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Effects of zooplankton carcasses degradation on freshwater bacterial community composition and implications for carbon cycling

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\textbf{Running title:} Effect of dead zooplankton on bacteria and C-cycle
In a recent comprehensive review on the role of zooplankton in the aquatic carbon cycle, Steinberg and Landry (2017) state that the carbon input via carcasses, in spite of their abundance, remains largely unknown. Our study is the first to cast light upon this important yet overlooked organic matter source for microorganisms in aquatic systems. Our study reveals that decomposing zooplankton carcasses could be a major driver of bacterial community composition in many aquatic ecosystems, e.g. in lakes with an inverted biomass pyramid and in common events of mass zooplankton mortality. Using stable isotope labeling we show that zooplankton carcasses are well degraded by heterotrophic prokaryotes indicating their labile nature, but do not significantly stimulate the degradation of more refractory organic matter such as humic matter. Thus, carcasses are important hotspots of microbial activity influencing the organic matter sinking flux and overall microbial diversity in aquatic ecosystems.
Summary

Non-predatory mortality of zooplankton provides an abundant, yet, little studied source of high quality labile organic matter (LOM) in aquatic ecosystems. Using laboratory microcosms, we followed the decomposition of organic carbon of fresh $^{13}$C-labelled *Daphnia* carcasses by natural bacterioplankton. The experimental setup comprised blank microcosms i.e. artificial lake water without any organic matter additions (B), and microcosms either amended with natural humic matter (H), fresh *Daphnia* carcasses (D) or both, i.e. humic matter and *Daphnia* carcasses (HD). Most of the carcass carbon was consumed and respired by the bacterial community within 15 days of incubation. A shift in the bacterial community composition shaped by labile carcass carbon and by humic matter was observed. Nevertheless, we did not observe a quantitative change in humic matter degradation by heterotrophic bacteria in the presence of LOM derived from carcasses. However, carcasses were the main factor driving the bacterial community composition suggesting that the presence of large quantities of dead zooplankton might affect the carbon cycling in aquatic ecosystems. Our results imply that organic matter derived from zooplankton carcasses is efficiently remineralized by a highly specific bacterial community, but doesn’t interfere with the bacterial turnover of more refractory humic matter.
Introduction

The global carbon cycle is one of the most important biogeochemical processes regulating the climate on our planet (Ward et al., 2013). In particular, carbon fluxes between aquatic and terrestrial ecosystems constitute a key component of global biogeochemical cycles (Pace et al., 2004; Battin et al., 2009; Ward et al., 2013). Nowadays, it is well known that a significant part of terrigenous organic matter drains from soils into aquatic ecosystems, especially in the boreal zone (Vachon et al., 2017). Freshwaters are considered hotspots of organic matter degradation, sustaining a shorter half-life of organic carbon compared to terrestrial and marine ecosystems (Catalán et al., 2015). In freshwaters, organic matter comprises a heterogeneous mixture of different carbon sources with varying degradability. Depending on their degradability by aquatic microbes, the drained terrigenous organic matter is buried to a variable extent in sediments of aquatic ecosystems (Tranvik et al., 2009). However, most of the terrigenous (allochthonous) organic carbon is transported into aquatic ecosystems in the form of refractory organic matter (ROM) resulting in a generally higher retention time due to its slow decomposition by aquatic microorganisms (Bianchi, 2011). A major part of this ROM in freshwater ecosystems is represented by humic matter (Rocker et al., 2012a).

Bacterial species differ in their response to various sources of carbon resulting in profound implications for aquatic carbon cycling. It has been demonstrated that the availability of organic matter promotes growth of both generalist species, which are able to degrade a wide range of substrates, as well as highly specialized populations degrading specific substrate fractions (Hutalle-Schmelzer et al., 2010). Dead zooplankton, which used to be generally neglected in aquatic ecology due to methodological limitations (Tang et al., 2009, 2014), is an overlooked and highly abundant source of labile carbon in most freshwater ecosystems. Zooplankton carcasses represent a high quality organic substrate for
heterotrophic bacteria due to their relatively low C:N:P ratio as compared to phytoplankton and detritus (Tang et al., 2014). Consequently, zooplankton carcasses are “hot spots” of activity of pelagic microorganisms consuming labile organic matter (LOM) as well as ROM (Tang et al., 2006; Grossart et al., 2007; Elliott et al., 2010; Kirillin et al., 2012). However, zooplankton carcasses provide not only a carbon source for microorganisms, but also surfaces for attachment. Microorganisms attached to particles are situated in close spatial proximity and can benefit from extracellular degradation enzymes released in the environment (Catalán et al., 2015). Thus, attached microorganisms have a higher capacity to degrade polymeric organic matter than their free living counterparts (Grossart, 2010). Consequently, zooplankton carcasses are selecting for specific, but yet uncharacterized microbial communities (Tang et al., 2010). The complex LOM of zooplankton carcasses constitutes a valuable source of nutrients and energy for microorganisms, thus implying effects on aquatic carbon cycling, in particular of the more refractory carbon pools. For instance, carcass LOM may induce a “priming effect” and facilitate the degradation of ROM (Bianchi, 2011).

Thus, our primary objective was to investigate consequences on bacterial community composition and carbon cycling in aquatic ecosystems after input of zooplankton carcasses. Since the quality of available organic matter can be a selective force for bacterioplankton community composition (Gómez-Consarnau et al., 2012), we tested the hypothesis that nutrient-rich LOM provided by Daphnia carcasses selects for generalist bacteria in contrast to C-rich ROM selecting to a larger extent for specialists. In a microcosms experiment, we observed the degradation of $^{13}$C-labeled carcasses by heterotrophic bacteria from a dystrophic humic bog lake in the presence of indigenous humic matter (treatment HD) to track the fate of carcass carbon (Fig. S1). In parallel, we followed three control treatments with either Daphnia carcasses (D) or humic matter (H) as a sole carbon source, and a blank treatment (B) containing solely a natural bacterial community. We used optical properties (specific UV absorbance at 254 nm – SUVA$_{254}$, humification index, etc.) and size exclusion
chromatography to analyze the influence of carcasses on the dissolved organic matter (DOM) pool and combined it with 16S rRNA gene Illumina amplicon sequencing to characterize the bacterial community composition in detail.

Results

Microbial dynamics and community composition

*Daphnia* carcasses showed a rapid decomposition during the first week of incubation, with visible changes in the state of carcasses over time (Fig. S2a-d). At the end of the experiment, the carcasses were still visible as disintegrated parts of the carapace. Dense bacterial colonization of the carcasses was observed (Fig. S2e), while protist grazers or autotrophic organisms were not detected indicating that protozoan grazers and large phytoplankton have been successfully removed by the pre-filtration step. No differences in bacterial counts were observed in the B microcosms between the start and the end of the experiment (Table 1). However, a clear increase in bacterial cells counts was observed in H, D and HD microcosms (43%, 654% and 617%, respectively; Table 1). This indicated a higher bacterial growth in the presence of *Daphnia* carcasses and a slower growth on humic matter alone. At the same time, bacterial cell counts did not significantly differ between HD and D microcosms (paired t-test, p > 0.05, Table 1).

After sequencing and performing a quality check for all samples, 595036 reads of 16S rRNA gene fragments were obtained that clustered into 1161 bacterial operational taxonomic units (OTUs). The identified OTUs belonged to 26 known phyla (Fig. 1). In the initial bacterial inoculum from Lake Grosse Fuchskuhle, *Proteobacteria* was the dominant phylum (48% of all sequences), with a high proportion of the class *Betaproteobacteria* (30% of all sequences). By the end of the experiment, the relative abundance of *Proteobacteria* increased...
in all treatments, especially in carcasses amended microcosms (HD and D; Fig. 1), with a dominance of the class *Gammaproteobacteria* (62 % and 50 % in HD and D respectively).

At the end of the experiment, the microcosms HD and D had a lower OTU richness and evenness compared to microcosms without added carcasses (H and B; Table 2). Microcosms H had lower species richness than B microcosms, but showed a higher evenness (Table 2).

In an unconstrained ordination (Fig. 2), all treatments were distinguishable from one another and from the initial inoculum (ANOSIM, $R = 0.835$, $p = 0.001$). The OTUs accounting for most of the difference between the treatments were identified using a SIMPER test (Table 3). The relative abundance of the most influential OTU (OTU1, *Pseudomonas* sp.) was significantly different between the start and the end of the incubation for all treatments (ANOVA $F_{4,15} = 102.89$, $p < 0.001$). The distribution of different OTUs in all treatments is discussed in more details in the Supplementary Information.

