.*, 2013; Bengtsson *et al.*, 2014; Rier *et al.*, 2014; Wagner *et al.*, 2017). The results of this work suggest that these variations are due to additional factors that shape the nature and strength of photo-heterotrophic interactions and, consequently, their relevance to the turnover of leaf C at the ecosystem scale. Factors include differences in the composition of photo- and heterotrophic communities, in the availability of nutrients, and indirect effects from variations in physicochemical environmental conditions.

*– Variations in the nature of photo-heterotrophic interactions –*

Recent evidence exists, that algal C is selectively associated with respiration, while terrestrial C is favorably associated with biosynthesis and thus assimilated (Guillemette *et al.*, 2016). These results are partly confirmed in the studies of this thesis **(Paper II, III)**. However, the availability of dead, particulate algal biomass primarily stimulated the respiration of leaf C **(Paper I)**, while dissolved algae derivatives particularly promoted incorporation of leaf C into cell compartments **(Paper II)**. Observed differences could be related to variations in the composition of the algae material. Carbohydrates are the major constituents of both particulate and dissolved algae OM (Myklestad, 1995; Espeland *et al.*, 2001; Kragh and Søndergaard, 2004). Nevertheless, the carbohydrate fraction in algal POM is two to three times higher than in algal DOM, the latter containing a higher level of nutrients and low molecular weight compounds (Biddanda and Benner, 1997). Therefore, algal DOM is likely to be generally more bioavailable and has a higher energy value than algae POM and, consequently, more strongly promote the assimilation of the low energy leaf material.

On the other hand, the observed differences in the metabolism of leaf C could also be due to variations in nutrient availability such as phosphate (**Paper I**, Rier *et al.*, 2014), as these differed greatly between the two studies **(Paper I, II)**. This accounts in particular for promoting effects of algal OM on the assimilation of leaf C into microbial biomass **(Paper I)**, as microorganisms sustain a C to nutrient supply in a fixed stoichiometric ratio for biomass production (Elser *et al.*, 2000; Danger *et al.*, 2016). The C: N ratio of the leaf material is well below the needs of the microorganisms. Whether C from the catabolized leaf compounds is exclusively mineralized for energy production or additionally assimilated is therefore controlled by the availability of nutrients. Respectively, I could only observe a positive effect of algae OM on the assimilation of leaf C under adequate nutrient supply **(Paper II, III)**. In addition, an increase in P content in the particulate algal material resulted in a decrease in leaf fraction on C mineralization **(Paper I)**. Thus, while it may be speculated that the energy provided by the algal material promotes degradation of leaf molecules through a promoted enzyme production (Romaní and Sabater, 2000; Rier *et al.*, 2014), their use in cellular metabolism depends on other factors, such as the molecular composition of the algal substrate and the availability of additional nutrient sources in the ecosystem.

Another influential factor is the composition and diversity of the heterotrophic community. Organisms prefer different organic substrates for a particular metabolic process, due to their different physiological properties (del Giorgio and Cole, 1998). Our method approach did not allow us to disentangle catabolic processes between the different heterotrophic organism groups. Yet, results underline that the strength of the algae material stimulating the assimilation of leaf C into microbial biomass relates to the composition of the microbial community **(Paper I, III)**. This assumption is even further supported by the fact that neither the use of the algae material nor the associated effects on the metabolism of leaf C were equally pronounced among bacterial species (**Paper II**).

*– Variations in the strength of photo-heterotrophic interactions –*

Light availability is the prerequisite for the occurrence of interactions as algal photosynthesis depends on light energy (Wetzel, 2001). Accordingly, light influences the quantity of available dissolved and particulate algal OM in the ecosystem and thus the occurrence of photo-heterotrophic interactions *per se* (Danger *et al.*, 2013; Rier *et al.*, 2014; Wagner *et al.*, 2017, **Paper II)**. In addition, light availability determines the structure and cell metabolism of phototrophic communities (Wetzel, 2001) and hence, secondarily influences the compound composition of algal OM, as it varies significantly between species, and in relation to the growth and physiological state of the algae (Granum et al., 2002, Biersmith and Benner, 1998). This in turn affects the affinity of heterotrophic communities for algae OM, as the different heterotrophic species prefer different algal compound classes (Sarmento *et al.*, 2016; Horňák *et al.*, 2017). Hence, algae OM is not equally available to microbial communities, depending on the composition of the

algae material and the species composition of the heterotrophic community (**Paper II**, Sarmento and Gasol, 2012; Horňák *et al.*, 2017). In addition, due to specific associations between algae and bacteria (Sarmento and Gasol, 2012; Horňák *et al.*, 2017), variations in the composition of both the communities most likely affect the extent of resource-driven changes in the heterotrophic processing of leaf C.

Given that leaf degradation is primarily occurring in the sediment, I experimentally examined how the sediment composition of streambeds alters the influence of photoautotrophic processes on the metabolism of leaf carbon in the microbial food web, and what consequences this has for the fate of leaf C in ecosystem processes. My findings underline that the sediment composition of the streambed may interact with light availability as a driving factor for photo-heterotrophic interactions during leaf turnover **(Paper III)**. Spatial variations in grain size distribution in streambeds stimulated the hyporheic transport of algal metabolites across the streambed and in turn extended the spatial availability of algal metabolites in the streambed beyond their production areas **(Fabian & Zlatanovic *et al.*, unpublished)**. Furthermore, grain size distribution in streambeds defines the composition of microbial streambed communities and, concomitantly, the strength and nature of the interaction between photo- and heterotrophic streambed communities **(**Zlatanović *et al.*, 2017, **Paper II, III)**. The results of this study generally emphasize that the environment has a significant impact on how relevant photo-heterotrophic interactions are for ecosystem level leaf disintegration.

## Summary and Outlook

Overall, my thesis provides a comprehensive insight into the functional role of algal OM in microbial leaf C turnover in aquatic environments, and how this role is modified by the prevailing abiotic and biotic conditions. The use of compound-specific stable isotope analysis proved to be beneficial in demonstrating that the contribution of bacteria, but not fungi, to leaf OM turnover is likely to be positively affected by algae OM. At the same time, however, findings also provide evidence that the various bacterial groups do not uniformly interact with algae OM and in turn emphasizes distinct shifts in their functional role in leaf litter breakdown. Furthermore, the results of my experimental investigations emphasize that algal OM influences both the assimilation and the respiration of leaf C, whereby the actual metabolic use is influenced by the abiotic and biotic environment, such as nutrient availability. Hence, environmental factors are likely to contribute to the actual fate of leaf C in microbial food webs by affecting both the strength of photo-heterotrophic interactions and metabolic use of leaf C.

1 Given the multitude of interactions by which microorganisms interact with their surroundings, multifactorial studies are needed to truly estimate the effect of algal OM on leaf C turnover in natural ecosystems. Only a sufficient understanding of how photo-heterotrophic interactions

vary according to simultaneous variations of multiple environmental factors allows a comprehensive understanding of the relevance of phototrophic-heterotrophic interactions for leaf C turnover in freshwaters.

2 Due to methodological restrictions, the focus of this work was on the contribution of filamentous fungi to leaf turnover, besides heterotrophic bacteria. However, much more fungal species are likely to be involved in leaf disintegration. For the most part, little is known about their ecological function in aquatic systems, but this does not necessarily mean that they do not functionally play a role in leaf degradation. Given observed differences between fungi and bacteria species, this work encourages the focus on other fungal species as well, such as *Chytridiomycota*, to allow an integrated response of the whole leaf degrading community to algal OM.

3 The same applies to bacterial groups. In this work mainly compound-specific analyses of phospholipids were applied. Although these allow a glimpse into the phylum level, from a taxonomic point of view, they provide a rather limited picture of detailed functional changes in the community. The combination of protein and / or DNA analysis with stable isotope tracers would be very promising to tackle resource driven variations in microbial functions at high taxonomic resolution.

4 Algal C and N are likely to be utilized differently. Leaf subsidies can be both an important C and nutrient source for the aquatic system. Therefore, this work recommends conducting studies in which both pools are labeled isotopically. Such an approach would significantly expand existing knowledge by giving not only insights into the fate of C but also in the fate of nutrients in biogeochemical cycles of freshwaters and how both are simultaneously affected by algae activities.

5 While the general functions of mycelia fungi and bacteria in leaf C turnover are well described, phyla-specific and especially species-specific functions remain blurry and poorly known. Yet, ample evidence is given, including the results of this work, that resource driven effects on leaf C turnover relate to functional shifts of microbial communities. This work therefore encourages further examination of species-specific association between algae and bacteria during leaf C turnover to better constrain resource driven variations in microbial functions to OM turnover.

6 And last, this work encourages further examination of algae induced interactions between fungal and bacterial species on spatial scale. There is growing evidence about intraspecific variations in interspecific interactions among microbial species that may relate to spatial associations between fungal and bacterial species or even among bacterial or fungal species.

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## STATEMENT OF ACADEMIC INTEGRITY

I hereby certify that the submitted thesis “Effects of algae on microbial carbon cycling in freshwaters - with focus on the utilization of leaf C by heterotrophic bacteria and fungi - ” 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,

Jenny Fabian

I **Fabian, J.**, Zlatanovic, S., Mutz, M., and Premke, K. (2017) Fungal-bacterial dynamics and their contribution to terrigenous carbon turnover in relation to organic matter quality. *ISME J.* **11**: 415–425.

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ORIGINAL ARTICLE

# Fungal–bacterial dynamics and their contribution to terrigenous carbon turnover in relation to organic matter quality

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**Ecological functions of fungal and bacterial decomposers vary with environmental conditions. However, the response of these decomposers to particulate organic matter (POM) quality, which varies widely in aquatic ecosystems, remains poorly understood. Here we investigated how POM pools of substrates of different qualities determine the relative contributions of aquatic fungi and bacteria to terrigenous carbon (C) turnover. To this end, surface sediments were incubated with different POM pools of algae and/or leaf litter. $^{13}C$ stable-isotope measurements of C mineralization were combined with phospholipid analysis to link the metabolic activities and substrate preferences of fungal and bacterial heterotrophs to dynamics in their abundance. We found that the presence of labile POM greatly affected the dominance of bacteria over fungi within the degrader communities and stimulated the decomposition of beech litter primarily through an increase in metabolic activity. Our data indicated that fungi primarily contribute to terrigenous C turnover by providing litter C for the microbial loop, whereas bacteria determine whether the supplied C substrate is assimilated into biomass or recycled back into the atmosphere in relation to phosphate availability. Thus, this study provides a better understanding of the role of fungi and bacteria in terrestrial–aquatic C cycling in relation to environmental conditions.**
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## Introduction

Freshwater ecosystems receive and process large amounts of terrigenous carbon (C) (Cole *et al.*, 2007), often in the form of leaf litter (Fisher and Likens, 1973; Tank *et al.*, 2010). Herein, microbial heterotrophs greatly contribute to terrestrial–aquatic C cycling (Battin *et al.*, 2008; Ward *et al.*, 2013). The terrigenous subsidy is either assimilated into biomass, providing energy and nutrients for higher trophic levels of the food web (Attermeyer *et al.*, 2013; Marcarelli *et al.*, 2011), or mineralized to $CO_2$, contributing substantially to global biogeochemical fluxes (Bardgett *et al.*, 2008; Rousk and Bengtson, 2014; Battin *et al.*, 2016).

Notably, the particulate forms of terrigenous C have a highly complex, aromatic chemical structure and large C to nutrient stoichiometry (McGroddy *et al.*, 2004; Lau *et al.*, 2008); they are therefore considered quite refractory toward microbial decomposition (Kleber *et al.*, 2011; Attermeyer *et al.*, 2014). Yet, modern research implies that environmental factors, rather than the chemical structure, control the microbial decomposition of terrigenous particulate organic matter (POM). Microbial communities respond rapidly to changes in their environment in two ways, either through variation in the species composition and/or metabolic activity (Comte and del Giorgio, 2011), both of which shape their performance in terrigenous POM cycling. Therefore, identifying drivers for the microbial turnover of terrigenous POM at the community level is fundamental to understand the role of aquatic decomposers in terrestrial–aquatic C cycling (Rousk and Bengtson, 2014).

Bacterial and fungal heterotrophs dominate microbial decomposer communities (Hieber and Gessner, 2002). However, these groups are phylogenetically distant and differ in their metabolic requirements and cellular capabilities (Mille-Lindblom and Tranvik, 2003). The typical filamentous structure of most fungi facilitates their penetration into particulate substrates, whereas bacteria are suspended in or attached to substrates (Baldy *et al.*, 1995). Fungal decomposers also have a lower metabolic nutrient

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demand (reviewed in Danger *et al.*, 2016) and wider enzymatic capabilities than bacteria, which allow them to mineralize low-quality substrates like particulate leaf litter (Güsewell and Gessner, 2009). However, bacteria are characterized by shorter turnover and higher metabolic activities (Bardgett *et al.*, 1999; Attermeyer *et al.*, 2013). Consequently, fungi and bacteria occupy different functional niches in POM decomposition: fungi act as primary degraders of particulate, predominately terrigenous C and bacteria act as rapid recyclers of simply structured nutrient-rich organic matter (OM) compounds (Gessner *et al.*, 2007; Krauss *et al.*, 2011).

The relative abundance of fungi and bacteria varies among different POM pools (Findlay *et al.*, 2002), which can have profound effects on ecosystem functioning (Rinnan and Bååth, 2009; Strickland *et al.*, 2009) with regard to their functional niche in the mineralization of terrigenous OM (De Graaff *et al.*, 2010). However, studies simultaneously evaluating the different ecological roles of both fungi and bacteria are scarce, especially in the relation to environmental factors (Schneider *et al.*, 2012), and such investigation is mainly limited to soil studies (Rousk and Bååth, 2007). Moreover, the contribution of fungi and bacteria to OM degradation is not solely a function of their abundance, but of their metabolic activity that varies with changes in POM quality (Meidute *et al.*, 2008; Brandstätter *et al.*, 2013).

The availability of leaf litter for microbial degradation increases in the presence of algae-derived OM (Danger *et al.*, 2013; Ward *et al.*, 2016) denoting that energy-rich OM aid in the microorganisms' metabolic capabilities to degrade more complex, low-quality C (Kuehn *et al.*, 2014). Yet, whether this newly available substrate is assimilated or mineralized seems to be determined by the phosphate (P) availability (Rier *et al.*, 2014). Hence, this study is aimed at deliberately exploring how POM pools of substrates of different qualities, such as algae and leaf litter, determine the relative contributions of fungal and bacterial groups to POM degradation. We hypothesized that (i) the quality of POM pools determines the microbial performance in POM decomposition, (ii) algal POM stimulates the metabolic activity of fungi and bacteria toward leaf litter POM decomposition rather than their abundance and (iii) the contribution of bacteria to terrigenous C turnover varies with the C:P stoichiometry of algal substrates.

Using a stable-isotope approach, we incubated natural streambed sediments with different POM pools. These pools were generated through the combination of high-quality (algal POM) and low-quality terrigenous (litter POM) substrates that were $^{13}$C-labeled to directly trace their microbial mineralization through continuous measurements of $^{13}CO_2$: total $CO_2$ production. We further combined microbial respiration measurements with the analysis of aquatic phospholipid-derived fatty acid biomarkers (PLFA) to link microbial activities to microbial community dynamics for fungi and bacteria. The combination of PLFA biomarker analysis with stable-isotope analysis further provides information on the acquisition of different substrates distinct in their isotopic signature. Thus, we were able to directly determine group-specific changes in substrate utilization (Neufeld *et al.*, 2007) in relation to the substrate composition of the applied POM pools.

Consequently, the results from this study provide important implications for our understanding of terrigenous C transformation in aquatic ecosystems and thus contribute to a better understanding of the role of microbial decomposers in terrestrial–aquatic C cycling.

## Materials and methods

*Experimental procedures*

Microbial sediment communities were incubated for 46 days under stable climate conditions (in the dark at 20 °C) together with POM pools that differed in their quality composition (Figure 1a). Therefore, natural sandy sediments were incubated in gastight sealed cylinders (acrylic glass, $d = 11.5$ cm, $h = 20$ cm) with artificial water (modified after Lehman, 1980, containing 20 mg $CaCl_2$ $l^{-1}$, 15 mg $MgSO_4 \cdot 7H_2O$ $l^{-1}$, 20 mg $NaHCO_3$ $l^{-1}$ and 20 µl $l^{-1}$ SL10 solution of trace elements mixed and autoclaved in bi-distilled water, without $PO_4^{-3}$ or $NO_3^{-2}$ addition) that recirculated through the sediment in the downward direction at a pore water velocity of $\sim 0.9 \times 10^{-4}$ m $s^{-1}$ (after Angermann *et al.*, 2012). Artificial water was allowed to enter the chamber through a side port in the chamber wall, ~2 cm above the water level, to ensure fast equilibration of $CO_2$ between water and the headspace needed for respiration analysis and to prevent anoxic conditions (Figure 1b). We chose artificial water as the percolation medium to avoid uncontrolled introduction of microbial communities and organic C substrates or other nutrients.

