1 **Effects of zooplankton carcasses degradation on freshwater bacterial community**

2 **composition and implications for carbon cycling**

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17 **Running title:** Effect of dead zooplankton on bacteria and C-cycle

Originality-Significance Statement

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.

48 **Introduction**

49   The global carbon cycle is one of the most important biogeochemical processes

50 regulating the climate on our planet (Ward *et al.*, 2013). In particular, carbon fluxes between

51 aquatic and terrestrial ecosystems constitute a key component of global biogeochemical

52 cycles (Pace *et al.*, 2004; Battin *et al.*, 2009; Ward *et al.*, 2013). Nowadays, it is well known

53 that a significant part of terrigenous organic matter drains from soils into aquatic ecosystems,

54 especially in the boreal zone (Vachon *et al.*, 2017). Freshwaters are considered hotspots of

55 organic matter degradation, sustaining a shorter half-life of organic carbon compared to

56 terrestrial and marine ecosystems (Catalán *et al.*, 2015). In freshwaters, organic matter

57 comprises a heterogeneous mixture of different carbon sources with varying degradability.

58 Depending on their degradability by aquatic microbes, the drained terrigenous organic matter

59 is buried to a variable extent in sediments of aquatic ecosystems (Tranvik *et al.*, 2009).

60 However, most of the terrigenous (allochthonous) organic carbon is transported into aquatic

61 ecosystems in the form of refractory organic matter (ROM) resulting in a generally higher

62 retention time due to its slow decomposition by aquatic microorganisms (Bianchi, 2011). A

63 major part of this ROM in freshwater ecosystems is represented by humic matter (Rocker *et*

64 *al.*, 2012a).

65   Bacterial species differ in their response to various sources of carbon resulting in

66 profound implications for aquatic carbon cycling. It has been demonstrated that the

67 availability of organic matter promotes growth of both generalist species, which are able to

68 degrade a wide range of substrates, as well as highly specialized populations degrading

69 specific substrate fractions (Hutalle-Schmelzer *et al.*, 2010). Dead zooplankton, which used to

70 be generally neglected in aquatic ecology due to methodological limitations (Tang *et al.*,

71 2009, 2014), is an overlooked and highly abundant source of labile carbon in most freshwater

72 ecosystems. Zooplankton carcasses represent a high quality organic substrate for

73 heterotrophic bacteria due to their relatively low C:N:P ratio as compared to phytoplankton

74 and detritus (Tang *et al.*, 2014). Consequently, zooplankton carcasses are "hot spots" of

75 activity of pelagic microorganisms consuming labile organic matter (LOM) as well as ROM

76 (Tang *et al.*, 2006; Grossart *et al.*, 2007; Elliott *et al.*, 2010; Kirillin *et al.*, 2012). However,

77 zooplankton carcasses provide not only a carbon source for microorganisms, but also surfaces

78 for attachment. Microorganisms attached to particles are situated in close spatial proximity

79 and can benefit from extracellular degradation enzymes released in the environment (Catalán

80 *et al.*, 2015). Thus, attached microorganisms have a higher capacity to degrade polymeric

81 organic matter than their free living counterparts (Grossart, 2010). Consequently, zooplankton

82 carcasses are selecting for specific, but yet uncharacterized microbial communities (Tang *et

83 al.*, 2010). The complex LOM of zooplankton carcasses constitutes a valuable source of

84 nutrients and energy for microorganisms, thus implying effects on aquatic carbon cycling, in

85 particular of the more refractory carbon pools. For instance, carcass LOM may induce a

86 "priming effect" and facilitate the degradation of ROM (Bianchi, 2011).

87   Thus, our primary objective was to investigate consequences on bacterial community

88 composition and carbon cycling in aquatic ecosystems after input of zooplankton carcasses.

89 Since the quality of available organic matter can be a selective force for bacterioplankton

90 community composition (Gómez-Consarnau *et al.*, 2012), we tested the hypothesis that

91 nutrient-rich LOM provided by *Daphnia* carcasses selects for generalist bacteria in contrast to

92 C-rich ROM selecting to a larger extent for specialists. In a microcosms experiment, we

93 observed the degradation of ^{13}C-labeled carcasses by heterotrophic bacteria from a dystrophic

94 humic bog lake in the presence of indigenous humic matter (treatment **HD**) to track the fate of

95 carcass carbon (Fig. S1). In parallel, we followed three control treatments with either *Daphnia*

96 carcasses (**D**) or humic matter (**H**) as a sole carbon source, and a blank treatment (**B**)

97 containing solely a natural bacterial community. We used optical properties (specific UV

98 absorbance at 254 nm – SUVA$_{254}$, humification index, etc.) and size exclusion

99   chromatography to analyze the influence of carcasses on the dissolved organic matter (DOM)

100 pool and combined it with 16S rRNA gene Illumina amplicon sequencing to characterize the

101 bacterial community composition in detail.