**DOM composition and fate of carcass carbon**

We aimed to test for the “priming effect” by comparing the predicted total organic carbon degradation rate ($\Delta$TOC) in HD microcosms, calculated from $\Delta$TOC in the control microcosms D, H, and B, to the measured $\Delta$TOC in HD microcosms (see Supplementary Methods for more details). The predicted $\Delta$TOC was $-1.015 \pm 0.115 \text{ mg L}^{-1}$ and did not differ significantly (paired t-test, $p > 0.05$) from the $\Delta$TOC measured in HD microcosms ($-1.094 \pm 0.057 \text{ mg L}^{-1}$).

During carcass decomposition (in HD and D microcosms only), the particulate organic carbon (POC) decreased approximately four-fold compared to the initial values (Table 1). Nevertheless, no significant differences in concentrations of dissolved organic carbon (DOC), high- and low-molecular weight substances (HMWS and LMWS, respectively) or humic substances were found throughout the experiment in any treatment (paired t-tests, $p > 0.05$ in
all tests, Table 1). However, in ROM supplemented microcosms (HD and H) we observed
trends of decreasing concentrations in polysaccharides, amphiphilic molecules, and building
blocks of humic substances (Table 1).

The SUVA\textsubscript{254}, spectral slopes and optical indices values were not different between
the humic-amended microcosms H and HD (Table 1), and they did not differ between D and
B microcosms (Table 1). An exception was the freshness index, being an indicator for
recently produced DOM (Hansen et al., 2016), which was lower in D compared to B
microcosms. Expectedly, the ratio of peakA to peakT, which is known as an indicator of the
ratio of humic-like (recalcitrant) to freshly produced (labile) organic matter (Hansen et al.,
2016), was higher in H microcosms compared to HD (Wilcoxon test, p-value = 0.03).

The carcasses had an average $^{13}\text{C}/^{12}\text{C}$ ratio of 0.168 ± 0.004 ($\delta^{13}\text{C} = 13945.3 ±$
303.9‰). According to this specific signature, the amount of processed carbon originating
from the carcasses was computed in all carcass-containing microcosms (i.e. HD and D). In
HD microcosms, 72.8% of POC and 2.2% of DOC originated from Daphnia carcasses,
against 82.1% of POC and 21.1% of DOC for the D microcosms (Fig. 3).

The bacterial respiration, measured as the increase in CO\textsubscript{2} and normalized to the
background respiration (i.e. respiration from the blank microcosms B) was higher in D
microcosms compared to H but lower compared to HD microcosms (Fig. S3). The respiration
per amount of initially added carbon and normalized to the background respiration was higher
in HD microcosms compared to H but lower compared to D microcosms, and was used to
confirm the labile character of the organic matter originating from the zooplankton carcasses.
All differences in CO\textsubscript{2} concentrations between the microcosms were significant (1-way
ANOVA $F = 241.6$, p < 0.001; Tukey post hoc test p < 0.01 for all pairs).

To test for the priming effect, the predicted $^{13}\text{C}/^{12}\text{C}$ ratio in the respired CO\textsubscript{2} of HD
microcosms was calculated from the values in the control microcosms D, H, and B, and
compared to the measured $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio in HD microcosms (see Supplementary Methods
for more details). The predicted $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio for HD (0.078 ± 0.003) did not differ significantly (paired t-test, p > 0.05) from the measured value (0.073 ± 0.003). In HD microcosms, 87.8 ± 6.3 % of respired CO$_2$ originated from zooplankton carcasses when normalized to the background respiration of microcosms B.

**Interactions between microbial community and DOC quality**

The interactions between bacterial community composition and DOC quality in microcosms HD, H, and D revealed specific patterns in bacterial substrate preferences (Fig. 4). The connections between bacterial genera and DOC qualities significantly differed between microcosms H and D (Fig. 4a). Thus, bacteria positively interacting with DOC concentration as well as humification and fluorescence indices are the ones thriving in the presence of humic matter (Fig. 4a). On the contrary, bacteria negatively associated with these parameters are favored by carcasses (Fig. 4a). Similarly, genera positively correlated with DOC concentration, fluorescence index and A/T peak ratio (Fig. 4b, comparing microcosms HD and D) seem to be favored by humic matter when *Daphnia* carcasses are available. However, those genera negatively correlated with these parameters are suppressed by humic matter in the presence of carcasses.

**Discussion**

The main objective of this study was to track the degradation of zooplankton carcasses, as a so far largely neglected but common and labile carbon source (Tang *et al.*, 2014). Our study reveals selection of defined bacterial communities in the presence of carcasses strongly related to the specific DOM quality released from carcasses. However, carcass-induced availability of LOM and related shifts in bacterial community composition did not result in significant changes in the turnover of the added ROM pool. Consequently, our data do not support a “priming effect” (Bianchi *et al.*, 2011) of refractory humic matter
removal in the presence of relatively labile carbon from *Daphnia* carcasses. Nevertheless, the bacterial community composition was greatly affected by the presence of carcass carbon. Thus, our study adds new quantitative and qualitative data on bacterial carbon utilization related to changes in the community composition induced by changes in substrate quality, i.e. addition of zooplankton carcasses, and adds new insights in microbial-organic matter interactions.

**Bacterial community composition depending on carbon source**

As outlined above, we did not measure any quantitative changes in organic matter degradation between HD microcosms and the predicted values calculated based on the parameters of single-carbon source microcosms H and D. Consequently, the presence of LOM from *Daphnia* carcasses did not change the degradation of humic matter but rather influenced the bacterial community.

According to our data on beta-diversity of bacterial assemblages (Fig. 2), the type of treatment strongly affected the bacterial community composition in each microcosm. In microcosms with no extra organic matter addition (B), the bacterial community remained similar to the initial inoculum indicating that experimental changes in environmental conditions did not modify the bacterial community composition drastically (Fig. 2). Overall, the addition of carcass LOM was the main driver of the bacterial community composition in the D and HD microcosms (Fig. 2). Bacterial taxa introduced into the microcosms with the carcasses may also have an effect on bacterial community composition and richness. However, according to literature data, zooplankton carcasses are not primarily decomposed by their native-associated bacterial communities, but rather by ambient bacteria (Bickel and Tang, 2010). This notion is also reflected by the fact that bacteria richness is the lowest in the HD and D treatments. Thus, in natural waters with a high amount of dead zooplankton, carcasses can be a primary factor driving bacterioplankton community composition with
potential effects for carbon cycling. In many lakes with an “inverted” biomass pyramid, zooplankton biomass is higher than phytoplankton biomass (Heathcote et al., 2016). We suppose that the same pattern for dead biomass would indicate that zooplankton and not algal LOM, which is usually in the researcher’s focus (Hoikkala et al., 2016; Landa et al., 2016), could be the major driver for bacterioplankton community composition in such ecosystems. In lakes with a “normal” biomass pyramid, zooplankton may still play an important role for determining bacterial community composition in the occasional events of mass zooplankton mortality (Tang et al., 2014). It would be interesting to test this presupposition in further studies. The co-presence of ROM also contributed to specific bacterial communities by selecting for a number of specific OTUs. The observed, significant difference in bacterial community composition between all treatments points to a pronounced effect of substrate quality on bacterial community composition and might result in functional differences.

The prevalence of Betaproteobacteria in the initial inoculum and in microcosms B and H (Fig. 1) was in accordance with previous studies on Lake Grosse Fuchskuhle (Grossart et al., 2008; Hutalle-Schmelzer et al., 2010). Indeed, Betaproteobacteria are among the most numerous bacteria in the upper layers of freshwater lakes, in particular of peat bog lakes (Newton et al., 2011). Moreover, the dominance of Gammaproteobacteria in carcass-amended microcosms (Fig. 1) was also expected according to previous studies (Tang et al., 2009; Shoemaker and Moisander, 2015). Gammaproteobacteria include many species with a copiotrophic lifestyle that can grow faster than the average lake bacterioplankton, especially under nutrient-rich conditions as can be found on carcasses (Newton et al., 2011). Interestingly, a more distinct community pattern emerged in the different microcosms when taking the level of individual OTUs into account. This indicates a close relationship between organic carbon quality and bacterial community structure (Attermeyer et al., 2014, 2015).
Links between organic matter quality and microbial community composition

A number of uncultivated bacterial taxa belonging to the order *Sphingobacteriales* were positively selected solely in the presence of carcasses only (Fig. 4a). Many members of *Sphingobacteriales* express chitinolytic activity (Kämpfer, 2015), but no information is available in particular about the ecological role of the uncultivated representatives found in the present study. High abundances of the NS11-12 marine group were previously associated with an increase in chlorophyll *a* concentration (Meziti *et al.*, 2015) and number of particles, while it is negatively correlated with nitrate concentration (Henson *et al.*, 2016). Moreover, the uncultured bacterial group OPS 17 was previously found not to respond to terrestrial DOM additions (Lindh *et al.*, 2015).