We sampled the sediments from a ripple region (upper 2 cm) of a lowland stream (Rheinsberger Rhin, 52°34'25"N 14°6'12"E, Brandenburg, Germany) characterized by regular sediment movement as well as intensive vertical water exchange and thereby intrusion of oxygen. The sampling area is surrounded by mixed forests and characterized by low-nutrient loading and low OM sandy sediments. After sampling, we sieved the sediment with stream water through 90- and 1000-µm meshes to remove the silt and clay as well as the coarse POM fraction. We let the resulting sediment slurries acclimate from *in situ* (2 °C) to experimental conditions in a stepwise (1–1.5 °C $d^{-1}$), 2-week-long procedure while allowing percolation by artificial water. After acclimation, we mixed the organic substrates into the sediment according to our treatments and subsequently filled the resulting slurries into the

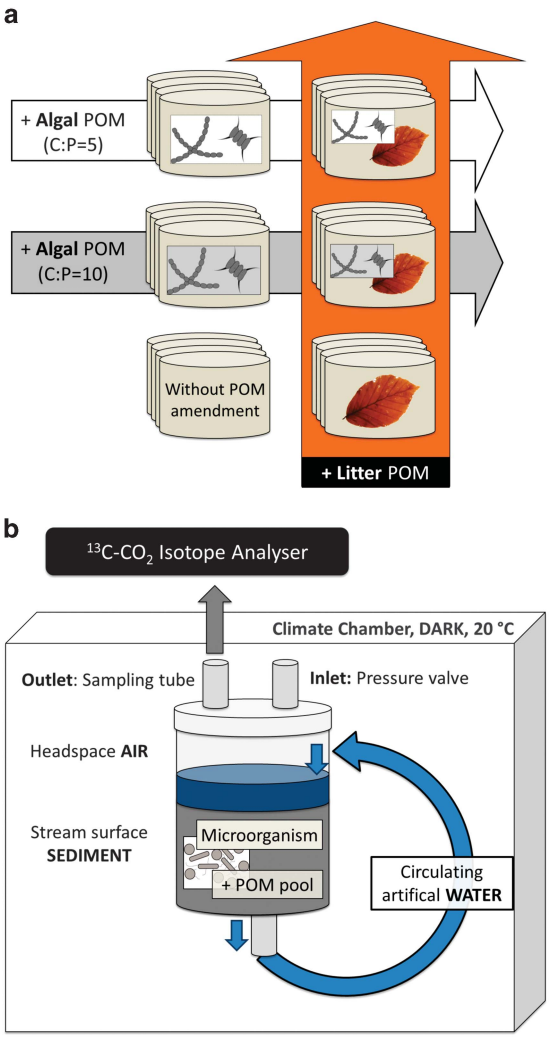


**Figure 1** (**a**) Treatments applied on the sampled sediments with respect to organic C modifications. Beech litter, algae (High C:P) and algae (Low C:P) were added as single-substrate modifications (algae or beech modification) or a mixture of these was used for the mixed modifications (mixed modifications: algae+beech). (**b**) Experimental set-up of incubation chambers with a focus on the flow path for artificial water.

incubation cylinders (four replicates each). The slurry approach was essential to ensure equal starting conditions for all treatments with respect to the composition of the microbial community and POM treatments and is commonly used in studies interrelating environmental effects on microbial performance (for example Marotta *et al.*, 2014; Dyksma *et al.*, 2016).

The different sediment POM pools were generated through the combination of three $^{13}$C-labeled POM substrates that differed in quality with respect to chemical structure and nutrient content. These were: pre-leached beech litter (1–2 mg per g sediment dry weight, $\delta^{13}C = 4500‰$) as a proxy for a low-quality, stream C substrate of terrestrial origin, characterized by a highly complex aromatic structure and low-nutrient content (C:N:P = 187:2:1); two algal substrates (6–12 µg per g sediment dry weight) as proxy for high-quality, stream C of aquatic origin. Both algal substrates differed in their P content and are referred to as algae HighCP (C:N:P = 80:10:1, $\delta^{13}C = 503‰$) and algae LowCP (C:N:P = 36:5:1, $\delta^{13}C = 488‰$) throughout the manuscript. Compared with the beech litter, the two algal substrates are characterized by a less-complex molecular structure and higher nutrient content that is assumed to be easily available for microbial decomposition. A detailed description of the applied OM substrates, including their preparation, has been provided in the Supplementary Methods. We amended the sediment with these three substrates in five different ways, resulting in five treatments that differed in their substrate composition of the sediment POM (Figure 1a): sediment with algae HighCP; sediment with algae LowCP; sediment with beech litter; sediment with beech litter and algae HighCP; and sediment with beech litter and algae LowCP. In addition to our treatments, we incubated four chambers with non-amended sediment (control) and applied the same handling to the entire preparation and incubation process.

We monitored microbial C respiration by sampling the headspace $CO_2$ every 6 h throughout the incubation. In addition, we sampled the water and sediment at the beginning (day 0) and the end (day 46) to obtain information on differences in the composition of the microbial decomposers, their metabolic activity (respiration and substrate specific assimilation), and nutrient consumption. During sampling, we collected the sediment in sterile falcon tubes using a laboratory spoon and the water in an Erlenmeyer flask through a port in the chambers' sidewall. For the start sample (day 0), we collected the sediment and water ~6 h after amendment with OM substrates and filling into incubation chambers to allow settling of the initially suspended sediment particles before the sampling.

*Community composition of the microbial decomposers*

Information on the composition of microbial decomposers was obtained through PLFA analysis from sampled sediments at days 0 and 46. PLFA are present in the membranes of all living cells and rapidly degrade to neutral lipids upon cell death (reviewed in Willers *et al.*, 2015), hence offer sensitive and reproducible measurements for characterizing microbial communities (for example, Boschker and Middelburg, 2002; Weise *et al.*, 2016). We extracted total lipids from 15 g of freeze-dried sediment using a well-established modified method described by Frostegard *et al.* (1991) and Steger *et al.* (2011). A detailed description on the extraction and analysis of PLFA from the sediments is given in the Supplementary Methods. The concentration of each identified microbial PLFA was corrected to the total concentration of microbial PLFA to compensate for variations resulting from PLFA extraction. We identified 15 PLFAs that occur

in the membranes of bacteria (i15:0, a15:0, i17:0), fungi (18:2ω6) and both microbial groups (14:0, 15:0, 16:1ω9c/7c, 16:1ω9t, 17:0, cy17:0, 18:0, 18:1ω9c, 18:1ω9t/7c, 18:3ω6/3, cy19:0). Certain PLFA were assigned to separate fungi (18:2ω6) from heterotrophic bacteria (i15:0) based on previous research (Boschker and Middelburg, 2002; De Carvalho and Caramujo, 2014) to disentangle their metabolic activity with respect to C assimilation from the introduced substrates.

*Microbial C mineralization*

Microbial C mineralization (C min), and thereby microbial activity, was measured as gaseous $CO_2$ emitted ($emCO_2$) during a 6 hour incubation period (equation 1). Total C mineralization was calculated from the sum of water total inorganic carbon ($\Delta TIC_{Water}$) and $emCO_2$ produced during 46 days (d) of incubation, expressed as a percentage of sediment total organic carbon (TOC), according to equations 2 and 3.

$$C\ \mathrm{min} = \frac{\mathrm{emCO_2}}{\mathrm{h}} = \frac{[CO_2]^{t=6h}_{headspace} - [CO_2]^{t=0h}_{headspace}}{\Delta \mathrm{t}} \quad (1)$$

$$\Delta TIC = TIC^{t=day\,46}_{water} - TIC^{t=day\,0}_{water} \quad (2)$$

$$\mathrm{Total}\ C\ \mathrm{min} = \frac{\left(\sum_{d=0}^{46} \frac{emCO_2}{24h}(d) + \Delta TIC\right)}{sediment\ TOC} \times 100 \quad (3)$$

The concentration and isotopy of $emCO_2$ was measured every 6 h using an Off-Axis Integrated-Cavity Output Spectroscope (Off-Axis ICOS CCIA, Los Gatos Research, CA, USA) connected to the chambers' headspace through tubes. The superscripts 't = 6 h' and 't = 0 h' represent the end and start of the incubation period. Every 12–24 h, we calibrated the instrument against an internal standard gas (70% $N_2$, 30% $O_2$ and 0.15% $CO_2$, Airliquide, Germany) and against the international standard Vienna Pee Dee Belemnite values to correct for isotope drifting, yielding a precision of 2‰ for $\delta^{13}C$ and 1 ppm for $CO_2$ concentration. The detailed description of headspace $CO_2$ analysis is provided in the Supplementary Methods. TIC was analyzed immediately after sampling according to DIN EN 1484 (DEV, H3) on a multi N/C 2100 Analyzer (Jena Analytics, Jena, Germany).

*Food source elucidation of the respired ($emCO_2$) and microbial fixed C (PLFA)*

For single OM modifications, we applied a two-source mixing model approach proposed by Cheng (1996) on $emCO_2$ to separate the sediment OM-derived $CO_2$ emission ($C_{SED}$) and algae-derived $CO_2$ emission ($C_A$) using equations 4 and 5:

$$C_{SED} = emCO_2 \times \frac{\left(\delta^{13} emCO_2 - \delta^{13} C_A\right)}{\left(\delta^{13} C_{SED} - \delta^{13} C_A\right)} \quad (4)$$

$$C_A = emCO_2 - C_{SED} \quad (5)$$

For mixed OM modifications (beech and algae together), we applied a two-source mixing model approach (equations 6 and 7) to separate for sediment +algae OM-derived $CO_2$ respiration ($C_{SED+A}$) and beech litter-derived $CO_2$ respiration ($C_{BEECH}$). In this approach, we treated the sediment and algal C as one C source and beech litter C as the second possible C source.

$$C_{SED+A} = emCO_2 \times \frac{\left(\delta^{13} emCO_2 - \delta^{13} C_{BEECH}\right)}{\left(\delta^{13} C_{SED+A} - \delta^{13} C_{BEECH}\right)} \quad (6)$$

$$C_{BEECH} = emCO_2 - C_{SED+A} \quad (7)$$

We obtained $\delta^{13} emCO_2$ from Keeling plot analyses of headspace $CO_2$ (Pataki, 2003). $\delta^{13} C_A$, $\delta^{13} C_{SED+A}$ and $\delta^{13} C_{BEECH}$ were assumed to be equal to $\delta^{13} C$ of the POM, based on the report by Mary *et al.* (1992), where fractionation during microbial degradation processes was shown to be negligible.

We used the same mixing model approach on specific PLFA biomarkers to separate for sediment OM and substrate-derived C assimilated into the different biomarkers. We obtained $\delta^{13} C$ for each specific PLFA biomarkers after correcting using a fractionation factor of 5‰, which is an average according to Boschker and Middelburg (2002). Fractionation factors may vary slightly between 1 and 3‰ for different PLFA biomarkers and according to environmental conditions; however, these variations are negligible because of the high enrichment in $^{13}C$ achieved with our tracer approach.

*Nutrient analysis*

Prior to analysis, we freeze-dried and grinded the sediments sampled at days 0 and 46. For TP analysis, we applied the ammonium molybdate spectrometric method (DIN EN ISO 6878 2004). Determination of total organic carbon (TOC) and total nitrogen were performed according to DIN EN 1484 1997 using an Elementar Vario EL cube (Elementar Analysensysteme GmbH, Hanau, Germany).

Dissolved nutrients were analyzed from water sampled at day 0 and 46 after filtration through a 0.45 µm cellulose acetate filter (Sartorius Stedim Biotech GmbH, Goettingen, Germany). Soluble reactive phosphorus was determined photometrically on a UV/VIS-Photometer CARY 1E (VARIAN, Darmstadt, Germany) according to DIN EN ISO 11732 modified after Murphy and Riley (1962). Dissolved inorganic nitrogen (sum of $NO_3^{2-}$ and $NH_4^+$) was analyzed according to DIN EN 1189 on a CFA-Photometer Skalar SAN (Skalar Analytical B.V.,

Breda, The Netherlands) and dissolved organic carbon according to DIN EN 1484 on a multi N/C 3100 Analyzer (Jena Analytics).

*Statistical analysis*

To evaluate and visualize changes in the composition of microbial communities according to our treatments, we performed a non-metric dimensional scaling (NMDS, three dimensions) ordination and a non-parametric multivariate analysis of variance (PERMANOVA) on computed pairwise Bray–Curtis dissimilarities on proportional lipid data (after Hall *et al*., 2010). We also performed a principal component analysis (PCA) to identify important fatty acids that showed different results between our POM treatments at day 46. PCA was performed on a correlation matrix generated from proportional lipid data and the ratio of bacterial to fungal PLFA biomarker (i15:0 to 18:2ω6) from sampling day 46. We further applied non-parametric Kruskal–Wallis rank sum tests with subsequent *post hoc* Dunn´s tests with Bonferroni correction to test for treatment effects ($n = 3$–4) with regard to the consumption of nutrients, assimilation of beech C into microbial PLFA, and changes in fungal (18:2ω6) and bacterial (i15:0) group-specific PLFA biomarkers. We determined the significances of differences between treatments for cumulative respiration of beech-derived C throughout the final incubation period ($30 \leqslant t \leqslant 46$) by computing a linear mixed-effects model. The fixed structure was set as the interaction for treatment+control (6 levels) and sampling time (11 levels), and for the random structure, we allowed different intercepts for each replicate. Each linear mixed-effects model 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. When the interaction was significant, we analyzed each sampling time individually. The linear mixed-effects models were followed by the conservative Turkey's *post hoc* test to test significant differences between treatments. Further, we performed single linear regression analysis to relate C turnover to the ratio of group-specific biomarkers. All statistical analyses were performed in the statistic software R using packages dunn.test, MASS, multcomp, nmle, vegan and psych at a significance level of $P \leqslant 0.05$ (Team R, 2010).

## Results

Sediment POM modifications resulted in five POM pools (treatments) that differ in their composition of labile and refractory substrates (Figure 1a) and nutrient content (Table 1).

*Composition of microbial decomposers in relation to POM pools*

NMDS ordination based on Bray–Curtis dissimilarity on proportional PLFA data (Figure 2a) showed a significant shift in the composition of all the identified PLFA after our treatment (PERMANOVA, Pseudo-F = 2.901, $P = 0.003$). Therein, the PLFA composition significantly differed between POM pools at the end of the incubation period (day 46) and was related to both the presence of algae as well as beech litter substrates within POM pools (PERMANOVA, Pseudo-F = 4.237, $P = 0.003$). In addition, PCA on proportional PLFA data (Figure 2b) indicated that variation in the ratio of the abundance of fungi (18:2ω6) to heterotrophic bacteria (i15:0) explains 42.4% of the observed interrelation between POM pools and PLFA composition. Thus, the abundance of fungal PLFA (Supplementary Figure S1b) increased in the presence of beech litter (Kruskal–Wallis, $\chi^2_{(5)} = 15.663$, $P = 0.01$), which was most pronounced for sediments incubated without algae (Beech, Dunn's test, $P = 0.01$) and was independent of algal C:P stoichiometry in sediments amended with beech (Beech+Algae, Dunn's test, $P = 1$). Further, PCA revealed that another 22.5% of the variation in PLFA data relates to the abundance of bacterial-specific PLFA i15:0 but was not related to our POM modifications (Supplementary Figure S1a, Kruskal–Wallis on i15:0, $\chi^2_{(5)} = 8.666$, $P = 0.12$).

*Mineralization of C substrates in relation to POM pools*

Figure 3 and Supplementary Figure S2 show the resulting changes in C mineralization after our

**Table 1** Overview of sediment nutrient content at the start of incubation (day 0) given in total amount and nutrient stoichiometry

| *Treatment* | *TN (mg gDW$^{-1}$)* | *TOC (mg gDW$^{-1}$)* | *TP (µg gDW$^{-1}$)* | *C:N* | *C:P* | *N:P* |
|---|---|---|---|---|---|---|
| Beech | 0.1 (0.0) | 0.6 (0.1) | 109.4 (22.1) | 10.5 | 14.9 | 1.4 |
| Beech+Algae HighCP | 0.1 (0.0) | 0.6 (0.1) | 110.9 (20.4) | 7.5 | 13.5 | 1.8 |
| Beech+Algae LowCP | 0.1 (0.0) | 0.6 (0.0) | 153.4 (41.2) | 9.3 | 10.8 | 1.2 |
| Algae HighCP | 0.1 (0.0) | 0.5 (0.0) | 65.1 (21.5) | 7.9 | 21.4 | 2.7 |
| Algae LowCP | 0.1 (0.0) | 0.5 (0.0) | 95.5 (17) | 7.9 | 14.6 | 1.9 |
| Control | 0.1 (0.0) | 0.6 (0.1) | 67.0 (19.0) | 8.2 | 21.6 | 2.6 |

Data are presented as mean of replicates ($n = 3$–4) with s.d. given in brackets. DW, sediment dry weight; T, total; N, nitrogen; P, phosphorus; O, organic; C, carbon.

