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Organic matter quality structures benthic fatty acid patterns and the abundance of fungi and bacteria in temperate lakes

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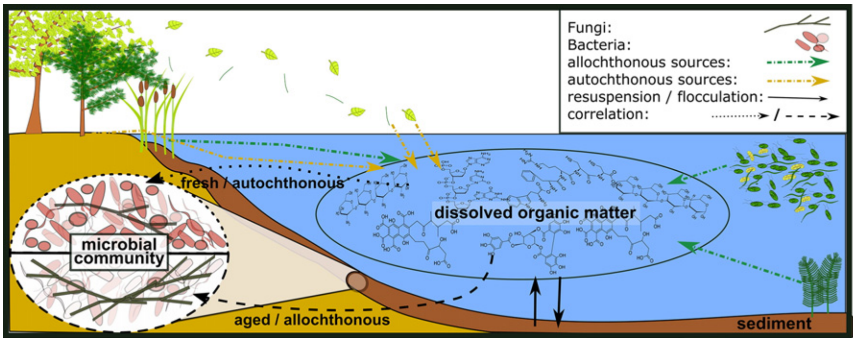
HIGHLIGHTS

- Correlation between dissolved organic matter quality and benthic microbial community is shown.
- Relative quantities of fungi and bacteria were calculated from PLFA with a Bayesian mixed model.
- Fungi are more abundant in sediments of lakes with aged and allochthonous DOM.
- Bacterial dominance increases with organic matter freshness and allochthony.

GRAPHICAL ABSTRACT



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ABSTRACT

Benthic microbial communities (BMCs) play important roles in the carbon cycle of lakes, and benthic littoral zones in particular have been previously highlighted as biogeochemical hotspots. Dissolved organic matter (DOM) presents the major carbon pool in lakes, and although the effect of DOM composition on the pelagic microbial community composition is widely accepted, little is known about its effect on BMCs, particularly aquatic fungi. Therefore, we investigated the composition of benthic littoral microbial communities in twenty highly diverse lakes in northeast Germany. DOM quality was analyzed via size exclusion chromatography (SEC), fluorescence parallel factor analyses (PRAFACs) and UV–Vis spectroscopy. We determined the BMC composition and biomass using phospholipid-derived fatty acids (PLFA) and extended the interpretation to the analysis of fungi by applying a Bayesian mixed model. We present evidence that the quality of DOM structures the BMCs, which are dominated by heterotrophic bacteria and show low fungal biomass. The fungal biomass increases when the DOM pool is processed by microorganisms of allochthonous origin, whereas the opposite is true for bacteria.

1. Introduction

Several studies have recently examined the relationship between the quality and quantity of organic matter (OM) and the composition, activity and physiological state of aquatic heterotrophic microbial communities (Lehman et al., 2010; Strickland and Rousk, 2010; Lange et al.,

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Abbreviations: BMC, benthic microbial community; SEC, size exclusion chromatography; PARAFAC, parallel factor analysis; HMWS, high molecular weight substances; HS, humic substances; SUVA, specific ultraviolet absorption.

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2015; Fabian et al., 2016). In freshwater ecosystems, both the quality and quantity of OM, which is predominantly composed of dissolved organic matter (DOM), have been shown to control the metabolic activity and composition of microbial organisms (Likens et al., 2009; Attermeyer et al., 2014; Kuehn et al., 2014). Quality is further defined as chemical composition relating to molecular size and molecular complexity. This relationship was confirmed by Findlay et al. (2003), who observed increased metabolic activities accompanied by changes in the benthic microbial community (BMC) when highly bioavailable OM was added. Microbial processing and photodegradation of DOM can cause DOM to flocculate, which may represent an important source of OM in sediments together with sinking particulate organic matter (POM) (Meyers and Ishiwatari, 1993; von Wachenfeldt et al., 2008). DOM can originate from internal primary production within an ecosystem, i.e., autochthonous OM, or from terrestrial inflow and precipitation, i.e., allochthonous OM. In most lentic ecosystems, allochthonous DOM predominates over autochthonous DOM (Wilkinson et al., 2013). An increased abundance of allochthonous DOM causes increases in dissolved organic carbon (DOC) concentrations, decreases in pH values (Roth et al., 2013, 2014; Larson et al., 2014), and alterations in microbial OM processing (Gudasz et al., 2012) and the structure of bacterial communities (Kritzberg et al., 2006; Ruiz-Gonzalez et al., 2015).

To date, heterotrophic bacteria have been considered the main components of planktonic and benthic carbon cycling in freshwater ecosystems; thus, they have been investigated intensively (Docherty et al., 2006; Judd et al., 2006; Amaral et al., 2016). However, the important role of aquatic fungi was revealed in studies on the degradation of leaf and plant litter, particularly in streams (Gessner et al., 2010; Fabian et al., 2016). In the case of leaf degradation, antagonistic effects of both fungi and bacteria have been shown (Mille-Lindblom and Tranvik, 2003); at the same time, however, bacterial activity and growth are promoted by fungal extracellular enzymatic activities that provide intermediate decomposition products (Romaní et al., 2006).