Another group of bacteria favored by zooplankton carcass LOM were ubiquitous chemoorganotrophs belonging to genera *Brevundimonas* and *Aeromonas* (Segers *et al.*, 1994).

Among the bacteria favored by humic matter (Fig. 4b), genera involved in nitrogen fixation (*Bradyrhizobium*, *Rhizobacter*, unclassified *Rhizobiales*) (Kuykendall, 2005; Goto, 2015), organic pollutants degraders *Rhodococcus* (Bell *et al.*, 1998), unclassified *Sphingomonadales* and methylotrophs (uncultured strain PRD01a011B from *Methylophilaceae* (Doronina *et al.*, 2014)) were detected. To a large extent the same bacterial genera were favored by humic matter regardless whether zooplankton carcasses were present. In contrast, bacteria suppressed by humic matter in the presence of carcasses were almost exclusively chemoorganotrophic generalists (Johansen *et al.*, 2005; O'Sullivan *et al.*, 2005; Song *et al.*, 2008; McBride, 2014; Evtushenko, 2015).

Besides selecting for certain bacterial populations, zooplankton carcasses strongly decreased the species richness and evenness of the bacterial community (Table 2). Therefore it appears that the availability of a high quality and abundant LOM source can reduce the biodiversity by favoring a small number of copiotrophs dominating the community: in the
present study about half of all sequences in the HD and D microcosms belonged to a single OTU (OTU 1, *Pseudomonas sp.*). In a similar study of Blanchet et al. (2017), the bacterial diversity was not affected by amino acid additions, possibly because free amino acids are simple compounds which can be consumed simultaneously by many members of the community (Trusova et al., 2012). Thus, this pattern can be best explained by the fact that zooplankton carcasses provide microbial habitats and complex, yet labile carbon sources shifting the overall bacterial community towards a less diverse, more uneven, and more copiotrophic community.

**Microbial carcass decomposition in relation to carbon quality**

An abrupt increase of LOM availability following mass zooplankton mortality commonly observed in natural waters (reviewed by Tang et al. 2014), leads to a substantial input of both DOC and POC. In agreement with a previous study (Tang *et al.*, 2006) *Daphnia* carcasses lost their mostly labile internal tissues rapidly, whereas the chitin-based carapace was more resistant to dissolution and microbial decomposition (Fig S2).

However, in our study the leached fraction was rapidly consumed by numerous ambient bacteria (Table 1), and did not increase the DOC concentration significantly in the microcosms with zooplankton carcasses (D and HD). This statement is supported by our observation that adding $1.334 \pm 0.038 \text{ mg C L}^{-1}$ with *Daphnia* carcasses resulted in only $0.055 \pm 0.011$ and $0.065 \pm 0.010 \text{ mg C L}^{-1}$ of carcasses-derived DOC in D and HD microcosms, respectively (Fig. 3).

On the other hand, the chitin-based structure of the carapace was only partially degraded and also used a surface for attachment. At the end of the experiment, $0.270 \pm 0.014$ and $0.258 \pm 0.007 \text{ mg C L}^{-1}$ originating from *Daphnia* carcasses (19-20% of the initial quantity) remained in the POC fraction of the D and HD microcosms, respectively (Fig. 3), mainly represented by the remaining carapace and the bacterial biomass (Fig. S2). This is
further confirmed by the finding that bacterial taxa degrading chitin were greatly favored in the presence of carcasses at the end of the incubation (Fig. 4). The difference between non-carcass-derived POC in microcosms D and HD (0.059 ± 0.003 and 0.096 ± 0.003 mg L⁻¹, respectively; Fig. 3) presumably occur due to humic matter aggregation converting DOC into POC. This assumption is supported by a two-fold POC increase in the H microcosms compared to the initial value (Table 1).

In our experiment we used 40 Daphnia carcasses per liter, a high but still natural value (Dubovskaya et al., 2003). Most of the organic carbon originating from carcasses was respired by the bacterial community within the two weeks of incubation. Therefore, in natural systems, the considerable amount of LOM released by zooplankton carcasses (Tang et al., 2014), can directly affect the functioning of the ecosystem by accelerating microbial carbon turnover and respiratory carbon losses to the atmosphere at short time scales. The more recalcitrant part of the carapace may persist for a longer time, and eventually escapes the water column to be further processed in the sediments (Tang et al., 2014), being also important for carbon sequestration. Consequently, the balance between microbial degradation of zooplankton carcasses and organic matter storage in sediments has a great influence on the aquatic carbon cycle.

**Priming, a concept under debate in aquatic sciences**

Humic matter was chosen as a recalcitrant carbon source for its ubiquity in aquatic ecosystems and as an important part of the carbon pool in the global carbon cycle. Humic matter can represent up to 80% of the total DOM in freshwaters (Rocker et al., 2012a). Although humic matter is considered as recalcitrant, it can at least partially be decomposed by bacteria (Hutalle-Schmelzer et al., 2010; Rocker et al., 2012a; Kisand et al., 2013). Furthermore, the degradation of humic acids by marine and estuarine bacterial communities seems to be favored by specific environmental conditions, e.g. along a salinity gradient.
Consequently, recalcitrance and lability of organic matter are not *per se* intrinsic chemical characteristics (Schmidt et al., 2011), and may only account for specific environmental settings (Bianchi et al., 2015).

In our experiment, we incubated *Daphnia* carcasses and humic matter in different combinations to test for a priming effect of bacterial degradation of ROM induced by the addition of carcass LOM. The addition of a mixture of natural humic matter and *Daphnia* carcasses resulted in the degradation of organic matter and an isotopic ratio of the respired CO$_2$ similar to what we predicted based on our linear addition model with humic matter or carcasses as the sole carbon source. Moreover, based on DOM characterization (Table 1), the natural humic matter from Lake Grosse Fuchskuhle was only little degraded by bacteria, irrespective of LOM addition via *Daphnia* carcasses. Consequently, and in agreement with previous studies (Bengtsson et al., 2014; Catalán et al., 2015; Dorado-García et al., 2015), we could not detect any quantitative changes in bacterial ROM degradation when using carcass LOM as a potential primer (Table 1).

Recently, a number of studies have investigated the prevalence of a priming effect in aquatic ecosystems (e.g., van Nugteren et al., 2009; Guenet et al., 2013; Kuehn et al., 2014; Steen et al., 2015). Various types of ecosystems (marine, lentic, lotic) and habitats (pelagic, hyporheic, sediments) have been tested, as well as different sources of LOM (carbohydrates, algae leachate, gastropod mucus, etc.) and ROM (terrestrial plant tissues, lignocellulose, humic matter, etc.) have been used. Although some authors have found support for ROM priming by more labile organic matter, mainly of algal origin (van Nugteren et al., 2009; Guenet et al., 2013; Hotchkiss et al., 2014; Bianchi et al., 2015; Gontikaki et al., 2015), others did not reveal any evidence for a positive priming effect (Bengtsson et al., 2014; Catalán et al., 2015; Dorado-García et al., 2015; Blanchet et al., 2017), or even found a negative priming effect (Gontikaki et al., 2013) with ROM being decomposed slower in the presence of a labile carbon source. Thus, it appears that the absence or presence of the
The degradation of ROM (such as humic matter) is a combination of two main processes: microbial and photochemical decomposition (Amado et al., 2015). The most efficient humic matter microbial degraders in aquatic systems are fungi (Grinhut et al., 2007). Generally, fungi have a higher capacity than bacteria to synthesize the extracellular oxidative enzymes involved in ROM degradation and thus more readily and successfully initiate humic matter degradation (Rojas-Jimenez et al., 2017). In contrast, bacteria join the process later as degraders of humic matter metabolites (Grossart and Rojas-Jimenez, 2016; Rojas-Jimenez et al., 2017). Due to our pre-filtration step to avoid the presence of protozoan grazers and large phytoplankton as has been frequently done in similar incubation experiments (Fonte et al., 2013; Guenet et al., 2013; Attermeyer et al., 2014, 2015; Blanchet et al., 2015), fungi, which could potentially constitute an important component of the aquatic priming effect, were removed.