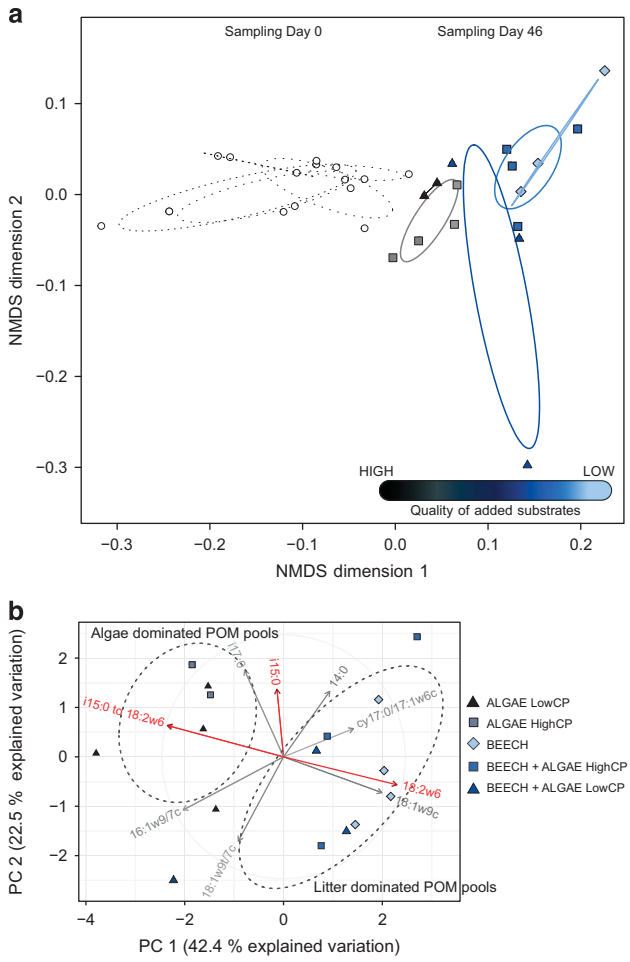


**Figure 2** (**a**) Non-metric multidimensional scaling ordination based on Bray–Curtis dissimilarity on PLFA data, Kruskal's stress = 0.02. Open dots (dashed ellipses) and colored squares or triangles (solid ellipses) separate sediment samples taken 6 h and 46 days after organic matter modifications, respectively. Colors separate the samples according to the modification: black = algae (LowCP); gray = algae (HighCP); dark blue = algae (LowCP) with beech litter; blue = algae (HighCP) with beech litter; light blue = beech litter. Ellipses represent 95% intervals around centroids for each treatment. (**b**) Principal component analysis of fatty-acid profiles of sediments sampled at day 46 reveal a main shift in PLFA biomarkers for heterotrophic bacteria (i15:0) versus fungi (18:2ω6) based on POM pool quality.

POM modifications. Total C mineralization was significantly related to the composition of POM pools (Supplementary Figure S2a, Kruskal–Wallis, $\chi^2_{(3)} = 11.514$, $P = 0.01$). Throughout the final incubation period (day 30–46), C mineralization patterns in sediments amended with algal substrates (Supplementary Figure S2a) were significantly related to algal nutrient stoichiometry (Dunn's test, $P < 0.01$). Further, C mineralization was significantly related to algae C:P stoichiometry in sediments amended with beech litter (Kruskal–Wallis, $\chi^2_{(2)} = 7$, $P = 0.03$). Significantly higher mineralization rates were observed for POM pools of beech litter and P-enriched algae (LowCP, Dunn's test, $P = 0.018$).

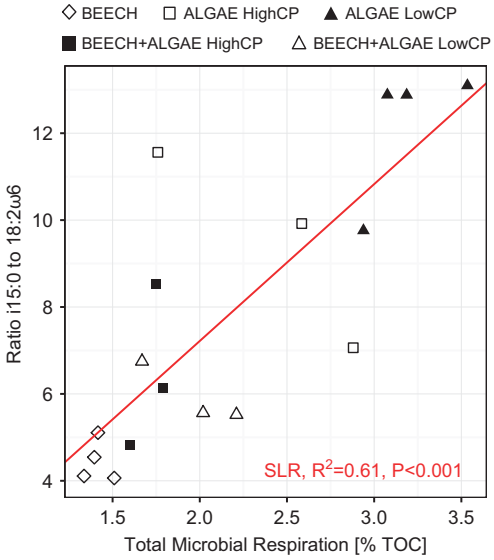


**Figure 3** Community dynamics versus microbial respiration. Fungal (18:2ω6)–bacterial (i15:0) dynamics relate to changes in C mineralization, where higher turnover rates were observed in the sediments with OM pools of higher quality.

Stable-isotope analysis of the respired $CO_2$ provides information on OM substrates metabolized by the microbial community. Unfortunately, we lost the isotope data for the first 29 days of incubation owing to technical problems. However, the available data indicate a general increase in the mineralization of beech litter POM and a decrease in the mineralization of algal POM throughout the incubation period (data not shown).

Figure 4 gives an overview of the effect of algal POM on the mineralization of beech litter. Thus, two-end member mixing model analysis of $emCO_2$ revealed a significant relation between POM pools and the proportion of beech-derived C in mineralized C (Figure 4b, Kruskal–Wallis, $\chi^2_{(2)} = 7.86$, $P = 0.01$). Even so, the stimulative effect of algae OM on total C mineralization resulted in a higher amount of beech C respired throughout incubation (Figure 4a, linear mixed-effects model, LowCP: $P = 0.026$, High CP: $P = 0.058$), which was unrelated to algal nutrient stoichiometry.

The observed differences in C mineralization were significantly related to fungal–bacterial ratios (Figure 3; single linear regression, $R^2 = 0.61$, $P < 0.001$), that is, higher C mineralization, as measured for sediments with a higher ratio of bacterial to fungal PLFA, which was the case when algal POM, in particular that of high nutrient content, was added.

*Effect of algal POM on the assimilation of beech litter by fungi and bacteria*

$^{13}C$ isotope analysis of phospholipids provides information on the substrates metabolized by fungi and bacteria in relation to our POM treatments. Figure 5 provides an overview of the $^{13}C$ isotope enrichment ($\delta^{13}C$) in all the identified PLFA at day

46, which was for all treatments up to 10-fold higher in fungal- (18:2ω6) then in bacterial- and unspecific PLFA biomarkers. Incubation of sediments with POM pools of beech and beech+algae HighCP resulted in similar $^{13}C$ isotope enrichment in PLFA.

Total amount of beech C assimilated into PLFA specific for heterotrophic bacteria (i15:0) was related to the composition of POM pools (Figure 6, Kruskal–Wallis, $\chi^2_{(2)} = 5.6$, $P = 0.06$), more precisely stimulated in presence of High C:P algae (Dunn's test, $P = 0.04$). In contrast, the amount of beech C assimilated into fungal PLFA was independent of algal presence (Kruskal–Wallis, $\chi^2_{(2)} = 3.84$, $P = 0.15$).

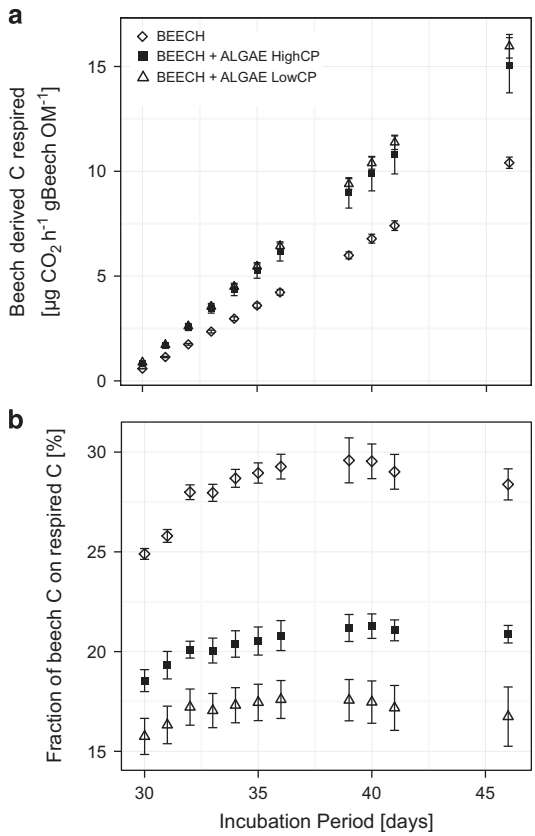


**Figure 4** Effect of algal OM on (**a**) overall and (**b**) fractional beech C turnover. Addition of algal OM high in P stimulated overall microbial respiration of beech-derived C but decreased the fractional utilization of beech C. Data are shown for the incubation period from day 30 to day 46. Data are presented as the mean of replicates ($n = 3$–4) with s.d.

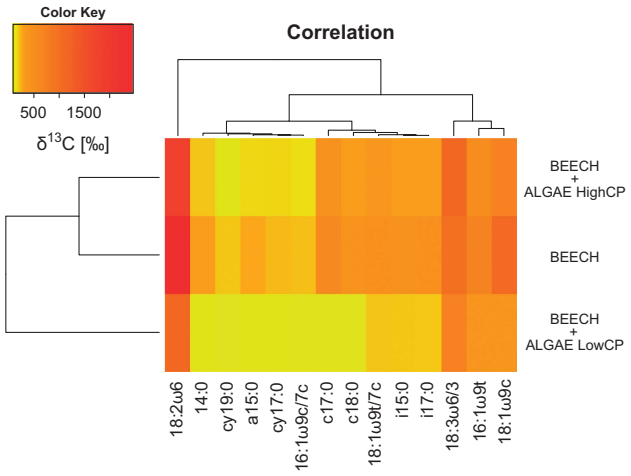


**Figure 5** Overview of $\delta^{13}C$ enrichment in microbial PLFA for all OM modifications including beech OM. Similar stable-isotope enrichment among PLFA is observed for OM modifications with only beech OM and in mixture with highCP algae OM. Data are presented as mean of replicates ($n = 3$–4).

*Consumption of dissolved inorganic and organic nutrients*

The consumption of soluble reactive phosphorus (Kruskal–Wallis, $\chi^2_{(5)} = 1.274$, $P = 0.94$), dissolved inorganic nitrogen (Kruskal–Wallis, $\chi^2_{(5)} = 9.016$, $P = 0.11$) and dissolved organic carbon (Kruskal–Wallis, $\chi^2_{(5)} = 7.179$, $P = 0.21$) was similar between all the treatments, except for lower consumption of dissolved inorganic nitrogen in the sediments modified using algae HighCP. Dissolved inorganic nitrogen was limiting at the end of incubation. For data, see Supplementary Table S1.

## Discussion

As expected, algae OM greatly stimulates microbial decomposition of terrigenous POM in aquatic ecosystems (Danger *et al.*, 2013; Ward *et al.*, 2016). Nevertheless, this study showed that microbial fungal and bacterial decomposers respond differently to substrate variations in POM pools and thus contributes to a better understanding of the role of microbial decomposers in terrestrial–aquatic C cycling.

*Pronounced contrasts in substrate quality drive fungal–bacterial occurrence within the degrader communities*

With regard to their distinct quality, algal and leaf litter are considered to differ in their availability to microbial decomposers (for example, Marcarelli *et al.*, 2011; Attermeyer *et al.*, 2014). This is reflected by the measured differences in the C-respiration rates observed for sediments amended with algae or beech litter (Figure 3 and S2a).

Thus, the interrelation between POM quality and bacterial to fungal occurrence observed in this study agree with the reported distinct metabolic capabilities of these organisms to break down substrates of different qualities (Rinnan and Bååth, 2009). Beech litter within POM pools favored the occurrence of fungi (Figure 3 and Supplementary Figure S1b), confirming that they dominate microbial communities when growing on refractory, nutrient-poor OM (Hieber and Gessner, 2002). However, amendments with algal OM favored the dominance of bacteria over fungi, denoting their competitive colonization and turnover of these high-quality substrates (for example, Bardgett *et al.*, 1999; Fontaine *et al.*, 2003).

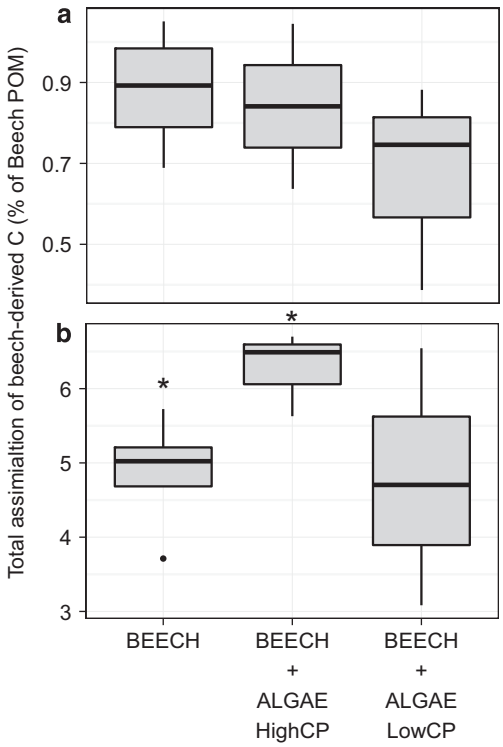


**Figure 6** Amount of beech-derived C fixed into microbial PLFA after 46 days of incubation for (**a**) fungal PLFA (18:2w6) and (**b**) bacterial PLFA (sum of all bacterial PLFA) given in % of beech used. The presence of algae HighCP enabled a 1.5–2% assimilation of beech C into bacterial PLFA but not into fungal PLFA. Asterisk boxes indicate significant differences between the marked treatments.

In addition, the lower abundance of fungal lipids in sediments amended with algal POM (Supplementary Figure S1b) indicates bacterial antagonism against fungi (Wargo *et al.*, 2006 and references therein), which was shown to be stimulated in the presence of high-quality OM (Mille-Lindblom *et al.*, 2006) such as our applied algal substrates.

Bacterial to fungal ratios did not differ between the algal P treatments (Figure 3), but NMDS on proportional data (Figure 2a) indicates a different composition of bacterial and fungal PLFA between the two algal C:P treatments. This mismatch between the results of the two analyses implies that the quantity of labile and refractory substrates within POM pools determines the ratio between fungi and bacteria, whereas other environmental factors such as the C:P ratio of the labile substrate might primarily affect the species composition within each group.

*Contribution of aquatic fungi and bacteria to terrigenous C turnover varies between POM pools*

In accordance with previous findings (for example, Danger *et al.*, 2013), algae POM stimulated the decomposition of beech litter (Figure 4a). However, our results support that the contribution fungi and bacteria to beech decomposition is likely not affected by algal POM in the same manner.

The more pronounced $^{13}C$ enrichment in the fungal compared with that in bacterial specific phospholipids (Figure 5) implies that fungi primary degrade beech litter, yet, fungal abundance was reduced in presence of algal OM. In result, a stimulated decomposition of beech litter by algal OM (Figure 4a) related to a more pronounced dominance of bacteria within the degrader communities (Figure 3) and thus resulted in an increased contribution of bacteria to beech C processing.

Bacterial abundance varied only slightly with our POM modification, implying that the additional C substrate was primarily expended for energy required for respiration, which is also supported by the observed less pronounced effect of algal POM on the assimilation of beech C into microbial lipids (Figure 6). In addition, algal POM did not increase the use of beech C in preference over other C substrates by microbial decomposers (Figure 4b), thus confirming that high-quality POM stimulated the contribution of bacteria to leaf breakdown through an increase in their metabolic activity rather than through affecting their abundance or substrate preference.

*Nutrient availability modifies the metabolic performance of bacteria in terrigenous C turnover*

The availability of nitrogen (N) and phosphorus (P) frequently controls ecological processes (Elser *et al.*, 2007) such as litter decomposition (Gulis and Suberkropp, 2003; Rier *et al.*, 2014). In the present study, N and P were almost exclusively provided in particulate form through algal and/or beech litter substrates, whereas the pool of external inorganic N and P remained to be small to limiting (Supplementary Table S1). In addition, the C to nutrient stoichiometry of our applied leaf litter by far exceeds the nutrient demands of fungi and bacteria in contrast to the applied algal substrates (reviewed in Danger *et al.*, 2016). Thus, we assumed that the additional N and P provided with the algal substrates determine whether the beech-derived C is assimilated into microbial biomass or mineralized to $CO_2$.