The role of fungi in lentic ecosystems is manifold and includes the degradation of leaves, macrophyte litter and pollen, as well as parasitism on algae (Jobard et al., 2010; Wurzbacher et al., 2010, 2014; Monchy et al., 2011; Taib et al., 2013), but these roles are often overlooked (Grossart and Rojas-Jimenez, 2016). However, fungi are present in all lake habitats, particularly in littoral sediments that represent biogeochemical hotspots of carbon cycling (Wurzbacher et al., 2016). Therefore, fungi and bacteria contribute significantly to microbial biomass, productivity and carbon flow (Buesing and Gessner, 2006).

Despite their primarily saprophytic lifestyle, fungi and bacteria are morphologically, physiologically and phylogenetically distinct, which explains their divergent preferences for environmental conditions and dominance in different niches (Grossart and Rojas-Jimenez, 2016). In this context, decreasing bioavailability and nutrient content, decreases in pH have been shown to increase the fungi:bacteria ratio (F:B) in soils and streams (Findlay et al., 2002; Bååth and Anderson, 2003; Rousk et al., 2009), suggesting that pH adaptation is most likely an important trait. Different F:B ratios have severe ecological consequences and may result in different carbon usage efficiencies (CUEs), i.e., the ratio of assimilated biomass carbon to the total carbon consumed, between both microbial groups (del Giorgio and Cole, 1998). Whether the ecological coherence between F:B ratios and OM characteristics and pH are also important in lake ecosystems remains largely unknown. Evidence of a similar relationship was obtained in a study of 49 Baltic rivers (Jørgensen and Stepanauskas, 2009) and an experiment on lake pollen degradation (Wurzbacher et al., 2014). Both suggest that fungal biomass is positively correlated with OM content.

The lack of simultaneous evaluations of aquatic fungal and bacterial biomass and analyses of their ecological role in aquatic ecosystems can be explained by methodological limitations. Ergosterol is a biomarker for fungi that does not occur in basal fungi such as Chytridiomycota, and it is still present after fungal cell death, which increases the difficulty of determining the relationships between aquatic fungi and environmental features (Mille-Lindblom et al., 2004). Although the analysis of phospholipid-derived fatty acids (PLFAs) allows for a reliable chemotaxonomic differentiation between fungi and bacteria and concurrent analyses of differential carbon sources via stable carbon isotopes in soil samples, aquatic samples are not easily analyzed. Although markers from soil bacteria can be assigned to aquatic bacteria, the soil fungal marker fatty acid C18:2n6,9 also appears in planktonic algae, which makes it unsuitable for reliable fungal biomass determinations in aquatic ecosystems per se. However, a Bayesian mixed model has been successfully applied to complex algal communities to overcome limitations posed by the single marker approach (Willers et al., 2015). For this approach, whole PLFA patterns of several cultured species of the same taxonomic groups need to be summarized. The Bayesian mixed model can then be used to calculate the relative contribution of the different groups in the environmental sample. Instead of focusing on one fatty acid, the model takes ratios between various fatty acids into account and allows for the analysis of taxa that do not possess a specific marker (De Carvalho and Caramujo, 2014; Strandberg et al., 2015). Therefore, extending this method to microbial communities in lake sediments holds the potential to quantitatively and simultaneously differentiate between the biomass of fungi, bacteria and phototrophic organisms.

Over the past several decades, carbon flow in freshwater food webs has been studied using stable isotope analyses (Jones et al., 1998; Grey et al., 2004; Premke et al., 2010). Stable carbon isotopes show minor trophic fractionation and can therefore be used to assess the carbon sources supporting heterotrophic organisms, provided that the potential food sources have distinct isotopic signatures (Fry and Sherr, 1989). Most studies have examined the stable isotope ratios in animals and, therefore, the carbon source used by these organisms, but only few studies have focused on the $^{13}C/^{12}C$-ratios in microorganisms in the form of PLFA (Boschker et al., 1999, Steger et al., 2011, Fabian et al., 2016).

Research on the interplay between fungi and bacteria and their different ecological roles within aquatic ecosystems, however, is still in a nascent stage. To identify factors that influence bacterial and fungal biomasses in littoral sediments as well as system-related differences, we investigated 20 lentic freshwater ecosystems along a DOC concentration gradient in the glacial landscape of northeast Germany. We hypothesized that DOM quantity and quality significantly affect BMC composition and activities and that benthic aquatic fungi represent an important microbial component in lake ecosystems. Therefore, the F:B ratio was assumed to increase in relation to DOC concentrations and sediment carbon content in lake ecosystems.

2. Materials and methods

2.1. Field sampling

Twenty lentic inland waters (lakes and kettle holes) in the glacial landscape of northeast Germany (Fig. 1, Table 1) were sampled between 6 October 2014 and 4 November 2014 along a DOC concentration gradient ranging between 5 and 42 mg C L^{-1}.

Temperature, pH and conductivity were measured in surface waters using a Multi 3430 multiprobe (WTW GmbH, Weilheim, Germany). In all lakes, water and sediment samples were collected in the littoral zone approximately 1 to 2 m in front of the reed belt or approximately 1.5 to 3 m from the lake shore if reed was absent. The effect of drought on the sample sites cannot be excluded for certain lakes but likely did not occur for at least the past two years. The sampling points in the kettle holes were distributed over the entire waterbody to account for their high internal spatial variability. Samples along the shoreline were collected at five different locations per lake, and three samples were collected for each sample location. Ultimately, three pools were formed containing one sample per location.

Surface water samples were collected with a 1 L plastic flask, and sediment cores were taken at a water depth of 0.5 to 2.5 m using a