Photochemical degradation can break down/oxidise recalcitrant DOM compounds, such as fulvic and humic substances, into more labile molecules (Spencer et al., 2009; Stubbins et al., 2010). It is likely that in natural systems photochemical and microbial degradation work synergistically and thus contribute to the priming effect. On the other hand, UV radiation can also affect biodegradability of LOM in the presence of humic matter (Tranvik and Kokalj, 1998). Our study was conducted in the dark as in other priming effect studies (e.g. Bianchi et al., 2015; Catalán et al., 2015) to avoid photosynthesis which could have obstructed the detection of differences in carbon oxidation between treatments and controls. Thus, it is clear that photodegradation of humic substances was not taken into
account in the present study and might have reduced our possibilities to measure a positive priming effect. However, our study is directly applicable to the situation when the zooplankton carcasses are sinking into the pelagic zone at depths with very low light penetration.

The incubation temperature in our experiment was relatively high (20°C) and not typical for deep waters. Nevertheless, if temperature might have a strong influence on bacterial community composition and its activity (Adams et al., 2010), a lower temperature only slows down the degradation process but not the biodegradability of carcasses. Therefore, in natural systems, the potential of zooplankton carcasses to release a consequential amount of carbon in the atmosphere and to select for specific bacterial community might happen over longer time scales than the ones observed in our experiment.

Another factor that can affect DOM degradation is the oxygen concentration, which usually decreases sharply with depth in the pelagic zone of humic lakes. In our experimental microcosms we maintained 100% oxygen saturation due to mixing with headspace air. However, Tang et al. (2006) observed only a small difference between microbial communities decomposing carcasses in anaerobic vs. aerobic conditions, presumably because zooplankton carcasses represent anoxic microenvironments even when oxygen is abundant in the surrounding waters (Glud et al., 2015).

Although the experimental set-up might have its limitations, our findings are relevant to natural environments such as the metalimnion of humic lakes and other deep water layers where the maximum percentage of dead zooplankton is found (Dubovskaya et al., 2018).

**Conclusions**

We observed a pronounced change in bacterial community composition in microcosms induced by the addition of *Daphnia* carcasses and humic matter. While the quality of both
added carbon sources played a role, zooplankton carcasses were the major driver of bacterial community assembly. Even though no priming effect was detected, it is critical to continue studies on ROM degradation in the presence of zooplankton carcasses to better understand microbial dynamics and thus potential changes in organic matter fluxes in freshwater ecosystems. In events of mass zooplankton mortality, the water column can be loaded with a considerable amount of carcass-derived LOM. By decreasing the bacterial diversity and selecting specialized bacteria, zooplankton carcasses potentially have further and so far likely unknown consequences on microbial dynamics and carbon fluxes. Indeed, our results suggest that a significant part of zooplankton carcasses is respired by bacteria. Therefore, our study provides evidence that quantifying the implication of zooplankton carcasses in the functioning of aquatic ecosystems is of primordial importance to understand the amount of carbon produced in and released from freshwaters.

**Experimental Procedures**

**Experimental setup**

The experiment was conducted in laboratory microcosms, which were set up in 1L acid-washed and muffled (4h, 450 °C) glass bottles half-filled with artificial lake water. The microcosms were inoculated with a concentrated bacterial community from the acidic bog lake Grosse Fuchskuhle (Northeastern Germany, 53°06'N 12°59'E; more detailed information is provided in the Supplementary Methods). Microcosms were sealed with PTFE-coated silicone septa, placed on a roller apparatus (Wheaton, USA), and incubated for 15 days in the dark at 20°C. The duration of the experiment, irradiance, temperature, and the rolling mode were chosen to fall within the range of natural conditions of sinking zooplankton carcasses in a water column (Tang *et al.*, 2009, 2014).
One set of microcosms (HD) was amended with humic matter and $^{13}$C-labeled *Daphnia* carcasses (Fig. S1). The detailed description of the amendments including their preparation is available in the Supplementary Methods. Carcasses control microcosms (D) were amended solely with $^{13}$C-labeled *Daphnia* carcasses in the same quantity and from the same batch of *Daphnia* as in HD microcosms. Humic matter control microcosms (H) were amended with humic matter only in the same quantity as in HD microcosms (Fig. S1). Further, blank microcosms (B) with no added organic matter were included to determine whether the bacterial community was capable of growing without the extra addition of organic carbon (Fig. S1). Each experimental treatment was conducted in five replicates.

**Bacterial counts and community composition**

Microbial abundances were determined after filtration of 5 mL of water through Nucleopore track-etched membrane filters with a pore size of 0.2 µm (Whatman, UK). Then, samples were stained with 4’,6-diamidino-2-phenylindole (DAPI) to monitor cell numbers using an imaging system linked to a Leica epifluorescence microscope. Pictures were taken from 30-50 fields and abundances were determined using the CellC software (Tampere University of Technology, Finland, [https://sites.google.com/site/cellcsoftware/](https://sites.google.com/site/cellcsoftware/)).

For DNA extraction, 150 mL of water was filtered through 0.22 µm GVWP filters (Millipore, Germany) and total DNA was extracted according to a modified protocol described by Nercessian *et al.* (2005) (see Supplementary Methods for details). DNA concentrations were determined with a Quantus fluorometer (Promega, USA), following the manufacturer’s instructions. PCR, library preparation and sequencing was done by LGC Genomics (Berlin, Germany). Briefly, the V3-V4 region was amplified using primers 341F-785R (Klindworth *et al.*, 2013), followed by library preparation (2x300 bp) and sequencing on a MiSeq Illumina platform. Sequences were quality checked and analyzed using Mothur v1.37.6 (Schloss *et al.*, 2009), see Supplementary Methods for the detailed workflow. The
sequence data was deposited in Genbank under the following accession number: PRJNA418906.

**Organic carbon concentration and composition**

Directly after sampling, water was passed through pre-combusted (4 h, 450°C) GF75 filters (Advantec, nominal pore size of 0.3 µm). Particulate organic carbon (POC) collected on the filters was measured with an Eltra SC 800 (Eltra, Germany). One subsample of filtrate was processed directly to measure the DOC concentration with a TOC-V_CPH (Shimadzu, Kyoto, Japan) as well as the phosphate concentration using a FIAstar 5000 (Foss, USA). Total organic carbon (TOC) concentration was estimated by summing up the DOC and POC concentrations.

Another subsample of filtrate was stored at 4°C for three weeks prior analysis with liquid chromatography - organic carbon detection – organic nitrogen detection (LC-OCD-OND, DOC Labor, Germany). The LC-OCD-OND allows distinguishing between HMWS, LMWS, and humic substances (Huber et al., 2011). Nevertheless, its sensitivity is not sufficient for analyzing samples with low DOC concentration (<0.2 mg L⁻¹). That was the case for the D and B microcosms which were therefore not analyzed by LC-OCD-OND.

**Spectral characteristics of DOM**

DOM optical characteristics were obtained with a UV-Vis spectrophotometer (Hitachi U2900, Germany) and a spectrofluorometer (Hitachi F7000, Germany). Absorbance spectra were recorded from 190 to 800 nm with an increment of 1 nm and used to compute the specific ultraviolet absorbance at 254 nm (SUVA₂₅₄) and absorption spectral slopes (Weishaar et al., 2003; Helms et al., 2008). SUVA₂₅₄ is an indicator of aromaticity and chemical reactivity while the absorption slopes are used as proxies for DOM molecular weight.
Excitation emission matrices (EEMs) were generated with excitation wavelengths ranging from 220 to 450 nm and emission wavelengths ranging from 230 to 600 nm, both with 5 nm increments. EEMs were corrected with a MilliQ water sample and for inner filter effect using the absorbance-based method (Christmann et al., 1980; Murphy et al., 2013).

Then, fluorescence, humification and freshness indices, as well as specific fluorescence intensity at various peaks were calculated as described by Hansen et al. (2016).

**Carbon stable isotope ratio**

Stable isotope analysis of the respired CO$_2$ provides information on carbon substrates metabolized by the microbial community (Fabian et al., 2017). The ratio of $^{13}$C/$^{12}$C in the dissolved CO$_2$ was measured directly in each microcosm by a membrane-inlet mass-spectrometer dissolved gas analyzer (HiCube pumping station, Pfeiffer Vacuum and Bay Instruments membrane, USA), controlled by Quickdata software. The sampling capillary was inserted through the PTFE-coated silicone septa prior to opening microcosms in order to avoid gas leakages. Concentrations of $^{12}$CO$_2$ and $^{13}$CO$_2$ were obtained from the ion currents sequentially recorded at mass to charge (m/z) ratios of 44 and 45, respectively.