In this respect, mineralization of beech litter was stimulated in presence of nutrient-rich, algal POM (Figure 4a), yet independent of algal C:P stoichiometry, implying that the quantity of algal POM primarily relates to the extent to which these labile C compounds stimulate the decomposition of refractory C substrates. With regard to the assimilation of beech C into biomass (Figure 6), we also did not observe a significant response to our algal treatments for fungal lipids, but a slight response for bacterial lipids was observed, confirming that both groups of organisms may acquire different amounts (Güsewell and Gessner, 2009) and pools of nutrients during litter decomposition. That is, fungi may fulfill their energy and nutrient demand directly from the decomposition of litter (Suberkropp and Chauvet, 1995; Gadd, 2006). In contrast, our results indicate

that the bacterial assimilation of low-nutrient, refractory C relies on additional nutrient pools, in particular with respect to P (Figure 6). This is further supported by the stronger variation in $^{13}C$ in bacterial than fungal specific PLFA with our POM treatment indicating that bacteria respond more sensitive to our algae treatments than fungi (Figure 5). As previously noted, the composition of POM pools with different algal C:P substrates resulted in distinct compositions of microbial decomposers (NMDS, Figure 2a). Thus, the higher amount of beech C assimilated into bacterial PLFA in the presence of high C:P algae may have resulted from a shift in the microbial community composition toward species that have a lower P demand and are more competitive in the assimilation of litter compounds.

## Conclusion

This study greatly improves our understanding of the effect of labile high-quality substrates on the fate of terrigenous OM in aquatic environments. We showed that the abundance of fungi and bacteria within degrader communities and their performance in metabolizing terrigenous OM are not intimately linked to each other, but largely depend on the composition of OM pools with respect to substrate quality. Clearly, the quantity of labile and refractory substrates determined the dominance of fungi and bacteria within the degrader communities and thereby the turnover of terrigenous C. However, the presence of specific environmental parameters such as P availability may also determine whether terrigenous substrates are assimilated and thereby transported into other trophic levels.

Our results suggest that aquatic fungi contribute to terrigenous C turnover by providing these C substrates for the microbial loop, whereas aquatic bacteria determine the amount of terrigenous C to be potentially recycled into the terrestrial environment through its emission into the atmosphere in the form of $CO_2$. However, given the large variety of POM pools in aquatic ecosystems and the few studies that exist on particulate C turnover, our results highlight the need for more sophisticated research to fully understand the contribution of fungi and bacteria to aquatic turnover of terrigenous C in relation to environmental conditions. Further, this study points out that the effects on both metabolic processes, assimilation and mineralization, have to be considered to fully understand these relations. Thus, the combinations of microbial and biogeochemical analyses with stable isotopes facilitated a more detailed observation of the underlying processes, and we highly recommend their application in further research.

## Conflict of Interest

The authors declare no conflict of interest.

## Acknowledgements

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Supplementary Information accompanies this paper on The ISME Journal website (http://www.nature.com/ismej)

# Supplementary Information

## Supplementary Methods

*Labelling of the substrates*

For the algal mixtures, 2 diatom algal species (*Navicula* sp. and *Cyclotella* sp.) and 5 green algal species (*Nannochloropsis limnetica* SAG18.99, *Mychonastes jurisii* SAG37.99, *Coelastrum microsporum* SAG2292, *Actinastrum hantzschii* SAG2015, and *Parachlorella beijerinchii* SAG2046) were used. The *Navicula* and *Cyclotella* species were grown in either Nicklisch (diatom) or Bonferroni media (green algae) under an 18:8 light:dark regime with about 100 µm photons $m^{-2}$ $s^{-1}$ fluorescence during the light conditions. To achieve algal materials of different cellular stoichiometries, the algae cultures were harvested by centrifugation at 1000 rpm for 20 min. The resulting pellet was then washed (resuspension in 0.9% NaCL + centrifugation) three times, resuspended in nutrient-free media, and split for cultivation on media containing two different inorganic N and P concentrations, herein referred to as repleted (0.5 mM $NaNO_3$ and 0.05 µM $KH_2PO_4$) and depleted (0.16 mM $NaNO_3$ and 0.005 µM $KH_2PO_4$) algal media. After 9 days of cultivation, the whole material of each strain was again harvested by centrifugation at 1000 rpm for 20 min and washed (resuspension in 0.9% NaCL + centrifugation) three times. All the strains grown on a common medium (depleted or repleted) were pooled together and fresh medium with the same nutrients was added to the culture, but with 20% of the $NaHCO_3$ (δ=-20 ‰) replaced by $Na^{13}C$-$HCO_3$ (99% purity, δ=1000 ‰) to increase the algae in $\delta^{13}C$. After 24-48 hours, the algal mixtures were harvested by centrifugation at 1000 rpm for 20 min, washed three times (resuspension in 0.9% NaCL + centrifugation), and subsequently resuspended in artificial

stream water. The algal mixtures were frozen, freeze-dried, and stored at -20°C until needed.

As a second carbon source, $^{13}$C-enriched beech leaves (*Fagus syslvatica, L.*) from trees grown under $^{13}CO_2$ atmosphere in greenhouses in Nancy, France, were used (for more details see Weise et al., 2016). The beech leaves were leached in artificial water for 48 hours prior to the start of the experiment and cut into small pieces (1-3 mm) after removing the middle vein. The leaves were then sieved through a 90-µm mesh to remove fine OM and frozen to allow similar treatments for both the C sources. We analysed the $^{13}$C isotopy of the particulate samples as described by Nitzsche et al. (2016).

*Measurement of the headspace $CO_2$*

Headspace $CO_2$ was sampled automatically. Therefore, the headspace was pumped out of the chamber through an outlet tube and flushed with outside air for 14 minutes through an inlet tube connected to the chamber's lid. A pressure-sensitive air-inlet valve in the chamber's inlet enabled automatic opening and closing. A small tube (length=7 cm, diameter=0.7 mm) connected from the inside to the inlet revealed that the outside air entered the chamber very close to the water-air interface, which caused a delay in the mixing of the outside air with the headspace. A stable-isotope carbon dioxide analyser (CCIA, Los Gatos Research, CA, USA) was placed between the pump and the chambers for analysing the concentration and isotopy of the headspace $CO_2$. An automated system comprising four multiport selectors (10-universal multiport selectors, Vici Valco, Houston, TX, USA) placed between the chambers and the analyser allowed automatic measurement for each chamber every 6 hours, four times a day. The selector-analyser network was flushed with outside air between each measurement. Every 12-24 hours, we ran a reference gas (70% $N_2$, 30% $O_2$

and 0.15% $CO_2$, Airliquide, Germany) to correct for isotope drifting of the instrument, yielding a precision of 2 ‰ for $\delta^{13}C$ and 1 ppm for $CO_2$ concentration. Data on isotopy is expressed relative to the international standard Vienna Pee Dee Belemnite (VPDB).

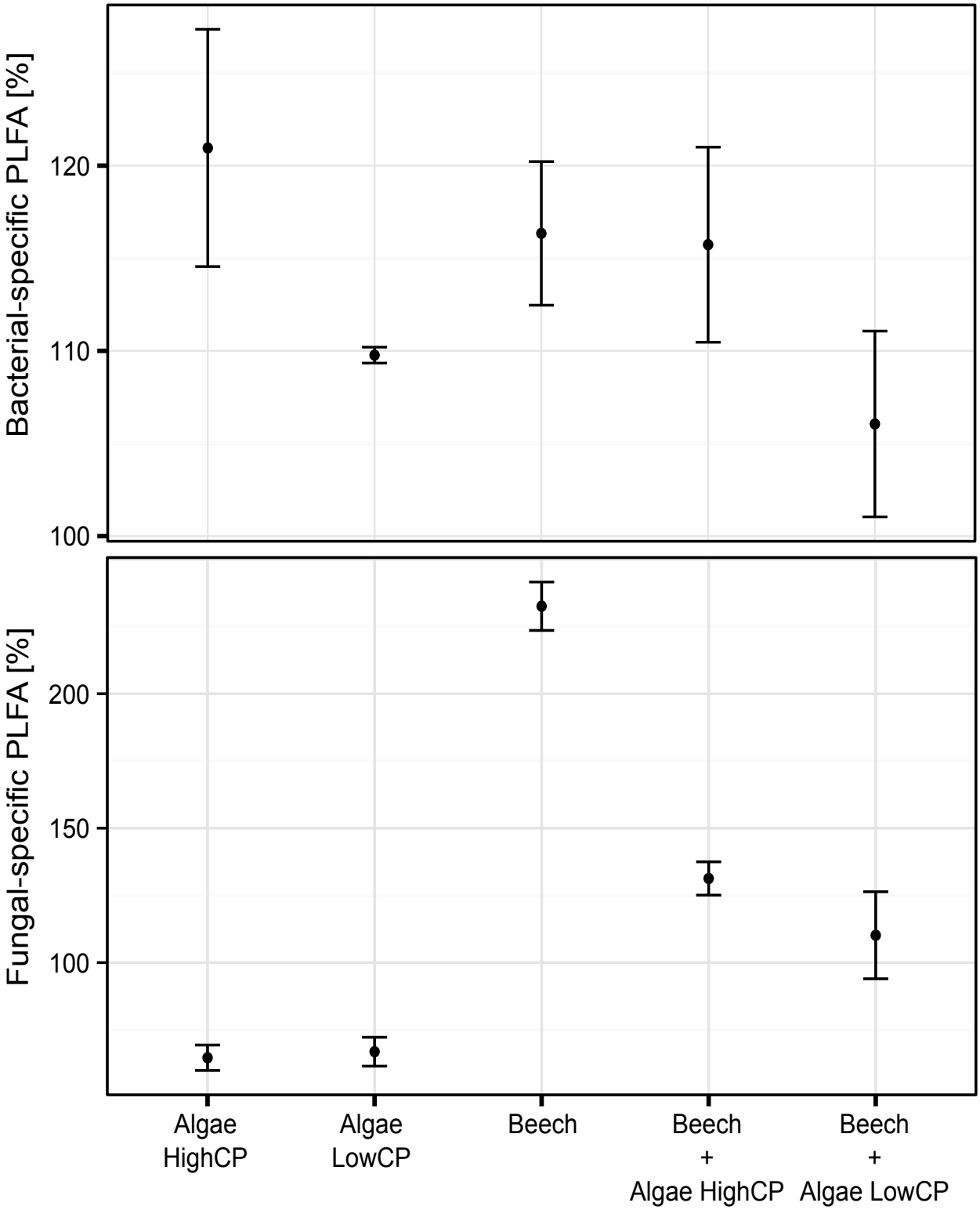
*Detailed description on PLFA extraction*

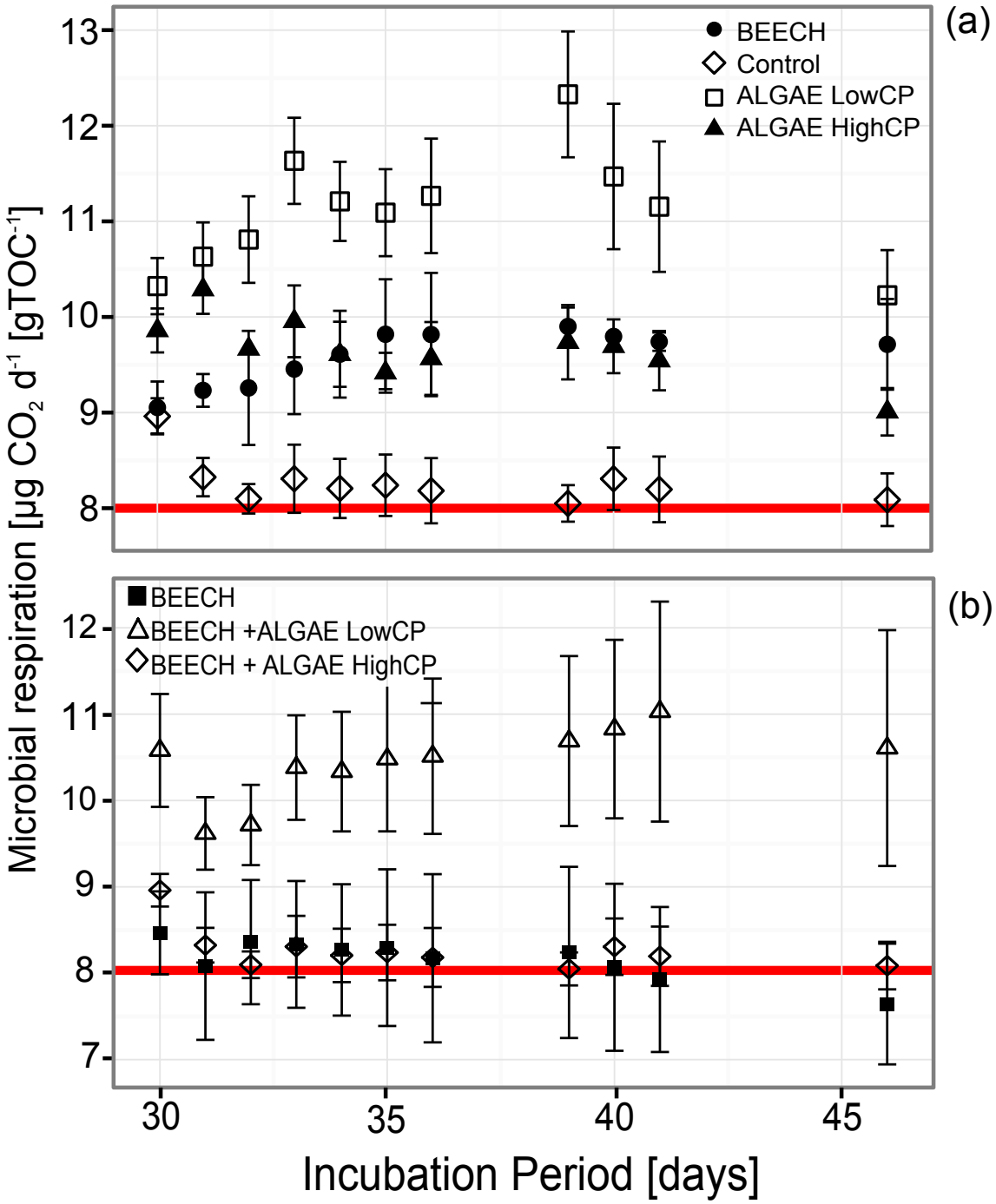
After extraction, the PLFA were separated from other lipids on silic acid columns (BondElut® LRC-Si, Altmann Analytic, Germany) using solid-phase extraction and methylated using mild alkaline methanolysis. The resulting PLFA methyl esters were dried over a N headspace and stored at -20°C until analyses. The concentration and $\delta^{13}C$ enrichment of the different PLFA were analysed by chromatography-combustion isotope ratio mass spectrometry (GC-C-IRMS) at the Stable Isotope Facility, University of California-Davis, USA. The compounds were separated chromatographically (Varian CP3800 gas chromatograph coupled to a Saturn 2200 ion trap MS/MS, Varian, Inc., Walnut Creek, CA U.S.A.), entirely combusted to gases ($CO_2$, $N_2$) and subsequently introduced into the isotope ratio mass spectrometer. Analyses were performed using a Thermo GC/C-IRMS system comprising a Trace GC Ultra gas chromatograph (Thermo Electron Corp., Milan, Italy) coupled to a Delta V Advantage isotope ratio mass spectrometer through a GC/C-III interface (Thermo Electron Corp., Bremen, Germany). A c12:0 fatty acid standard was added to each sample for quantification. The C isotope ratio of each compound was reported relative to the Vienna Pee Dee Belemnite standard (V-PDB) and corrected for the C-containing methyl group introduced during derivatization according to the method of Boschker & Middelburg (1999).

## Figure Legends

S 1 Abundance of PLFA biomarker for heterotrophic (a) bacteria (i15:0) and (b) fungi at sampling day 46 relative to starting conditions (sampling day 0) given in %.

S 2 Microbial respiration for single and mixed OM modifications with BEECH and ALGAL OM presented for the incubation period from day 30 to day 46. Data is presented as mean of replicates (n=3-4) with standard deviation. An orientation line for the comparison of figure panels a and b is given in red.


Bacterial-specific PLFA [%]
120
110
100
Fungal-specific PLFA [%]
200
150
100
Algae HighCP
Algae LowCP
Beech
Beech + Algae HighCP
Beech + Algae LowCP


(a)
BEECH
Control
ALGAE LowCP
ALGAE HighCP
(b)
BEECH
BEECH +ALGAE LowCP
BEECH + ALGAE HighCP
Microbial respiration [µg CO2 d-1 [gTOC-1]
Incubation Period [days]
13
12
11
10
9
8
7
30
35
40
45

II **Fabian, J.**, Klawonn, I., Musat, N., Stryhanyuk, H., Dippoldt, M., Grossart H.-P., and Premke, K.: Algal exudates impact microbial turnover of leaf litter in freshwater systems. *Manuscript*

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# Environmental Control on Microbial Turnover of Leaf Carbon in Streams – Ecological Function of Phototrophic-Heterotrophic Interactions

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In aquatic ecosystems, light availability can significantly influence microbial turnover of terrestrial organic matter through associated metabolic interactions between phototrophic and heterotrophic communities. However, particularly in streams, microbial functions vary significantly with the structure of the streambed, that is the distribution and spatial arrangement of sediment grains in the streambed. It is therefore essential to elucidate how environmental factors synergistically define the microbial turnover of terrestrial organic matter in order to better understand the ecological role of photo-heterotrophic interactions in stream ecosystem processes. In outdoor experimental streams, we examined how the structure of streambeds modifies the influence of light availability on microbial turnover of leaf carbon (C). Furthermore, we investigated whether the studied relationships of microbial leaf C turnover to environmental conditions are affected by flow intermittency commonly occurring in streams. We applied leaves enriched with a $^{13}$C-stable isotope tracer and combined quantitative and isotope analyses. We thereby elucidated whether treatment induced changes in C turnover were associated with altered use of leaf C within the microbial food web. Moreover, isotope analyses were combined with measurements of microbial community composition to determine whether changes in community function were associated with a change in community composition. In this study, we present evidence, that environmental factors interactively determine how phototrophs and heterotrophs contribute to leaf C turnover. Light availability promoted the utilization of leaf C within the microbial food web, which was likely associated with a promoted availability of highly bioavailable metabolites of phototrophic origin. However, our results additionally confirm that the structure of the streambed modifies light-related changes in microbial C turnover. From our observations, we conclude that the streambed structure

influences the strength of photo-heterotrophic interactions by defining the spatial availability of algal metabolites in the streambed and the composition of microbial communities. Collectively, our multifactorial approach provides valuable insights into environmental controls on the functioning of stream ecosystems.

**Keywords: algae, bacteria, microbial interactions, $^{13}C$ stable isotopes, PLFA, terrestrial carbon, streambed structure, light**

## INTRODUCTION

The decomposition of organic matter (OM) by microbial sediment biofilms contributes substantially to energy flow and nutrient cycling in fluvial ecosystems (Cole and Caraco, 2001; Hotchkiss et al., 2014). Besides *in situ* OM production, terrestrial OM often dominates OM input in streams, with falling leaves being the major form of external particulate OM input (Fisher and Likens, 1973). Owning to its complex, aromatic molecular structure and distinct chemical composition, the bioavailability of terrestrial OM to microbial communities is considered to be limited (Farjalla et al., 2009; Guillemette et al., 2013). Yet, multiple factors, including climate, streambed hydrology, primary production, nutrient availability, and microbial biomass and composition, modulate the relevance of molecular properties for the availability of organic compounds (e.g., Tank et al., 2010; Ziegler and Lyon, 2010; Singer et al., 2011; Rier et al., 2014). Therefore, it is widely accepted that microbial decomposition of OM is related to environmental conditions rather than the molecular structure of the substrate (Schmidt et al., 2011; Marín-Spiotta et al., 2014).

Biofilms dominate microbial life in streams and form highly diverse and complex matrix-enclosed communities in and upon the streambed as well as on OM fragments (Battin et al., 2007). In subsurface streambeds, i.e., the hyporheic zone, biofilms are dominated by heterotrophic species. In light mediated streambed areas, i.e., the benthic zone, eukaryotic algae and cyanobacteria, together with heterotrophic bacteria and fungi form diverse biofilms that favor potential metabolic interactions (Rier et al., 2007; Danger et al., 2013). Algal exudation of dissolved OM provides a highly bioavailable substrate to microbial heterotrophs that is selectively removed from the OM pool (Guillemette et al., 2013). These organic compounds of phototrophic origin are rich in energy as well as nutrients and therefore are assumed to stimulate the metabolic capability of fungi and heterotrophic bacteria to decompose less bioavailable terrestrial OM (Kuehn et al., 2014). Accordingly, interactions among microbial photo- and heterotrophs significantly alter the turnover of terrestrial OM in aquatic ecosystems (Rier et al., 2007; Danger et al., 2013). Yet, additional research implies that fungi and bacteria do not equally respond to the availability of algal OM, with stimulating effects on activity and biomass mainly observed for bacteria (Mille-Lindblom and Tranvik, 2003; Frossard et al., 2012; Fabian et al., 2017). Nevertheless, although environmental conditions apparently influence the appearance and strength of photo-heterotrophic interactions (Rier et al., 2014; Hotchkiss and Hall, 2015), it remains unclear how multiple environmental factors synergistically affect their significance for microbial turnover of terrestrial OM in streams.

In stream ecosystems, microbial turnover of OM is significantly influenced by hyporheic flow, that is the flow of surface water through the pore space of the streambed in flow paths that return to surface water (Battin et al., 2003; Boano et al., 2014; Perujo et al., 2017). The hyporheic flow varies according to the distribution and spatial arrangement of sediment grain sizes (Bear, 1972), or in other words the structure of the streambed. In natural ecosystems, the arrangement of the different sediment grain sizes varies both vertically and horizontally and poorly conductive habitats of sand or silt commonly alternate with well-conducting habitats of gravel or cobbles (Powell et al., 2005; Bridge, 2009). These spatial variations in hydraulic conductivity cause up-and-down welling of water across the streambed surface, thus increasing the hyporheic flow, which in turn promotes the transport of dissolved compounds through the streambed. (Malard et al., 2002; Salehin et al., 2004).

Accordingly, streambed structure influences the hyporheic transport of dissolved organic resources from the benthic zone into the pore space of deeper sediment layers (Battin, 2000; Navel et al., 2011). Previous research underlines that changes in the spatial distribution of resources cause variations in resource use efficiency and organic carbon (C) turnover in streambed biofilms (Battin et al., 2003; Singer et al., 2010, 2011). Hence, streambed structure likely determines the extent to which streambed heterotrophs interact with OM of phototrophic origin during C turnover through associated changes in the hyporheic flow of phototrophic compounds through the streambed.

Given the multitude of environmental factors whose influence on microbial C turnover has been reported for streambed biofilms, the ecological relevance of photo-heterotrophic interactions for the degradation of terrestrial OM may vary greatly among different ecosystem types (Wagner et al., 2017). In intermittent streams, flow disruption provides a strong influence on microbial function in ecosystem processes (Febria et al., 2015; Duarte et al., 2017) and the turnover of terrestrial OM is largely determined by the characteristic dry-wet cycles (Abril et al., 2016; Weise et al., 2016). It is therefore likely that light conditions, as well as associated photo-heterotrophic interactions, are less important for the turnover of terrestrial OM in intermittent streams than assumed for perennial streams. However, given the significant contribution of intermittent streams to global C fluxes (Tockner and Stanford, 2002), it is important to understand how recognized functions of photo-heterotrophic interactions for terrestrial OM turnover in perennial streams can be transferred to these temporary waters.

This study investigated how light and streambed structure interactively alter the cycling of terrestrial OM through the microbial food web in temporary stream ecosystems with a focus on possible metabolic interactions between microalgae and

heterotrophic bacteria. In experimental streams, we manipulated hyporheic flow and phototrophic activity by applying different levels of streambed structure and light intensities, respectively. Fragments of leaf litter enriched in $^{13}C$ stable isotopes were applied as a proxy for terrestrial OM, whose cycling through the microbial food web was followed via isotope analysis of different C pools: microbial biomass [phospholipid derived fatty acids (PLFA), dissolved organic carbon (DOC), and respired C]. The findings were related to treatment-related variations in overall biofilm community composition derived from PLFA, as well as bacterial biofilms, as analyzed by terminal restriction fragment length polymorphism (T-RFLP) profiling. Based on findings in perennial ecosystems, we expected that bacterial assimilation of leaf C is stimulated by phototrophic activities, and therefore, related to light conditions. In addition, we assume that streambed structure defines the hyporheic transport of phototrophic metabolites through the streambed. Accordingly, we hypothesize that effects of light on leaf C turnover correlate with streambed structure.

## MATERIALS AND METHODS

### Study Design

Applying a 2 × 2 factorial design, we manipulated streambed sediment composition and light in 16 outdoor, experimental streams, i.e., flume mesocosms (400 × 12 × 10 cm, **Figure 1A**), installed beneath a white tent to prevent uncontrolled input of coarse OM and rainwater. Streams were filled with a 3-cm streambed of sand and gravel, which were rinsed with 10% hydrochloric acid and tap water prior to use to avoid uncontrolled microorganism input. As a proxy for terrestrial OM, 31.25 g C $m^{-2}$ of $^{13}C$-labeled beech leaf fragments were mixed into the upper 1 cm of the streambed. A surface-water column of 2.9 ± 1 cm [tap water + nitrogen (4,54 mg $L^{-1}$ $N\text{-}NO_3^-$) and phosphorus (542 µg $L^{-1}$ $P\text{-}PO_4^{-3}$) + 0.3 ml/L SL−10 trace element solution, according to Lehman (1980)] was circulated at a constant flow velocity of 2.5 ± 0.03 cm $s^{-1}$ using an aquarium pump (EHEIM GmbH & Co. KG, Deizisau, Germany). Evaporated water was replaced with deionized water.

Streambed sediments were inoculated with a microbial community (16.45 mg $CL^{-1}$) sampled from a first-order rarely intermittent stream (Waldbach, Bad Saarow, Germany, N52° 16.4578 E14° 3.2794) located in a forest catchment. The inoculum was generated from a suspension in stream water of randomly collected surface sediments, leaf litter, and woody fragments for 48 h followed by filtration through a 125-µm mesh to exclude macro-invertebrates and larger OM particles. After 7 weeks of colonization, we ran a 6-week dry/2-week rewet cycle to generate conditions of an intermittent stream ecosystem (Larned et al., 2010). Primary production was manipulated by two levels of light intensity: ambient (outdoor conditions) vs. reduced light. For the latter condition, ambient light intensity was reduced by 54 ± 8% by placing 3 layers of black mosquito net (grid size 1.29 × 1.13 mm) 20 cm above the stream. Differences in streambed structure and associated changes in hyporheic flow were generated by varying the structural arrangement of gravel (2–10-mm grain size, d50 = 4.75 mm) and sand (0.2–2-mm grain size, d50 = 0.57 mm) in the streambed. Solute transfer in gravel greatly exceeds that in sand and sand-gravel (Mendoza-Lera et al., 2017). As a result, alternating patches of sand and gravel intensify up- and down-welling, leading to enhanced vertical water exchange (VWE) and thus, solute transport into the pore spaces of sorted as compared to homogenously mixed streambeds (Malard et al., 2002). Accordingly, we generated two levels of streambed structure by filling 8 streams with a homogeneous mixture of sand and gravel (Mixed Streambed, reduced solute transport) and 8 streams with 8 alternating blocks (45 cm) of gravel and sand (Sorted Streambed, enhanced overall solute transport).

The combination of both factors, light intensity and streambed structure, added up to 4 different treatments, with 4 replicates each (**Figure 1B**). Treatment-related effects on microbial C respiration and DOC production, relate to whole stream scale and were measured weekly throughout the entire experimental phase (**Figure 1C**). Treatment-related effects on the function and composition of biofilm community relate to sediment scale, thus gravel, sand, and sand-gravel sediments were sampled and analyzed separately. Biofilm community composition was measured at the end of the colonization and rewet period. The stable isotope composition of biofilm fatty acids was measured at the end of the rewet period.

### Monitoring of Treatment Conditions

Light intensity at the streambed and water temperature were monitored every 10 min (ONSET HOBO Pendant® Temperature/Light 64K Data Logger, PCB Synotech GmbH, Hückelhoven, Germany). To monitor solute transfer through the streambed, surface water flow and VWE were estimated by using the conservative fluorescent tracer uranine, following Mutz et al. (2007). A small pulse of concentrated tracer solution (3 µg $L^{-1}$) was added to the stream at the discharge side and the tracer concentration in the water column was measured every 15 min for 6 h at the opposite end of the stream with a fluorometer (LLF-M, Fiber-Optic fluorometer; Gotschy Optotechnik, Adnet, Austria). Surface water flow was calculated based on the tracer's mean travel time. VWE was determined from the decrease in uranine concentration resulting from down-welling of the tracer into the streambed and upwelling into the free water. Measurements were made at night and under stable pH to avoid photochemical decay and quenching of the tracer.

### Quantification and Stable Isotope Analysis of DOC and Respired C

For DOC quantification and isotope analysis, filtered surface water samples (cellulose acetate, 0.45 µm; Sartorius Stedim Biotech GmbH, Göttingen, Germany) were fixed with 22 mmol $L^{-1}$ $ZnCl_2$ and stored at 4°C until analyzed on an OI Analytical Aurora 1030W TIC-TOC analyzer (OI Analytical, College Station, TX, United States) coupled in continuous flow mode to a Thermo Scientific Delta V *plus* IRMS. Analysis of internal laboratory standards enabled an analytical precision of <±0.3‰ for $\delta^{13}C$-DOC (±1σ). Isotope ratios are expressed as

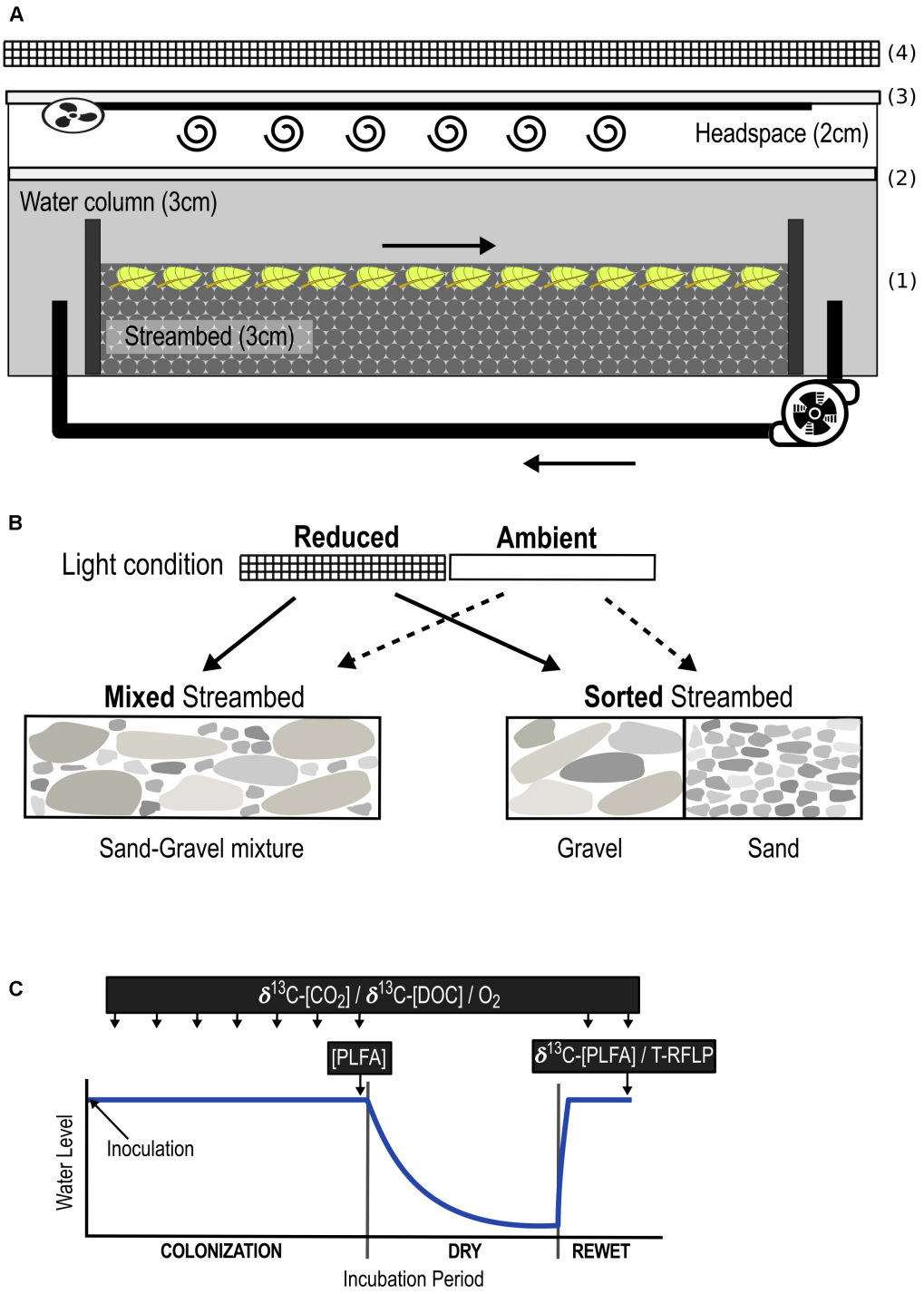


**FIGURE 1 |** Overview of the experimental set-up. Experimental outdoor streams **(A)** were filled with a streambed layer of gravel and sandy sediments in two different arrangements: sorted and mixed **(B)**. $^{13}$C-enriched leaf fragments were mixed into the upper 1 cm of the streambed (1). Streams were sealed gas-tight without headspace for oxygen measurements (2), and with headspace for the analysis of headspace $CO_2$ (3). A fan attached to the lid and connected to a 3-m tube facilitated mixing of the headspace air. Streams were exposed to ambient (outdoor condition) and reduced (54 ± 8% of ambient) light intensities, the latter achieved by placing 3 layers of black mosquito net 20 cm above the streams (4). The combinations of both light availability and streambed structure added up to 4 different treatments: (1) Sorted Streambed + Reduced Light, (2) Sorted Streambed + Ambient Light, (3) Mixed Streambed + Reduced Light, (4) Mixed Streambed + Ambient Light. **(C)** Streams were inoculated with a natural microbial community and then incubated for a 7-week colonization period, followed by a 6-week dry and a 2-week rewet period. Quantity of respired C and DOC were measured over the entire incubation period. The composition of the biofilm community (PLFA/T-RFLP) was measured at the end of the colonization and rewet period. The stable isotopic composition of biofilm fatty acids was measured at the end of the rewet period.