For the carbon stable isotope ratio in POC and DOC, 100 ml of water was passed through GF75 filters (Advantec, USA) and both the flow-through and the filter (all five replicates were pooled onto one filter to have enough material for subsequent analyzes) were collected and freeze-dried. Moreover, we dried 1 mg of acid-killed *Daphnia* and approximately 1 mg of extracted humic matter for carbon stable isotope ratio analysis. The samples were analyzed with an Elemental Analyzer (Thermo Flash EA 2000), coupled to a continuous-flow isotope ratio mass spectrometer (Thermo Finnigan Delta V) via an open split interface (Thermo Finnigan Conflow IV) in the IRMS Laboratory of the Leibniz Institute for Baltic Sea Research Warnemünde (Germany), and with a PDZ Europa ANCA-GSL elemental
analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the UC Davis Stable Isotope Facility (USA).

**Decomposition calculations**

To test for a possible priming effect, we used an addition model as in Hannides and Aller (2016). Initial and final total organic carbon concentrations in the humic control, i.e. TOC$_i$ (H) and TOC$_f$ (H), respectively, in the carcass control, i.e. TOC$_i$ (D) and TOC$_f$ (D), and in the blank, i.e. TOC$_i$ (B) and TOC$_f$ (B), respectively, were used to predict the degradation of total organic carbon ($\Delta$TOC) in the HD microcosm. Thereby, we assumed that the degradation rates of carbon sources observed in the control microcosms are conserved when humic matter and carcasses are incubated together. The predicted $\Delta$TOC(H+D) was compared with the measured $\Delta$TOC(HD) to detect any enhanced degradation of organic carbon caused by a potential “priming effect”. Similarly, data on CO$_2$ concentration and isotope ratio ($^{13}$CO$_2$/^{12}$CO$_2$) in the microcosms was used to calculate respiration of recalcitrant and labile carbon pools. We assumed that if a priming effect is present, there should be a difference in the observed isotope ratio between measured and predicted CO$_2$, based on the sum of control values.

The carcass carbon fraction ($F_c$) in DOC, POC and CO$_2$ was calculated via a stable isotope mixing model (Hopkins and Ferguson, 2012). We also applied Keeling plot analyses of dissolved CO$_2$ (Pataki et al., 2003) to estimate the $\delta^{13}$C of the carbon source respired in the respective microcosms. Descriptive calculation formulas are available in the Supplementary Methods.

**Statistical analyses**

For alpha-diversity calculations of bacterial communities, individual samples were rarefied to the lowest number of reads in a sample (10080), with 10 iterations per sample.
using QIIME (Caporaso et al., 2010). Shannon biodiversity index was calculated to estimate richness and evenness of the microcosm communities. Beta-diversity of bacterial communities was calculated by using the Bray-Curtis similarity coefficients (Bray and Curtis, 1957) using QIIME.

All analyses described below were run via R version 3.3.3 (R Development Core Team, 2006). R package vegan (vegan: Community Ecology Package, 2017, https://CRAN.R-project.org/package=vegan) was used to carry out principal coordinate analysis (PCoA) and to calculate ANOSIM and SIMPER (Clarke, 1993).

Bacterial community composition was linked to DOC characteristics which significantly differentiated microcosms. We used the least absolute shrinkage and selection operator (LASSO) to select genera that were influenced by a variation in DOM composition (Traving et al., 2016). Before designing the LASSO models, we summed the abundances of OTUs belonging to the same genus and performed a centered log-ratio transformation (Gloor and Reid, 2016) of the absolute abundances of all genera. To visualize the outcome, we depicted the selected genera in two different networks using Cytoscape software (Shannon et al., 2003).

All differences between treatments were tested using a one-way analysis of variance (ANOVA) and post-hoc Tukey tests. Pairwise comparisons between initial and final parameters of microcosms and between two treatments were done using Student’s t-test and Wilcoxon rank sum test. Normality was checked by Shapiro–Wilks tests when necessary.

**Acknowledgements**

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Scientific Schools (grant NSh-9249.2016.5). OVK was supported by Michail-Lomonosov-Programme-Linie A, 2015 (57180771) funded by the Ministry of Education and Science of the Russian Federation and the German Academic Exchange Service (DAAD). The authors thank Uta Mallok for DOC and phosphate concentration measurements. We are grateful to Maren Voss and Iris Liskow in the IRMS Laboratory of the Leibniz Institute for Baltic Sea Research Warnemünde (Germany) for stable isotope analysis. We greatly appreciate the useful advices of Jenny Fabian and Isabell Klawonn on stable isotope sample preparation and data handling. We thank two anonymous reviewers for carefully reading the manuscript and suggesting substantial improvements.

The authors declare no conflict of interests.

References


**Table and Figure legends**

**Table 1.** Chemical and optical parameters of experimental microcosms varying in carbon sources: \( B_i \) – blank initial, \( B_f \) – blank final, \( H_i \) – humic matter initial, \( H_f \) – humic matter final, \( D_i \) – carcasses initial, \( D_f \) – carcasses final, \( HD_i \) – humic matter + carcasses initial, \( HD_f \) – humic matter + carcasses final. Parameters in \( D_i \) and \( HD_i \) were not measured directly but calculated from parameters in \( B_i \) and \( H_i \). Values are given as means of five replicates ±
standard errors, except for the initial DNA concentration values obtained from a single measurement. nd = not determined.

**Table 2.** Alpha-diversity of bacterial communities in the microcosms with additions of humic matter (H), *Daphnia* carcasses (D), humic matter and carcasses together (HD), and blank with no carbon sources (B). Values are given as means of five replicates ± standard errors, except for the initial inoculate (I) obtained from a single measurement, and B microcosms (four replicates due to too low DNA content in one sample).

**Table 3.** Abundances (in %) of the quantitatively prominent OTUs contributing the most to the dissimilarity among the inoculum sample (I) and the end points of the microcosms with additions of humic matter (H), *Daphnia* carcasses (D), humic matter and carcasses together (HD), and blank with no carbon sources (B). Values are given as means of five replicates ± standard errors, except for the inoculum sample obtained from a single measurement, and B microcosms (four replicates due to low DNA content of two samples which were pooled).

**Fig. 1** Relative abundance of major bacterial phyla and classes of Proteobacteria in the initial sample (I) and the end points of microcosms with additions of humic matter (H), *Daphnia* carcasses (D), humic matter and carcasses together (HD), and blank with no carbon sources (B).

**Fig. 2** Principal coordinate analysis of the initial sample (I) and the end points of microcosms with additions of humic matter (H), *Daphnia* carcasses (D), humic matter and carcasses together (HD), and blank with no carbon sources (B), based on Bray-Curtis community similarity, calculated as relative abundance of operational taxonomic units (OTUs).

**Fig. 3** Carbon fractions originated from *Daphnia* carcasses at the end of the experiment in pools of dissolved (DOC) and particulate organic carbon (POC) as well as in
dissolved CO$_2$ in microcosms with humic matter and _Daphnia_ carcasses (HD) and carcasses only (D). The area size of diagrams is relative to carbon concentration.

**Fig. 4** Network of bacterial genera connected with major DOM parameters, significantly different in pairs of microcosms: a – microcosms H – humic matter, and D – carcasses; b – microcosms HD – humic matter + carcasses, and D – carcasses. Nodes that interact positively are connected by solid black edges, nodes connected by dashed edges have negative interactions. Legend: DOC – concentration of DOC; HIX – humification index, FIX – fluorescence index, fresh – freshness index, SUVA254 – Specific UV absorbance at 254 nm, A/T – ratio between the specific fluorescence at peak A (excitation 240-260 nm/emission 400-500 nm, UVC humic-like fluorescent component) and peak T (excitation 270-285 nm/emission 340-380 nm, tryptophan-like fluorescent component).
Table 1. Chemical and optical parameters of experimental microcosms varying in carbon sources: B_i – blank initial, B_f – blank final, H_i – humic matter initial, H_f – humic matter final, D_i – carcasses initial, D_f – carcasses final, HD_i – humic matter + carcasses initial, HD_f – humic matter + carcasses final. Parameters in D_i and HD_i were not measured directly but calculated from parameters in B_i and H_i. Values are given as means of five replicates ± standard errors, except for the initial DNA concentration values obtained from a single measurement. nd = not determined.