$\delta^{13}C$ in per mil (‰) (Coplen, 2011) relative to the Vienna PeeDee Belemnite (VPDB) standard.

Microbial C respiration was calculated from day–night dynamics of $O_2$ in the water column. Streams were sealed headspace-free with acrylic glass lids, and $O_2$ was measured every 30 min for 24 h using a multichannel fiber oxygen optode system (Oxy 10 mini; Presens, Regensburg, Germany). C respiration rates were calculated and standardized to 20°C according to Mendoza-Lera and Mutz (2013) and expressed as C equivalents assuming a respiratory quotient of 0.85 (Bott, 2007). The isotopic composition of respired C was obtained by Keeling plot analysis of $CO_2$ emitted from the water column into the headspace during night incubations (10 PM to 4 AM) following Pataki et al. (2003). To this end, streams were sealed gas-tight with acrylic glass lids and equipped with an automatic sampling system for headspace $CO_2$ connected to a $CO_2$-isotope analyzer (Off-Axis ICOS CCIA; Los Gatos Research, San Jose, CA, United States) that facilitated online monitoring of $CO_2$ concentration and $\delta^{13}C$-$CO_2$ values every 2 h throughout incubation, modified after Fabian et al. (2017). A fan (ACT-RX Technology Corporation, Taiwan; airflow capacity of 116 L $min^{-1}$) attached to the insides of the lids and connected to a 3-m tube enabled homogenous mixing of the headspace during incubations. Regular measurements of an internal laboratory $CO_2$ gas standard (0.15% $CO_2$ in 70% $N_2$ + 30% $O_2$) allowed for data correction with respect to instrumental drift and linearity during measurements, yielding a precision of 2‰ for $\delta^{13}C$-$CO_2$.

## Biofilm Community Composition Analysis

PLFA are present in the membranes of all living cells, but rapidly degrade to neutral lipids upon cell death (White et al., 1979; Willers et al., 2015). Hence, monitoring the variation in PLFA allows sensitive and reproducible characterization and quantification of microbial communities (Boschker and Middelburg, 2002; Yao et al., 2015). PLFA were extracted from 0.2 g of freeze-dried leaf fragments sampled at the end of the experiment, followed by solid-phase separation from other lipids (Bond Elute LRC cartridge 500 mg; Agilent Technologies, Santa Clara, CA, United States) and mild alkaline methylation to fatty acid methyl esters (FAMEs) (White and Tucker, 1969; Frostegård et al., 2011; Steger et al., 2011). FAMEs were quantified on a GC-MS system (Agilent 6890/Agilent 5973, Agilent Technologies, Germany) using a CP SIL 88 column (100 m, ID: 0.25 mm, film: 0.2 μm; Agilent Technologies), as described in Boëchat et al. (2014). A standard mix (Supelco 37 Component FAMEs Mix) was applied for system calibration. FAMEs were identified by their mass spectra and retention times, which were validated with standard FAMEs or by comparing their equivalent chain lengths with literature reports for the applied column (Eder, 1995; Santercole et al., 2012).

For more detailed profiling of bacterial biofilm communities, DNA was extracted from freeze-dried leaf fragments sampled at the end of the experiment using the NucleoSpin Soil kit (Macherey-Nagel, Germany). T-RFLP profiling was performed as described by Weise et al. (2016). GeneMapper software version 5.0 (Applied Biosystems) was used for data analysis. T-RFLP profiles were normalized according to Dunbar et al. (2001).

## Stable Isotope Analysis of Biofilm Lipids

FAME isotope ratios, expressed as $\delta^{13}C$ in ‰ relative to VPDB, were measured on a HP5890 GC (Agilent Technologies, Palo Alto, CA, United States) connected to a Delta plus XL IRMS via a combustion interface GC/C III (both Thermo Scientific, Bremen, Germany) following Kramer and Gleixner (2008). Based on previous culture observations (de Carvalho and Fernandes, 2010; Akinwole et al., 2014; Arce Funck et al., 2015; Strandberg et al., 2015), cell membrane fatty acids, of which we could analyze the carbon isotope ratios, were grouped into bacterial (*iso*14:0, *iso*15:0, *anteiso*15:0, *iso*16:0, *iso*17:0, *cyclic*17:0), algal (C20:4w6, C20:5w3, C22:5w6, C22:6n3), or fungal + algal (C17:1w9, C18:1w7, C18:2w6, C18:3w3, C18:3w6, C18:1w9) PLFA biomarker. Further, we identified several unspecific, universal PLFA (C14:0, C15:0, C16:0, C16:1w5, C16:1w7, C17:0, C18:0). Consequently, to link identity and function of microbial decomposers, we focused on the biomarkers specific for heterotrophic bacteria, as biomarkers for fungi overlap with those for algae.

## Calculation of the Proportion of Leaf C in Organic and Respired C Pools

The fraction of leaf C in organic and respired C pools ($F_{Leaf}$) was calculated by applying a two end-member mixing model as follows (1), assuming two C substrate pools in the system:

$$F_{Leaf}[\%] = \frac{\left(\delta^{13}C_{Sample} - \delta^{13}C_{Atmospheric}\right)}{\left(\delta^{13}C_{Leaf} - \delta^{13}C_{Atmospheric}\right)} \times 100 \quad (1)$$

The first C pool ($\delta^{13}C_{Atmospheric}$) comprised all C substrates that originally entered the ecosystem C cycle through photosynthetic fixation of atmospheric $CO_2$, representing natural isotope composition. $\delta^{13}C_{Atmospheric}$ was therefore assumed to equal 1‰ and −23‰ in calculations for the respired and organic C pools, respectively (Peterson and Fry, 1987; Finlay, 2004). The second C substrate pool ($\delta^{13}C_{Leaf}$) comprised C that originally entered the ecosystem C cycle through the decomposition of leaf OM enriched in $^{13}C$ isotope. $\delta^{13}C_{Leaf}$ was obtained from isotope analysis of 6 subsamples taken from the initial pool of leaf fragments and equaled 804 ± 12‰.

For DOC pools, $\delta^{13}C_{Sample}$ was obtained by isotope analysis assuming isotope fractionation during microbial C degradation to be negligible (Mary et al., 1992). For biofilm C respiration, $\delta^{13}C_{Sample}$ was obtained by Keeling plot analysis (Keeling, 1961; Pataki et al., 2003) of respired $CO_2$, corrected according to Mook et al. (1974). For biofilm C assimilation, $\delta^{13}C_{Sample}$ was obtained by isotope analysis of PLFA corrected according to Boschker and Middelburg (2002). The quantity of leaf derived C ($[C_{Leaf}]$) for each C pool was obtained by multiplying $F_{Leaf}$ with the total quantity of the respective C pool ($[C_{Sample}]$), according to

$$[C_{Leaf}] = [C_{Sample}] \times F_{Leaf}. \quad (2)$$

## Statistical Analysis

Analyses were performed with the statistical software (R Core Team, 2016) using the packages nlme, vegan, MASS, multcomp, and psych, at a significance level of $P \leq 0.05$. Significance of environmental factors for the composition of organic C pools as well as biofilm composition and C metabolism was evaluated by computing a linear mixed-effects model for each studied parameter. We included the treatments "Light" (2 levels: Reduced versus Ambient) and either "Streambed Structure" (2 levels: Sorted versus Mixed) or "Sediment" (3 levels: Gravel, Sand, Sand-Gravel) as fixed effects. For time series (DOC/C respiration), we included "Sampling Day" as a random effect to correct for pseudo-replication, i.e., re-sampling of the same stream at different dates. To test for an interaction effect between the drought event and the treatments on the respective parameter, we included "Period" as a fixed effect. In the respective model, we further included "Stream" as a random effect to allow a different intercept for each replicate. Interactions among fixed effects were tested for each model and were included if significant. The statistical significance of each fixed effect was tested using a likelihood-ratio (LR) test by comparing the model with and without the target effect.

Relationships between the composition of the microbial (PLFA) and bacterial (T-RFLP of the 16S rRNA gene) communities and the environmental variables (light availability, habitat) and respired C were investigated by distance-based redundancy analysis (dbRDA). Accordingly, a distance matric was generated by calculating pairwise Bray–Curtis dissimilarities between individual replicate samples from square root-transformed PLFA or T-RFLP data, followed by principle coordinate analysis (PCoA). The eigenvalues obtained in PCoA were then visualized in a redundancy analysis (RDA). In the following, the significance of the contribution of a particular variable to the explained variation was tested by permutational multivariate analysis of variance (PERMANOVA), applying 500 permutations and a significance level of $P > 0.05$. The resulting pseudo-*F*-values and significance level were reported for each test result.

# RESULTS

## Treatment Conditions

Mean light availability was significantly higher under ambient, (44.2 ± 4.4) μmol $m^{-2}$ $h^{-1}$, than reduced, (26.2 ± 1.0) μmol $m^{-2}$ $h^{-1}$, light conditions. Pore water exchange in gravel blocks occurred within 10 min after tracer addition, which was below the 15-min measuring interval. Therefore, VWE in sorted streambeds was calculated based on the pore water exchange in the sandy sediment habitats. The VWE was significantly higher in the sandy sediments of sorted streambeds, (3.80 ± 0.65) $dm^3$ $m^{-2}$ $h^{-1}$, than in the sand-gravel sediments of homogenously mixed streambeds, (3.08 ± 0.16) $dm^3$ $m^{-2}$ $h^{-1}$, ($LR = 6.50$, $P = 0.01$). Variations in VWE were independent of light conditions ($LR = 0.01$, $P = 0.91$).

## Biofilm Cycling of Leaf C Within Respired C and DOC Pools

Quantitative changes in microbial C turnover were derived from variations in DOC concentrations and C respiration rates. In addition, variations of the leaf C fraction in the DOC and respired C pools, $F_{Leaf}$, provide information about the proportional use of leaf C compared to other C sources. Accordingly, the combination of quantitative analyses with stable isotope analyses provides information on whether a change in C turnover is associated with altered use of leaf C within the microbial food web.

The rate to which leaf C was respired was significantly regulated by light availability (**Figures 2A,B**; $LR = 16.72$, $P < 0.0001$), independent of streambed structure (**Figures 2E,F**; $LR = 0.02$, $P = 0.88$). On the contrary, we observed a significant interaction between light availability and streambed structure on $F_{Leaf}$ in respired C (**Figures 3A,B**; $LR = 18.89$, $P < 0.0001$). Hence, the proportion of leaf C in respired C was higher under ambient than under reduced light conditions in sorted streambeds, whereby opposing effects were observed in mixed streambeds. Furthermore, under ambient light, the proportion of leaf C in respired C was significantly higher in sorted than in mixed streambeds (**Figures 3E,F**; $P < 0.001$).

Similar to the respired C pool, we observed a significant interaction between streambed structure and light availability for treatment-induced changes in $F_{Leaf}$ in the DOC pool (**Figures 3C,D**; $LR = 11.57$, $P < 0.001$). Accordingly, the proportion of leaf C in the DOC pool was significantly lower under ambient than under reduced light conditions in homogenously mixed streambeds (**Figures 2C,D**; $P = 0.001$). On the contrary, the proportion of leaf C did vary according to light availability among DOC pools of sorted streambeds ($P = 0.5$). Under ambient light conditions, the proportion of leaf C in DOC was significantly higher in sorted than in mixed streambeds (**Figures 3G,H**; $LR = 5.21$, $P = 0.02$).

Observed treatment effects on the rate of leaf C respiration and on the quantity of leaf DOC did not differ before and after the drought event (*Light* × *Phase:* $LR = 0.15$, $P = 0.70$, *Streambed* × *Phase:* $LR = 0.03$, $P = 0.86$). The same accounts for the effect of our treatments on the proportion of leaf C in the respired C and the DOC pool.

## Biofilm Assimilation of Leaf C

Carbon isotopes could be analyzed from 22 identified PLFA biomarkers. Within the group of non-specific PLFAs and bacteria-specific PLFAs, variations in $F_{Leaf}$, hence the proportion of leaf C in PLFA biomass, were similar in terms of light availability and streambed structure (**Figure 4**). In contrast, $F_{Leaf}$ values of algae-specific PLFA biomarkers varied differently relative to the treatment conditions.

In bacteria-specific PLFAs, $F_{Leaf}$ varied between the sediments. These differences were not equally pronounced under ambient than under reduced light conditions. Under ambient light conditions, the proportion of leaf C was lowest in PLFAs from sand-gravel sediments of the mixed streambeds and highest in sand and gravel sediments of the sorted streambeds. Under

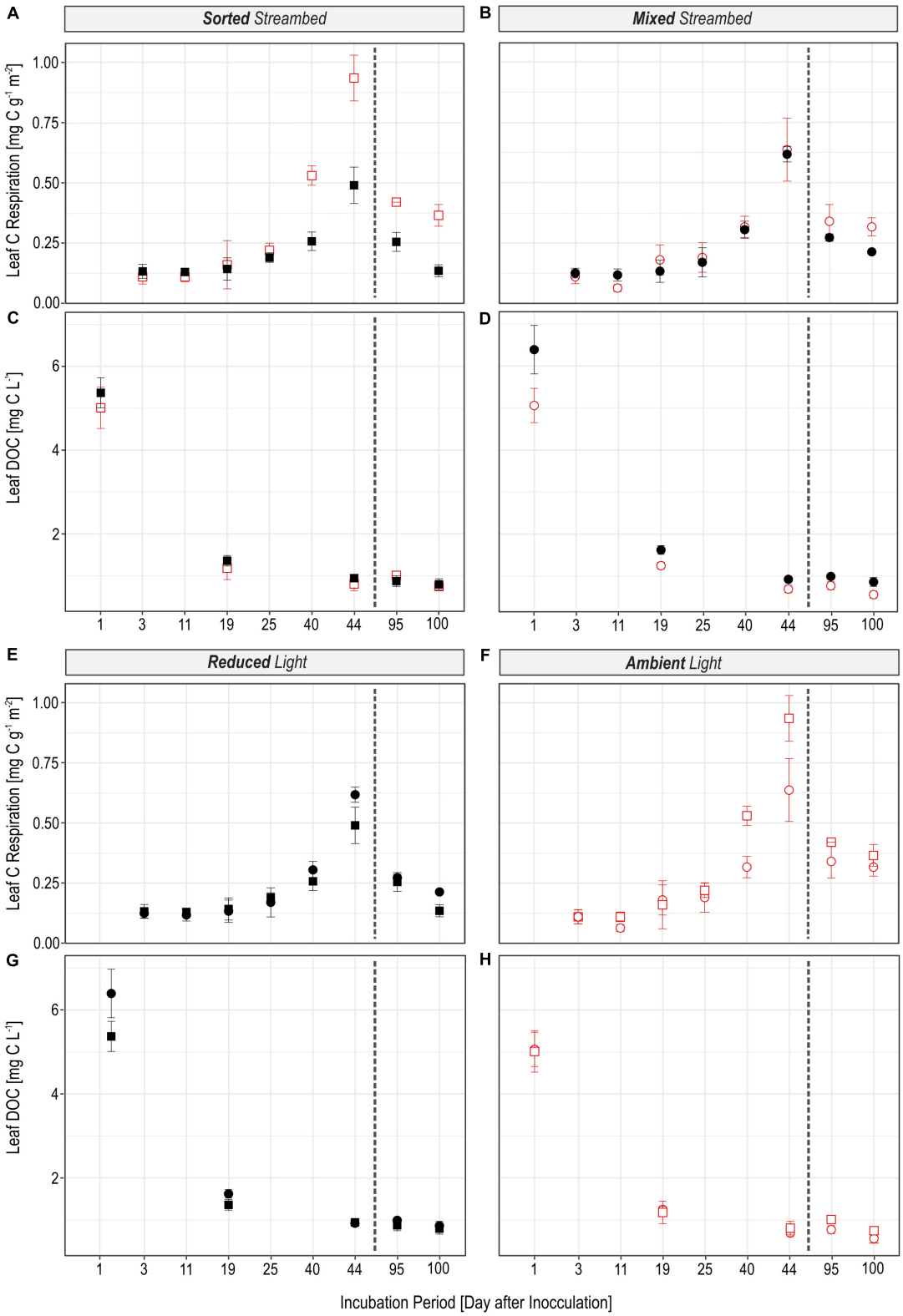


**FIGURE 2 |** Figures illustrate the effect of the light availability **(A–D)** and the effect of the streambed structure **(E–H)** on the magnitude of microbial leaf C turnover. The amount of leaf C which was respired per hour **(A,B,E,F)** and which was bound in the DOC pool **(C,D,G,H)** was different in experimental streams depending on whether the streambed structure was sorted (squares) or homogeneously mixed (circles) and whether streams were incubated under reduced (black) or ambient light conditions (red). The dashed line separates pre- and post-drought phases.

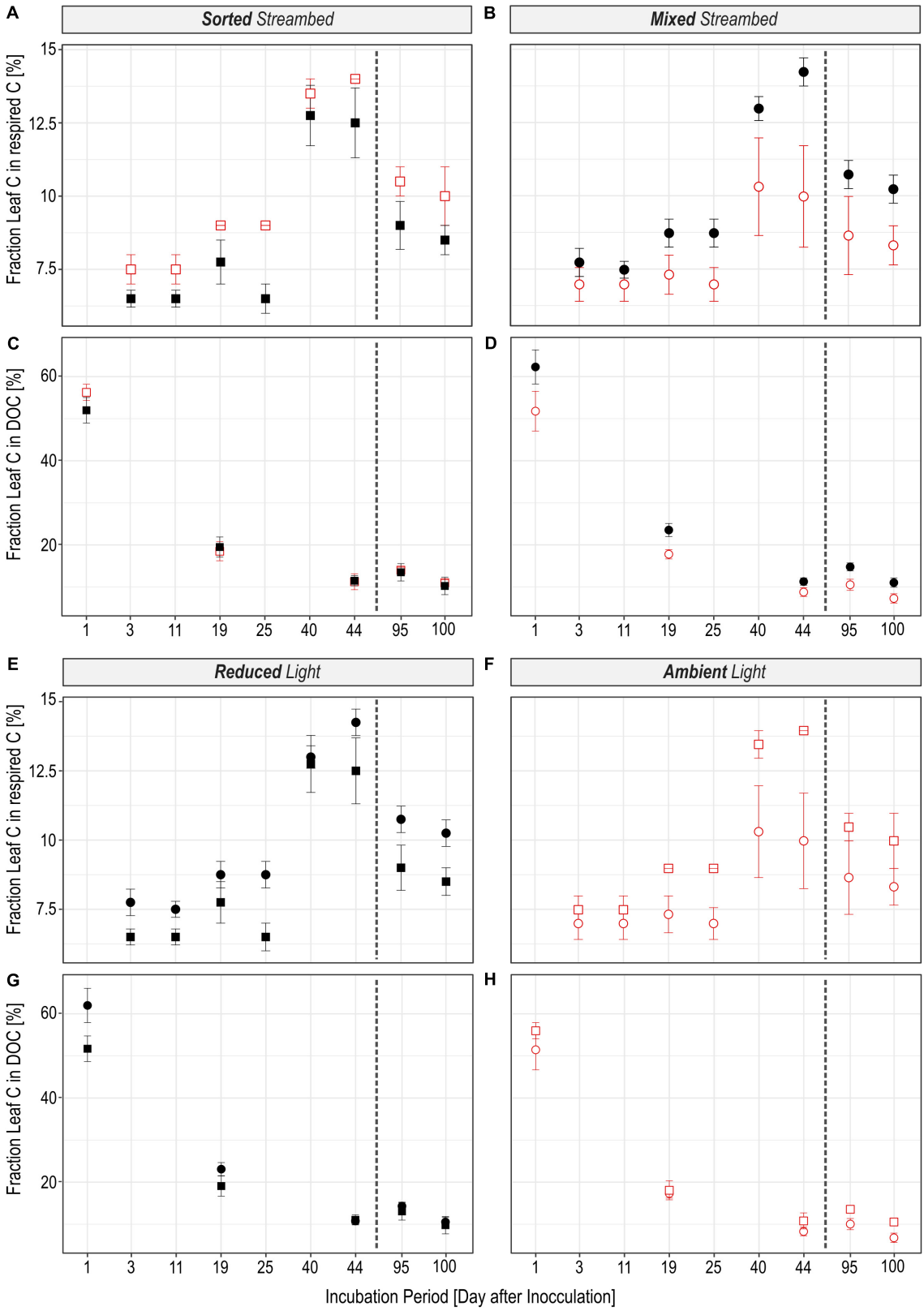


**FIGURE 3 |** An overview is given about the fraction by which leaf C is utilized in streams with a sorted (squares) or homogenously mixed (circles) streambed structure that were either incubated under reduced (black) or ambient (red) light conditions. **(A–D)** Illustrate the effect of the light availability and **(E–H)** the effect of the streambed structure on the fractional use of leaf C in respired C **(A,B,E,F)** and in DOC **(C,D,G,H)**. The dashed line separates pre- and post-drought phases.

reduced light conditions, however, no significantly different $F_{Leaf}$ values were measured between gravel and sand-gravel sediments, with $F_{Leaf}$ values being significantly higher in PLFAs from sandy sediments. Consequently, light-induced variations in the proportion of leaf C on PLFA biomass were sediment-specific. In sand and gravel sediments, we found a generally higher proportion of leaf C under ambient than under reduced light conditions. Thereby, the $F_{Leaf}$ gap between both light treatments was most pronounced for bacterial PLFAs from gravel habitats. On the contrary, the proportion leaf C in bacterial PLFAs from sand-gravel sediments did not vary significantly between both light treatments. In addition, the extent to which our treatments induced variations in the proportion of leaf C in bacterial PLFAs differed between the different PLFA types (**Figure 5A**).

## Environmental Shifts in PLFA and T-RFLP Profiles

Distance based redundancy analysis (dbRDA) of PLFA profiles (**Figure 6**) indicated a significant shift in the composition of biofilm communities from pre- to post-drought conditions ($F = 49.75$, $P < 0.001$), which explained 40% of the variation in the data set. Additionally, PLFA profiles significantly varied according to light availability (*habitat:* $F = 2.81$, $P = 0.001$) but not among the sediment habitats (*habitat:* $F = 1.19$, $P = 0.19$). Combined results of PLFA and respiration analysis reveal that the rate of leaf C respiration was significantly correlated with light-induced shifts in PLFA profiles ($F = 1.80$, $P = 0.03$).

T-RFLP profiling of the bacterial 16S rRNA gene (**Figure 5B**) revealed highly diverse profiles of microbial communities, which varied considerably between replicate samples. In total, 289 T-RFs were identified after normalization. The profiles indicated significant variation in bacterial community structure in relation to treatment conditions ($F = 1.58$, $P = 0.037$). dbRDA revealed a greater influence of the sediment habitat (*habitat:* $F = 1.29$, $P = 0.09$) than light availability (*habitat* × *light:* $F = 1.15$, $P = 0.24$) on bacterial community structure, explaining 12.5 and 5.28% of variance in the data set, respectively. Distinct bacterial community composition in relation to light availability were only observed for gravel habitats (*habitat* × *light:* $F = 1.37$, $P = 0.04$). Microbial respiration of leaf C did not clearly correlate with the composition of the bacterial community (*Respiration-leaf C:* $F = 1.29$, $P = 0.13$, $F_{Leaf}$*:* $F = 0.81$, $P = 0.75$). Nevertheless, the results indicate a significant relationship between leaf C respiration and light-induced shifts in the composition of the bacterial communities of gravel habitats ($F = 2.16$, $P = 0.02$), but not sandy habitats ($F = 0.84$, $P = 0.73$) and sand-gravel habitats ($F = 0.69$, $P = 0.87$).

# DISCUSSION

In this study, we present evidence that streambed structure interacts with light as a driving factor for photo-heterotrophic interactions during leaf decomposition. Our results emphasize that the structure of the streambed defines the spatial availability of phototrophic metabolites in the streambed, as well as the composition of microbial streambed communities. Both are known to define the extent to which phototrophs and heterotrophs interact. Accordingly, our results emphasize that the structure of the streambed may influence the relevance of photo-heterotrophic interactions for leaf degradation. Furthermore, results confirm the fundamental influence of light availability and streambed structure on microbial functions in temporary streams.

## Interrelating Effects of Light and Streambed Structure on Microbial C Turnover

Phototrophic activities were strongly related to light availability as primary production was significantly higher under ambient than under reduced light conditions (see Zlatanović et al., 2017 for detailed discussion). Accordingly, we hypothesized that the decomposition of leaf C increases with increasing light availability because of a previously postulated stimulating effect of phototrophic activities on microbial leaf breakdown (Danger et al., 2013; Kuehn et al., 2014; Rier et al., 2014). Indeed, leaf C respiration rates were higher under ambient than under reduced light conditions, which agreed well with our expectations. On the contrary, stable isotope analyses of the respired C pool do not imply a generally promoted utilization of leaf C with increasing light availability, but instead highlight an interaction between our light and streambed treatments. While in sorted streams the proportion of leaf C in respired C was higher under ambient rather than reduced light conditions, in mixed streams the opposite was true. The stable isotope composition of the respired C pool and, consequently, the proportion of leaf C in the respired C pool, is the sum of heterotrophic and phototrophic activities (Fry and Sherr, 1989). At the ecosystem level, the phototrophic activities did not differ between the two streambed treatments (Zlatanović et al., 2017). Accordingly, the observed interactive effect of the streambed structure and the light availability on the composition of the respired C pool must underlie changes in the breakdown of leaf litter by heterotrophic activities. This assumption is further supported by the stable isotope analysis of bacterial PLFA biomarkers corresponding to the treatment-induced changes in the stable isotope ratios of the respired C pool. In gravel and sandy sediments (sorted streambeds), the higher proportion of leaf C in bacterial PLFA under ambient than under reduced light conditions indicates a higher utilization of leaf C by bacteria with increasing light availability. On the contrary, for bacterial PLFA from sand-gravel sediments (mixed streambeds), no significant differences between the light conditions were observed. Consequently, our results support that the streambed structure influences the proportional utilization of leaf C within the microbial food web, but also demonstrate that streambed structure had only a marginal influence on the magnitude of leaf C respiration. One possible explanation for these contradictory results could be the significant contribution of algae to leaf C respiration in our approach (Zlatanović et al., 2017). As previously mentioned, phototrophic and heterotrophic activities were not equally affected by our streambed treatments. Moreover, $F_{Leaf}$ values of the algae-specific fatty acid biomarkers varied

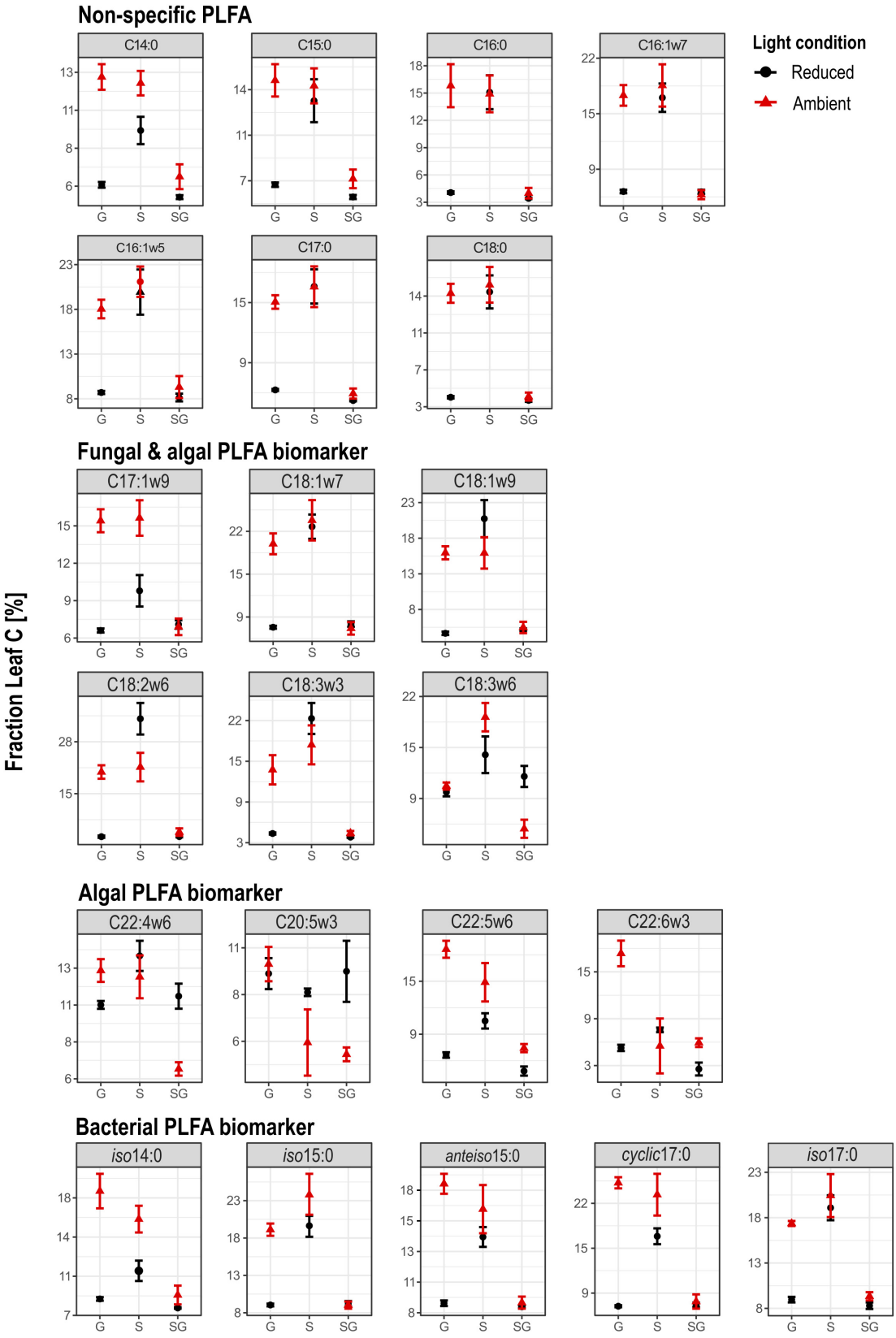


**FIGURE 4 |** Overview on the fraction of leaf C in PLFA biomarkers of heterotrophic bacteria, microalgae and fungi. G, Gravel; S, Sand; SG, Sand-Gravel.

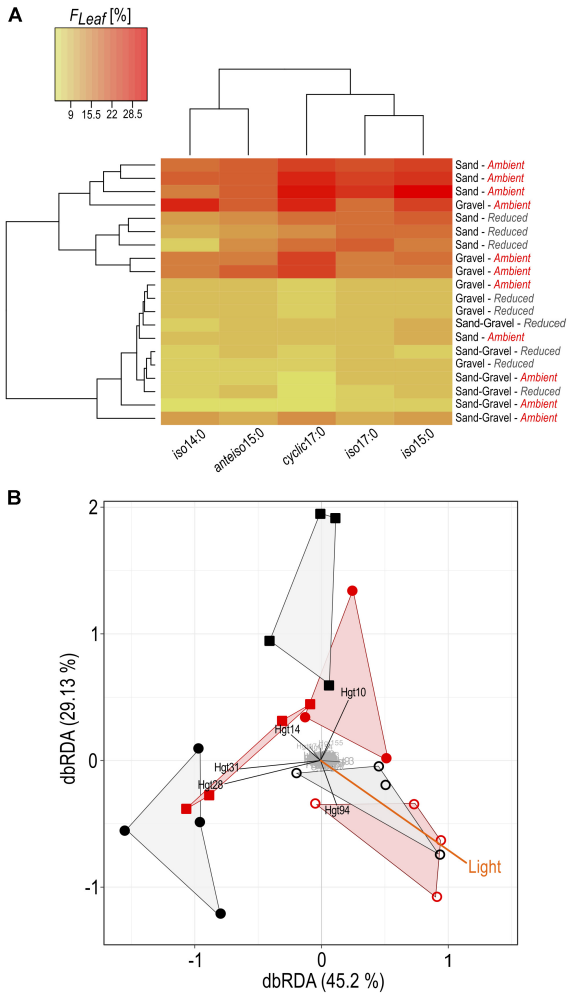


**FIGURE 5 |** Alterations in bacterial community composition and C assimilation in relation to environmental conditions. **(A)** The proportion of leaf C in bacterial PLFA is given, expressed in %. All treatment replicates are presented on sediment level (sand, gravel, sand-gravel) sampled from streambeds incubated under reduced or ambient light. **(B)** Distance-based redundancy analysis of bacterial community fingerprints and environmental data. A hull connects replicates of sand (open circles), gravel (circles) and sand-gravel (squares) sediments incubated under reduced (black) or ambient light (red). T-RF's with marginal influence on underlying differences between the samples are highlighted in gray.

differently according to light availability and streambed structure compared to bacteria-specific fatty acid biomarkers (**Figure 4**). Hence stable isotope analysis of fatty acids implies distinct effects of our treatments on the resource utilization of algae compared to bacteria. It is therefore possible that the high contribution of algae to C respiration superimposed the effect of our treatments on the magnitude of leaf C respiration by the heterotrophic community.