<table>
<thead>
<tr>
<th>Chemical parameters</th>
<th>B_i</th>
<th>B_f</th>
<th>H_i</th>
<th>H_f</th>
<th>D_i</th>
<th>D_f</th>
<th>HD_i</th>
<th>HD_f</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOC mg·L⁻¹</td>
<td>0.221 ± 0.003</td>
<td>0.216 ± 0.009</td>
<td>3.025 ± 0.070</td>
<td>2.952 ± 0.109</td>
<td>0.221 ± 0.003</td>
<td>0.269 ± 0.016</td>
<td>3.025 ± 0.070</td>
<td>2.944 ± 0.017</td>
</tr>
<tr>
<td>POC mg·L⁻¹</td>
<td>0.044 ± 0.006</td>
<td>0.028 ± 0.004</td>
<td>0.033 ± 0.002</td>
<td>0.067 ± 0.010</td>
<td>1.378 ± 0.044</td>
<td>0.333 ± 0.015</td>
<td>1.367 ± 0.040</td>
<td>0.354 ± 0.007</td>
</tr>
<tr>
<td>Humic substances (mg·L⁻¹)</td>
<td>nd</td>
<td>nd</td>
<td>2.065 ± 0.034</td>
<td>2.130 ± 0.015</td>
<td>nd</td>
<td>nd</td>
<td>2.065 ± 0.034</td>
<td>2.125 ± 0.009</td>
</tr>
<tr>
<td>Building blocks of humic substances (mg·L⁻¹)</td>
<td>nd</td>
<td>nd</td>
<td>0.185 ± 0.020</td>
<td>0.156 ± 0.010</td>
<td>nd</td>
<td>nd</td>
<td>0.185 ± 0.020</td>
<td>0.174 ± 0.012</td>
</tr>
<tr>
<td>Low molecular-weight acids (mg·L⁻¹)</td>
<td>nd</td>
<td>nd</td>
<td>0.020 ± 0.002</td>
<td>0.019 ± 0.001</td>
<td>nd</td>
<td>nd</td>
<td>0.020 ± 0.002</td>
<td>0.019 ± 0.002</td>
</tr>
<tr>
<td>Amphiphilic molecules (mg·L⁻¹)</td>
<td>nd</td>
<td>nd</td>
<td>0.290 ± 0.032</td>
<td>0.248 ± 0.043</td>
<td>nd</td>
<td>nd</td>
<td>0.290 ± 0.032</td>
<td>0.271 ± 0.022</td>
</tr>
<tr>
<td>Polysaccharides (mg·L⁻¹)</td>
<td>nd</td>
<td>nd</td>
<td>0.073 ± 0.002</td>
<td>0.056 ± 0.005</td>
<td>nd</td>
<td>nd</td>
<td>0.073 ± 0.002</td>
<td>0.069 ± 0.008</td>
</tr>
<tr>
<td>Phosphate (mg·L⁻¹)</td>
<td>0.332 ± 0.003</td>
<td>0.337 ± 0.003</td>
<td>0.339 ± 0.003</td>
<td>0.323 ± 0.002</td>
<td>0.332 ± 0.003</td>
<td>0.340 ± 0.001</td>
<td>0.339 ± 0.003</td>
<td>0.329 ± 0.002</td>
</tr>
<tr>
<td>DNA concentration (ng·mL⁻¹ of medium)</td>
<td>0.14</td>
<td>0.112 ± 0.043</td>
<td>nd</td>
<td>1.569 ± 0.348</td>
<td>nd</td>
<td>20.467 ± 2.250</td>
<td>nd</td>
<td>29.133 ± 0.879</td>
</tr>
<tr>
<td>Bacterial cell count (10⁶ cells·mL⁻¹)</td>
<td>1.343 ± 0.601</td>
<td>1.403 ± 0.627</td>
<td>nd</td>
<td>1.931 ± 0.864</td>
<td>nd</td>
<td>10.129 ± 4.530</td>
<td>nd</td>
<td>9.630 ± 4.307</td>
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</table>
Table 1 (continued).

<table>
<thead>
<tr>
<th>Absorbance measurements</th>
<th>$B_i$, $D_i$</th>
<th>$B_f$</th>
<th>$D_f$</th>
<th>$H_i$, $HD_i$</th>
<th>$H_f$</th>
<th>$HD_f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific UV absorbance at 254 nm [SUVA$_{254}$ (L mg-L$^{-1}$ m$^{-1}$)]</td>
<td>0.431 ± 0.085</td>
<td>0.188 ± 0.055</td>
<td>0.177 ± 0.022</td>
<td>3.826 ± 0.030</td>
<td>3.723 ± 0.050</td>
<td>3.775 ± 0.009</td>
</tr>
<tr>
<td>Spectral slope at 275–295 nm (nm$^{-1}$)</td>
<td>0.015 ± 0.000</td>
<td>0.029 ± 0.014</td>
<td>0.032 ± 0.007</td>
<td>0.014 ± 0.000</td>
<td>0.013 ± 0.000</td>
<td>0.013 ± 0.000</td>
</tr>
<tr>
<td>Spectral slope at 350-400 nm (nm$^{-1}$)</td>
<td>0.005 ± 0.000</td>
<td>0.003 ± 0.001</td>
<td>0.005 ± 0.000</td>
<td>0.008 ± 0.000</td>
<td>0.014 ± 0.001</td>
<td>0.013 ± 0.001</td>
</tr>
<tr>
<td>Spectral slopes ratio</td>
<td>0.691 ± 0.034</td>
<td>nd</td>
<td>0.811 ± 0.234</td>
<td>0.765 ± 0.008</td>
<td>0.821 ± 0.009</td>
<td>0.818 ± 0.009</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Fluorescence measurements</th>
<th>$B_i$, $D_i$</th>
<th>$B_f$</th>
<th>$D_f$</th>
<th>$H_i$, $HD_i$</th>
<th>$H_f$</th>
<th>$HD_f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescence index (FIX)</td>
<td>1.739 ± 0.218</td>
<td>1.524 ± 0.331</td>
<td>1.501 ± 0.073</td>
<td>1.808 ± 0.009</td>
<td>1.779 ± 0.020</td>
<td>1.798 ± 0.008</td>
</tr>
<tr>
<td>Humification index (HIX)</td>
<td>0.246 ± 0.018</td>
<td>0.226 ± 0.362</td>
<td>0.392 ± 0.018</td>
<td>0.887 ± 0.002</td>
<td>0.879 ± 0.003</td>
<td>0.877 ± 0.004</td>
</tr>
<tr>
<td>Freshness index ($\beta$:$\alpha$)</td>
<td>0.851 ± 0.138</td>
<td>1.231 ± 0.196</td>
<td>0.855 ± 0.034</td>
<td>0.556 ± 0.003</td>
<td>0.555 ± 0.003</td>
<td>0.550 ± 0.015</td>
</tr>
<tr>
<td>Specific fluorescence at peak A (RU L mg-C$^{-1}$)</td>
<td>0.008 ± 0.001</td>
<td>0.010 ± 0.002</td>
<td>0.015 ± 0.000</td>
<td>0.563 ± 0.008</td>
<td>0.579 ± 0.007</td>
<td>0.574 ± 0.008</td>
</tr>
<tr>
<td>Specific fluorescence at peak B (RU L mg-C$^{-1}$)</td>
<td>0.790 ± 0.011</td>
<td>0.330 ± 0.070</td>
<td>0.252 ± 0.011</td>
<td>0.808 ± 0.037</td>
<td>0.221 ± 0.009</td>
<td>0.343 ± 0.101</td>
</tr>
<tr>
<td>Specific fluorescence at peak C (RU L mg-C$^{-1}$)</td>
<td>0.005 ± 0.002</td>
<td>0.004 ± 0.001</td>
<td>0.007 ± 0.000</td>
<td>0.253 ± 0.004</td>
<td>0.264 ± 0.001</td>
<td>0.272 ± 0.003</td>
</tr>
<tr>
<td>Specific fluorescence at peak M (RU L mg-C$^{-1}$)</td>
<td>0.006 ± 0.000</td>
<td>0.008 ± 0.001</td>
<td>0.011 ± 0.000</td>
<td>0.303 ± 0.005</td>
<td>0.319 ± 0.002</td>
<td>0.338 ± 0.007</td>
</tr>
<tr>
<td>Specific fluorescence at peak T (RU L mg-C$^{-1}$)</td>
<td>0.071 ± 0.002</td>
<td>0.076 ± 0.003</td>
<td>0.083 ± 0.003</td>
<td>0.136 ± 0.003</td>
<td>0.145 ± 0.003</td>
<td>0.155 ± 0.003</td>
</tr>
</tbody>
</table>
Table 2. Alpha-diversity of bacterial communities in the microcosms with additions of humic matter (H), *Daphnia* carcasses (D), humic matter and carcasses together (HD), and blank with no carbon sources (B). Values are given as means of five replicates ± standard errors, except for the initial inoculate (I) obtained from a single measurement, and B microcosms (four replicates due to low DNA content in one sample).

<table>
<thead>
<tr>
<th>Microcosm</th>
<th>Observed OTUs</th>
<th>Shannon Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>313.4</td>
<td>5.00</td>
</tr>
<tr>
<td>B</td>
<td>234.0 ± 13.1</td>
<td>4.60 ± 0.27</td>
</tr>
<tr>
<td>H</td>
<td>217.0 ± 11.9</td>
<td>4.73 ± 0.20</td>
</tr>
<tr>
<td>D</td>
<td>189.1 ± 10.6</td>
<td>3.31 ± 0.24</td>
</tr>
<tr>
<td>HD</td>
<td>191.5 ± 6.8</td>
<td>3.08 ± 0.17</td>
</tr>
</tbody>
</table>
Table 3. Abundances (in %) of the quantitatively prominent OTUs contributing the most to the dissimilarity among the inoculum sample (I) and the end points of the microcosms with additions of humic matter (H), *Daphnia* carcasses (D), humic matter and carcasses together (HD), and blank with no carbon sources (B). Values are given as means of five replicates ± standard errors, except for the inoculum sample obtained from a single measurement, and B microcosms (four replicates due to low DNA content of two samples which were pooled).

<table>
<thead>
<tr>
<th>OTU</th>
<th>I</th>
<th>B</th>
<th>H</th>
<th>D</th>
<th>HD</th>
<th>Genus</th>
<th>Family</th>
<th>Class</th>
<th>Phyla</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>6.84</td>
<td>17.80</td>
<td>3.95</td>
<td>45.63</td>
<td>57.39</td>
<td><em>Pseudomonas</em></td>
<td><em>Pseudomonadaceae</em></td>
<td>Gammaproteobacteria</td>
<td>Proteobacteria</td>
</tr>
<tr>
<td>2</td>
<td>1.31</td>
<td>2.90</td>
<td>3.53</td>
<td>15.82</td>
<td>5.40</td>
<td><em>Duganella</em></td>
<td>Oxalobacteraceae</td>
<td>Betaproteobacteria</td>
<td>Proteobacteria</td>
</tr>
<tr>
<td>3</td>
<td>1.33</td>
<td>0.76</td>
<td>0.24</td>
<td>9.11</td>
<td>7.42</td>
<td><em>Flavobacterium</em></td>
<td>Flavobacteriaceae</td>
<td>Flavobacteria</td>
<td>Bacteroidetes</td>
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<tr>
<td>4</td>
<td>20.91</td>
<td>7.66</td>
<td>9.32</td>
<td>0.19</td>
<td>0.57</td>
<td><em>Polynucleobacter</em></td>
<td>Burkholderiaceae</td>
<td>Betaproteobacteria</td>
<td>Proteobacteria</td>
</tr>
<tr>
<td>5</td>
<td>0.08</td>
<td>0.06</td>
<td>0.09</td>
<td>5.83</td>
<td>4.13</td>
<td><em>Janthinobacterium</em></td>
<td>Oxalobacteraceae</td>
<td>Betaproteobacteria</td>
<td>Proteobacteria</td>
</tr>
<tr>
<td>6</td>
<td>0.29</td>
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Supplementary Information

Community composition in different microcosms

*Actinobacteria* and *Verrucomicrobia* had comparatively high abundances in the initial inoculum (19% and 18%, respectively), but their final abundance diverged in different treatments (Fig. 1). While in HD and D microcosms both phyla decreased down to 1-2% of total abundance, in H microcosms *Verrucomicrobia* increased to 24 %, and *Actinobacteria* slightly decreased to 14 % at the end of the incubation. In contrast, *Actinobacteria* increased in abundance (to 21 %) and *Verrucomicrobia* decreased (to 7 %) at the end of the incubation in the B microcosms (Fig. 1).

Initially, the relative abundance of *Bacteroidetes* was 7%, increasing to 15 % and 17 % in the HD and D microcosms, respectively (Fig. 1). At the end of the incubation, only 3 % of sequences in H microcosms and 2 % of sequences in B microcosms belonged to *Bacteroidetes*. Members of the candidate phylum *Parcubacteria* initially accounted for 3% of all sequences, but dropped in all treatments. All other identified phyla did not exceed a threshold of 1% in any sample.

In the bacterial inoculum from Lake Grosse Fuchskuhle, OTU4 *Polynucleobacter* sp. from the class *Betaproteobacteria* dominated (21 %, Table 3). OTU10 FukuN18 (*Verrucomicrobia*) was the second numerous OTU (16 %, Table 3) in the natural community. However, at the end of the experiment, both OTUs decreased in all microcosms, especially in the HD and D microcosms (<1%, Table 3).

In the B microcosms, OTU1 *Pseudomonas* from the class *Gammaproteobacteria*, which was also present in the initial sample with 7 %, became dominant (18 %), and OTU7 *Rhizobacter* (*Betaproteobacteria*) considerably increased from almost absent to 12 % (Table 3).

In the H microcosms, OTU4 *Polynucleobacter* decreased to 9 % of all sequences representing the second most abundant member of the community (Table 3). Instead, OTU6
vadinHA64 (*Opitutae, Verrucomicrobia*) became dominant (20%) in the H microcosm, but did not increase in any other microcosm. Microcosms H were characterized by higher fractions of OTU15 *Aquabacterium*, OTU16 *Alkanibacter*, OTU30 *Prosthecobacter* and OTU34 *Zoogloea* when compared to other microcosms.

In both D and HD microcosms, OTU1 *Pseudomonas* was dominant (46% and 57%, respectively; Table 3). In the D microcosm, OTU2 *Duganella* (*Betaproteobacteria*) was remarkably more abundant than in other treatments (16%). OTU3 *Flavobacterium* (*Bacteroidetes*) was also highly abundant in D and HD microcosms (9% and 7%, respectively). Other OTUs were enriched in the HD and D microcosms: OTU 5 *Janthinobacterium*, OTU11 uncultured Chitinophagaceae, OTU13 *Pseudomonas* (Table 3).

Interestingly, OTU19 *Bacteriovorax* was enriched only in the HD microcosms (Table 3).

Overall, the diversity of bacterial communities decreased with the total amount of available LOM (Table 2), indicating the copiotrophic nature of the dominant *Gammaproteobacteria*. Microcosms B (no OM addition) supported the bacterial community which remained most similar to the initial inoculum I (Fig. 2). The dissimilarity between the initial inoculum and microcosms B was mainly caused by the dominance of OTU4 *Polynucleobacter* (Table S1) in the initial sample. This group of bacteria represents a common genus of freshwater bacterioplankton communities (Hahn *et al.*, 2009), and has been described earlier for Lake Grosse Fuchskuhle (Hutalle-Schmelzer *et al.*, 2010). Several *Polynucleobacter* clades prefer acidic lakes and have been shown to assimilate the breakdown products of humic matter (Newton *et al.*, 2011), but not humic matter itself (Hutalle-Schmelzer *et al.*, 2010). At the end of the experiment, *Pseudomonas* represented the most dominant genus in microcosms D and HD, but was also abundant in microcosms B (Table 3). This particular genus contributed the most to the observed differences between HD, D and all other microcosms (OTU1, Table S1), but it represents a taxonomically and physiologically diverse group, including putrefactive (Pekhtasheva, 2012) and chitinolytic bacteria (Gooday,
1990). Our data suggest that Pseudomonas are greatly favored by the presence of the relatively labile organic carbon pool derived from zooplankton carcasses indicating the high potential of carcasses to select for specific bacterial communities different from those in Lake Grosse Fuchskuhle and other lakes.

Bacterial communities of microcosms HD and D were very similar (Fig. 2) and characterized by a high abundance of copiotrophs adapted to high nutrient availability, and chitinolytic bacteria, i.e. Flavobacterium (Gooday, 1990; Newton et al., 2011), Janthinobacterium, Duganella (Haack et al., 2016) and uncultured representatives of Chitinophagaceae (Kämpfer, 2015).

In the H microcosms, the dominant OTU6 vadinHA64 (Opitutae, Verrucomicrobia, Table 3) is of a particular interest due to its ability to benefit from humic matter additions. However, only limited information is available about this so far uncultivated and uncharacterized strain.

References


Supplementary Methods

Microcosm medium and added substrates

Artificial lake water

Artificial lake water was prepared following the protocol for acidic waters of Smith et al. (2002), but excluding the addition of aluminum chloride. The pH was corrected to 5.3 to match the original pH of Lake Grosse Fuchskuhle at the time of sampling. 1.6 mg L\(^{-1}\) of nitrate and 0.36 mg L\(^{-1}\) of phosphate were added to avoid nutrient limitation. Each microcosm was filled with 499 mL of artificial lake water.