## Streambed Structure Determines the Spatial Availability of Algal Metabolites

While phototrophic activities and thus the production of energy-rich OM by phototrophs are limited to the benthic zone, heterotrophic processes occur throughout the entire streambed (Battin et al., 2016). Accordingly, heterotrophic activities are significantly influenced by hyporheic flow, as it determines the solute supply with regard to organic substrates, nutrients and redox partners (Battin et al., 2003; Perujo et al., 2017). We hypothesized that a greater hyporheic flow and resultant greater vertical solute transfer across the streambed would expand the stimulatory function of phototrophic metabolites on heterotrophic leaf decomposition to deeper sediment areas. Light-related changes in the assimilation of leaf C into bacterial PLFAs were most pronounced in gravel sediments compared to sandy and sand-gravel sediments (**Figures 4**, **5A**). Gravel sediments are characterized by a much deeper photic zone and greater hydraulic conductivity than sand or sand-gravel sediments (Mendoza-Lera et al., 2017). Hence, consistent with our assumptions, the stronger hydraulic conductivity and the deeper benthic zone in gravel sediments could thus have favored the availability of algae metabolites into deeper sediment areas. Given the stimulating effect of algae metabolites on heterotrophic activities (Cole et al., 1982), this could have led to a greater proportion of the heterotrophic sediment community being mobilized in their activity, meaning that more leaf OM was actively degraded accompanied by a higher proportional use of leaf C within the microbial food web.

Our assumption that the streambed structure influences the effect of light on leaf C turnover by altering the spatial availability of algal metabolites is further supported by differences in leaf C utilization between sand and sand-gravel sediments. Indeed, both sediments provide similar habitat characteristics with respect to specific sediment surface area, a low depth of the photic zone, and low hydraulic conductivity, and thus share limited solute transport (Mendoza-Lera et al., 2017). Nevertheless, we observed stronger light-induced changes in the biomass proportion of leaf C in sandy than in sand-gravel habitats. Compared to findings by Mendoza-Lera et al. (2017), our VWE data indicated an increase in hydraulic conductivity in sandy sediments when sand and gravel were arranged in blocks (sorted streambed). Furthermore, in a related study, we demonstrate that the sorted arrangement of sand and gravel sediments provides a deeper vertical and lateral supply of benthic solutes from highly conductive gravel to low conductive sandy sediments (data unpublished). Accordingly, our findings provide evidence that a heterogeneous arrangement of high and low hydraulic sediments in the streambed enhances the effect of light on leaf degradation in low hydraulic sediments such as sand. In turn, our data underlines that recognized effects of light availability on leaf C turnover in microbial streambed biofilms are modified by the hyporheic flow in the streambed.

## Environmental Effects on Bacterial Community Composition

In addition to the spatial availability of algal metabolites, habitat conditions also influence the species composition of the microbial community (Zeglin, 2015). Indeed, T-RFLP profiling of bacterial DNA implies different species compositions among

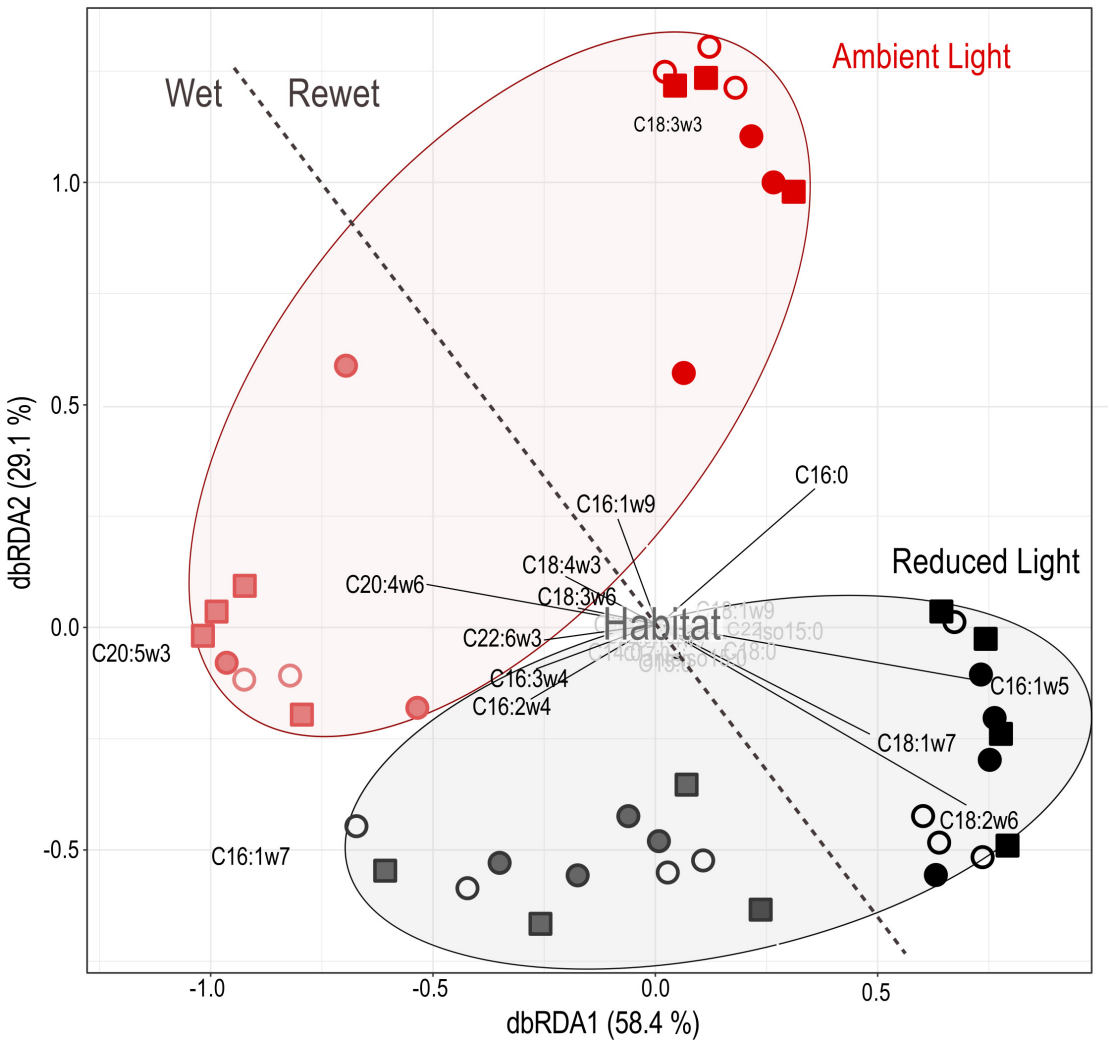


**FIGURE 6 |** Distance-based redundancy analysis of PLFA profiles of sand (open circles), gravel (closed circles) and sand-gravel (squares) sediments and environmental data to assess compositional differences. Samples taken before (Wet = end of the colonization period) and after (Rewet = end of the rewet period) the drought period are separated by the dashed line. Environmental factors and PLFA types with marginal influence on underlying differences between the samples are highlighted in gray.

sand, gravel and sand gravel habitats (**Figure 5B**). Previous research emphasizes that bacterial species largely differ in their metabolic capability to degrade leaf C (Hutalle-Schmelzer et al., 2010; Carlson and Hansell, 2015). The differing leaf C utilization among the bacterial communities on sand, gravel, and sand-gravel sediments could therefore be due to a different species composition and hence metabolic capability. In addition, interactions between bacteria and algae are species-specific (Sarmento and Gasol, 2012; Horňák et al., 2017), which implies that only certain bacterial strains are affected in their functional role in leaf degradation by algal OM. Observed differences in $F_{Leaf}$ among bacterial PLFAs support this notion (**Figures 4**, **5A**). Bacterial taxa produce the measured fatty acids in different proportions (Haack et al., 1994; Boschker et al., 1998). For example, *iso14:0* almost exclusively comprises in the membrane of the taxa *Actinobacteria* and *Firmicutes*, whereas *iso15:0* dominates in the membrane of *Bacterioidetes* species. Consequently, the observed interrelating effects of streambed structure and light availability on leaf C turnover could be linked to a different species occurrence within the communities in sand, gravel and sand-gravel habitats, which interact differently with algal OM. However, previous research also hints that induced changes in the function of a microbial community in carbon turnover underlie a change in the composition of the microbial community (Jones et al., 2009; Attermeyer et al., 2014; Blanchet et al., 2017). Accordingly, the light-induced changes in bacterial utilization of leaf C should have been accompanied by a change in community composition. This, however, cannot be fully proven by our measurements, as light had only an indirect influence on the composition of the T-RFLP profiles. Only for gravel sediments did we observe a significant shift in the composition of the community with varying light availability,

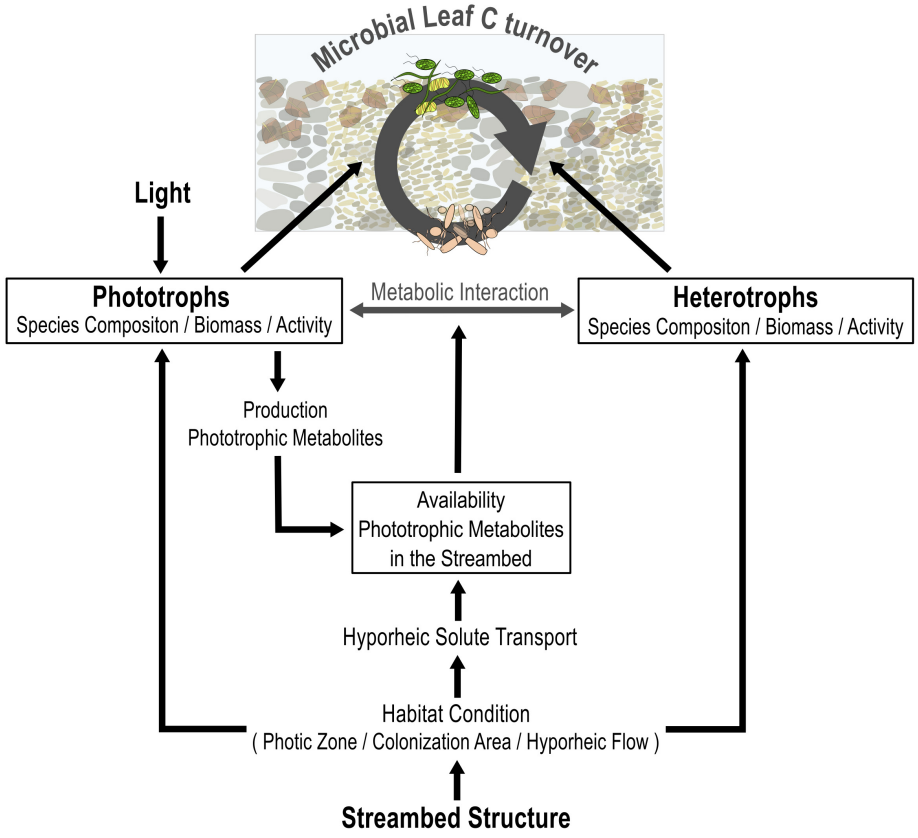


**FIGURE 7 |** Conceptual overview of how light availability and streambed structure interactively modify the contribution of microbial phototrophs and heterotrophs to leaf C turnover, including their interaction. The availability of light controls the contribution of biofilm phototrophs to C turnover and, in turn, the production of algal metabolites in the streambed. Streambed structure defines the contribution of both, the phototrophic and the heterotrophic community, to leaf C turnover by defining habitat conditions in terms of hyporheic flow, light penetration depth, and available colonization area. The hyporheic flow, in turn, defines the spatial availability of algal metabolites in the streambed.

which correlated well with the light-induced shift in leaf C utilization.

Consequently, PLFA and T-RFLP data suggested a species-related shift in bacterial function via photosynthetically produced OM and underline that communities are likely differentially stimulated by algal OM, depending on their species composition and/or additional habitat-related factors. Although environmental conditions may determine the composition of bacterial biofilm communities, light-induced shifts in microbial function did not clearly relate to shifts in community composition.

## Fundamental Importance of Light and Streambed Structure for the Functioning of Intermittent Streams

In view of the increasing proportion of intermittent stream ecosystems, it is important to understand how periodic drought alters environmental impacts on leaf decay. Recent findings emphasize that alternating wet–dry states disturb microbial activities with profound consequences for microbial function in the ecosystem C cycle (Febria et al., 2015). However, periodic desiccation of sediments has also been described to increase the bioavailability of leaf litter, thereby stimulating its microbial turnover (Abril et al., 2016; Weise et al., 2016). Indeed, drought disturbed microbial activity as discussed in detail by Zlatanović et al. (2017), accompanied by a lower respiration rate of leaf C (**Figure 2**). The decline in leaf C respiration accords well with previously reported adverse effects of drought on ecosystem function (Datry et al., 2014) associated with the death of the microbial cells from osmotic stress and consequently a reduction in microbial biomass (Birch, 1960; Rees et al., 2006). Hence, the observed reduction in biofilm activity from pre- to post-drought conditions likely resulted from an accompanying decrease in biofilm biomass. However, drought events have also been shown to affect microbial function through modification of community composition (Pohlon et al., 2013), which is in accordance with the differences in PLFA profiles between pre- and post-drought conditions observed in our study (**Figure 6**). Microbial species differ in their activity and thus recycle OM at different rates (Edwards and Meyer, 1986; Steward et al., 1996). Therefore, the combined effects of drought on biofilm biomass and community composition likely lead to a general reduction in biofilm activity and consequently, leaf C respiration. However, the effect of light

and streamed structure on the proportion of leaf C in the DOC and the respired C pools (**Figure 3**) remained similar before and after the drought event. Hence, in contrast to previous results (Abril et al., 2016; Weise et al., 2016), the dryness of the streambed did not seem to further stimulate the decomposition of leaf C in our approach. Thus, despite the negative impact of the drought period on the magnitude of leaf C respiration, no interference of flow intermittency with the effect of our treatments on microbial utilization of leaf C was observed. In view of increasing drought periods in future, not only in streams but also in lakes and ponds, our results indicate that flow intermittency may not fully diminish the effects of environmental drivers of terrestrial-aquatic C cycling that were reported for perennial aquatic systems. It is necessary, however, to keep in mind that flow intermittency was mimicked by only one full dry–wet cycle in this study. Sediment drying reportedly most significantly affects phototrophic biofilm communities, depending on the intensity and frequency of drought events (Ledger et al., 2008; Timoner et al., 2012). It is therefore likely that in intermittent streams frequently exposed to drought, photo-heterotrophic interactions become less important for leaf C turnover with increasing intensity of drought stress.

## CONCLUSION

In summary, the results from this study highlight that the availability of light and the structure of the streambed interactively modulate the cycling of leaf C within the microbial food web (**Figure 7**). Light availability determined the occurrence and thus functional importance of algae to leaf C cycling, including the stimulatory effect of algal metabolites on the activity of heterotrophic bacteria. Our results further suggest that the streambed structure determines the spatial availability of algal exudates within the streambed, as well as the species composition of bacterial communities. That in turn, defines the stimulatory function of phototrophic activities for the use of leaf C by heterotrophic bacteria. Accordingly, our results emphasize that environmental factors interactively influence the relevance of photo-heterotrophic interactions for leaf degradation.

In addition, our results highlight a prominent influence of light and streambed structure on microbial leaf C turnover, even under dynamic hydrological conditions. This, in turn, underlines the fundamental role of both environmental factors in the functioning of stream ecosystems.

Collectively, the results from this study provide valuable insights for future studies on the mechanisms by which environmental factors control terrestrial C turnover in stream ecosystems, particularly regarding the ecological functions of photo- and heterotrophic biofilm communities. Given the multitude of environmental factors potentially affecting biofilm C metabolism, in addition to invertebrates and protist grazers, we emphasize the need for additional *in situ* surveys to better estimate the relevance of photo-heterotrophic coupling for terrestrial C turnover in natural ecosystems.

## AUTHOR CONTRIBUTIONS

The study was designed and conducted by SZ, KP, MM, and JF. Stable isotope analysis of PLFA, respired C, DOC, and particulate OM, including data interpretation, was performed by RvG, GG, and JF. T-RFLP of bacterial DNA, including data interpretation, was performed by AU and JF. The combined data set was interpreted and discussed by KP, H-PG, and JF. The manuscript was written by JF. KP, MM, H-PG, SZ, AU, and RvG contributed to the writing and correction of the manuscript. All authors read and approved the final manuscript.

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