Bacterial community and humic matter

The bacterial source community was obtained from the north-eastern basin of the artificially divided lake Grosse Fuchskuhle (Grossart et al., 2008; Hutalle-Schmelzer et al., 2010), which is fed by waters of a surrounding bog, thus most of its dissolved organic matter is composed of humic matter. After preliminary filtration of lake water through 0.8 µm for elimination of grazers as previously described in other degradation studies (Hutalle-Schmelzer et al., 2010; Attermeyer et al., 2014), the bacterial community was concentrated by tangential flow filtration and further centrifugation (30 min at 8000 rpm at 4°C). The bacterial pellet was then transferred to PBS buffer and kept overnight at 4 °C prior inoculation of 1 mL into each microcosm as in Attermeyer et al. (2014).

Humic matter previously extracted from the south-western part of Lake Grosse Fuchskuhle by reverse osmosis was used as a ROM source (Hutalle-Schmelzer et al., 2010). The humic extract was diluted, filtered through 0.45 µm, and added to microcosms HD and H (mean final concentration 3.025 ± 0.070 mg C L\(^{-1}\)). The ROM added to the microcosms had a signature of \(\delta^{13}C = -6.28\%\).
\(^{13}\)C-labeled zooplankton carcasses

A culture of *Daphnia magna* was fed with *Scenedesmus quadricauda* grown in modified Z-medium (Schlösser, 1994), containing \(^{13}\)C-labeled sodium bicarbonate (Sigma Aldrich, 98 atom % \(^{13}\)C). Just before starting the experiment, daphnids were killed by short exposure to 10% acetic acid and thereafter rinsed several times in ultrapure water (Tang *et al.*, 2006). Carcasses were manually divided into equal groups by numbers and size, and adjusted to even out the weights of individual groups before inoculation to reach a homogenous distribution of both carcasses mass and length between all HD and D microcosms. We selected the number of carcasses (20 per microcosm) to be as close as possible to natural values (Dubovskaya *et al.*, 2003). The carbon content of a subset of acid-killed daphnids was detected by a carbon analyzer Eltra SC 800 (Eltra, Germany) to estimate the surplus of carbon introduced by carcasses supply.

**DNA extraction**

Zirconium and glass beads of various diameter and 0.6 mL CTAB (cetyltrimethylammonium bromide) buffer were added to the samples. Then 60 µl of 10% sodium dodecyl sulfate (w:v), 60 µl of 10% N-Lauroylsarcosin (w:v), and 0.6 mL of pH-neutral phenol-chloroform-isoamylalcohol mixture (25:24:1, v:v:v) were added. Samples were homogenized on a vortexer for 10 min at highest speed and then centrifuged at 16000×g for 10 min at 4 °C. The aqueous phase was transferred into new reaction tubes, washed with 1 volume of chloroform-isoamylalcohol (24:1, v:v) and centrifuged at 16000×g for 10 min at 4 °C. Again, the aqueous phase was transferred into new reaction tubes and mixed with 2 volumes of 30% polyethylene glycol (w:v) in 1.6 M NaCl. After incubation for 1.5 h at 4 °C samples were centrifuged at 17000×g for 60 min at 4 °C. The supernatant was removed and the pellet was washed with 1 mL of ice-cold 70% ethanol. After centrifugation at 17000×g for 10 min the
supernatant was removed and the nucleic acid pellet was air-dried and finally dissolved in 50 µL ultra-pure water.

**Sequencing data processing**

Sequences with an average quality of < 25 over a 50 bp window, that were shorter than 300 bp or which contained ambiguities and homopolymer stretches of more than 8 bases were excluded from further analysis. Chimera check was performed using UCHIME (Edgar *et al.*, 2011). Taxonomy assignment of the OTUs was done using a naïve Bayesian classifier (Wang *et al.*, 2007) and the SILVA reference database v128 with a confidence threshold of 80%. All sequences classified as unknown, eukaryote, mitochondrion, chloroplast and archaea were subsequently removed. Sequences were then clustered into operational taxonomic units (OTU) using VSEARCH (Rognes *et al.*, 2016); as implemented in Mothur with a minimum sequence similarity value of 97% and global singleton sequences were removed.

**Carbon decomposition calculations**

The initial amount of carbon in D microcosms is presumed to be equal to the initial blank plus carbon of the added *Daphnia* carcasses 1.334 ± 0.038 mg L\(^{-1}\). Accordingly, the initial amount of carbon in the HD microcosms, TOC\(_i\) (HD), equals to the sum of total organic carbon in H microcosms, TOC\(_i\) (H), and carbon of the added carcasses. Total carbon degradation throughout the experiment was calculated in each microcosm as a difference between the final and initial TOC concentration:

\[
\Delta \text{TOC} = \text{TOC}_f - \text{TOC}_i \quad (\text{Eq. 1})
\]

Predicted total degradation of carbon in HD microcosms was computed as:

\[
\Delta \text{TOC}(H+D) = \Delta \text{TOC}(H) + \Delta \text{TOC}(D) - \Delta \text{TOC}(B) \quad (\text{Eq. 2})
\]

The carcass carbon fraction (F\(_c\)) in DOC and POC was calculated from the stable isotope mixing model (Hopkins and Ferguson, 2012):

\[
F_c = (d_\alpha - d_\beta)/(d_c - d_\beta) \quad (\text{Eq. 3})
\]
where $d_x$ is $\delta^{13}C$ ($\%$) of the DOC or POC at the end of the experiment, $d_c$ is $\delta^{13}C$ ($\%$) of $Daphnia$ carcasses and $d_h$ is $\delta^{13}C$ ($\%$) of humic matter.

**Carbon respiration calculations**

In parallel, we computed $^{13}CO_2$ respiration rate as a result of carcass carbon degradation in D microcosms, representing the difference between final concentrations of $^{13}CO_2$ in D and B microcosms:

$$^{13}\Delta CO_2(\text{carcasses}) = ^{13}CO_2(D) - ^{13}CO_2(B)$$  \hspace{1cm} (Eq. 4)

Similarly, the quantity of $^{13}CO_2$ respired from humic matter degradation in H microcosms was obtained from:

$$\Delta^{13}CO_2(\text{humics}) = ^{13}CO_2(H) - ^{13}CO_2(B)$$  \hspace{1cm} (Eq. 5)

Then, we predicted $^{13}CO_2$ concentration at the final point of the experiment if carcasses and humic matter were degraded together in one microcosm without any priming effect:

$$^{13}CO_2(H+D) = \Delta^{13}CO_2(\text{carcasses}) + \Delta^{13}CO_2(\text{humics}) + ^{13}CO_2(B) = ^{13}CO_2(D) + ^{13}CO_2(H) - ^{13}CO_2(B)$$  \hspace{1cm} (Eq. 6)

The same calculations (Eq. 3-5) were done for $^{12}CO_2$, and the predicted ratio $^{13}CO_2(H+D)/^{12}CO_2(H+D)$ was computed. The result was compared with the observed ratio $^{13}CO_2(\text{HD})/^{12}CO_2(\text{HD})$.

For calculating the carcass carbon fraction ($F_c$) in the respired $CO_2$ of the HD microcosms we used the stable isotope mixing model:

$$F_c = (d_c' - d_h)/(d_c - d_h)$$  \hspace{1cm} (Eq. 7)

where $d_c'$ is the $\delta^{13}C$ ($\%$) of the dissolved $CO_2$ at the end of the experiment, $d_c$ is $\delta^{13}C$ ($\%$) of respired $CO_2$ originating from $Daphnia$ carcasses, and $d_h$ is $\delta^{13}C$ ($\%$) of respired $CO_2$ originating from humic matter.
References


Supplementary Figure Legends

**Fig. S1** Experimental setup with blank controls (right, B), single-source incubations (middle, D and H), and mixed treatments (left, HD). Microcosms were 1L glass bottles half-filled with artificial lake water and inoculated with a natural bacterial community. Each type of microcosm is represented by five replicates.

**Fig. S2** Microscopic observations of *Daphnia magna* carcasses decomposition during the first 5 days of the experiment: a – Day 0; b – Day 1; c – Day 2; d – Day 3-5. After day 3, differences in individual carcasses conditions are noticeable. After day 5, all carcasses fell apart into pieces; e – DAPI-stained carcass colonized by bacterial cells at day 15.

**Fig. S3** Change in concentrations of $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ compared to the blank microcosm in the final points of experimental microcosms with different organic carbon sources: humic matter (H), *Daphnia* carcasses (D), humic matter and carcasses together (HD).
Table S1. OTUs contributing the most to dissimilarity between the experimental microcosms according to SIMPER and significantly different at P < 0.05 after Tukey’s test for one-way ANOVA. Treatments abbreviations: I – initial inoculum, B – blank microcosms with no carbon sources, H - with humic matter, D – with Daphnia carcasses, HD - humic matter and carcasses combined together.

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