Results

103 **Microbial dynamics and community composition**

104 *Daphnia* carcasses showed a rapid decomposition during the first week of incubation,

105 with visible changes in the state of carcasses over time (Fig. S2a-d). At the end of the

106 experiment, the carcasses were still visible as disintegrated parts of the carapace. Dense

107 bacterial colonization of the carcasses was observed (Fig. S2e), while protist grazers or

108 autotrophic organisms were not detected indicating that protozoan grazers and large

109 phytoplankton have been successfully removed by the pre-filtration step. No differences in

110 bacterial counts were observed in the **B** microcosms between the start and the end of the

111 experiment (Table 1). However, a clear increase in bacterial cells counts was observed in **H**,

112 **D** and **HD** microcosms (43%, 654% and 617%, respectively; Table 1). This indicated a higher

113 bacterial growth in the presence of *Daphnia* carcasses and a slower growth on humic matter

114 alone. At the same time, bacterial cell counts did not significantly differ between **HD** and **D**

115 microcosms (paired t-test, $p > 0.05$, Table 1).

116 After sequencing and performing a quality check for all samples, 595036 reads of 16S

117 rRNA gene fragments were obtained that clustered into 1161 bacterial operational taxonomic

118 units (OTUs). The identified OTUs belonged to 26 known phyla (Fig. 1). In the initial

119 bacterial inoculum from Lake Grosse Fuchskuhle, *Proteobacteria* was the dominant phylum

120 (48% of all sequences), with a high proportion of the class *Betaproteobacteria* (30% of all

121 sequences). By the end of the experiment, the relative abundance of *Proteobacteria* increased

122   in all treatments, especially in carcasses amended microcosms (**HD** and **D**; Fig. 1), with a

123   dominance of the class *Gammaproteobacteria* (62 % and 50 % in **HD** and **D** respectively).

124   At the end of the experiment, the microcosms **HD** and **D** had a lower OTU richness

125   and evenness compared to microcosms without added carcasses (**H** and **B**; Table 2).

126   Microcosms **H** had lower species richness than **B** microcosms, but showed a higher evenness

127   (Table 2).

128   In an unconstrained ordination (Fig. 2), all treatments were distinguishable from one

129   another and from the initial inoculum (ANOSIM, R = 0.835, p = 0.001). The OTUs

130   accounting for most of the difference between the treatments were identified using a SIMPER

131   test (Table 3). The relative abundance of the most influential OTU (OTU1, *Pseudomonas* sp.)

132   was significantly different between the start and the end of the incubation for all treatments

133   (ANOVA $F_{4,15}$ = 102.89, p < 0.001). The distribution of different OTUs in all treatments is

134   discussed in more details in the Supplementary Information.

135   **DOM composition and fate of carcass carbon**

136   We aimed to test for the "priming effect" by comparing the predicted total organic

137   carbon degradation rate (ΔTOC) in **HD** microcosms, calculated from ΔTOC in the control

138   microcosms **D**, **H**, and **B**, to the measured ΔTOC in **HD** microcosms (see Supplementary

139   Methods for more details). The predicted ΔTOC was -1.015 ± 0.115 mg L^{-1} and did not differ

140   significantly (paired t-test, p > 0.05) from the ΔTOC measured in **HD** microcosms (-1.094 ±

141   0.057 mg L^{-1}).

142   During carcass decomposition (in **HD** and **D** microcosms only), the particulate organic

143   carbon (POC) decreased approximately four-fold compared to the initial values (Table 1).

144   Nevertheless, no significant differences in concentrations of dissolved organic carbon (DOC),

145   high- and low-molecular weight substances (HMWS and LMWS, respectively) or humic

146   substances were found throughout the experiment in any treatment (paired t-tests, p > 0.05 in

147   all tests, Table 1). However, in ROM supplemented microcosms (**HD** and **H)** we observed

148   trends of decreasing concentrations in polysaccharides, amphiphilic molecules, and building

149   blocks of humic substances (Table 1).

150   The $SUVA_{254}$, spectral slopes and optical indices values were not different between

151   the humic-amended microcosms **H** and **HD** (Table 1), and they did not differ between **D** and

152   **B** microcosms (Table 1). An exception was the freshness index, being an indicator for

153   recently produced DOM (Hansen *et al.*, 2016), which was lower in **D** compared to **B**

154   microcosms. Expectedly, the ratio of peakA to peakT, which is known as an indicator of the

155   ratio of humic-like (recalcitrant) to freshly produced (labile) organic matter (Hansen *et al.*,

156   2016), was higher in **H** microcosms compared to **HD** (Wilcoxon test, p-value = 0.03).

157   The carcasses had an average $^{13}C/^{12}C$ ratio of 0.168 ± 0.004 ($\delta^{13}C$ = 13945.3 ±

158   303.9‰). According to this specific signature, the amount of processed carbon originating

159   from the carcasses was computed in all carcass-containing microcosms (i.e. **HD** and **D**). In

160   **HD** microcosms, 72.8% of POC and 2.2% of DOC originated from *Daphnia* carcasses,

161   against 82.1% of POC and 21.1% of DOC for the **D** microcosms (Fig. 3).

162   The bacterial respiration, measured as the increase in CO_2 and normalized to the

163   background respiration (i.e. respiration from the blank microcosms **B**) was higher in **D**

164   microcosms compared to **H** but lower compared to **HD** microcosms (Fig. S3). The respiration

165   per amount of initially added carbon and normalized to the background respiration was higher

166   in **HD** microcosms compared to **H** but lower compared to **D** microcosms, and was used to

167   confirm the labile character of the organic matter originating from the zooplankton carcasses.

168   All differences in CO_2 concentrations between the microcosms were significant (1-way

169   ANOVA F = 241.6, p < 0.001; Tukey post hoc test p < 0.01 for all pairs).

170   To test for the priming effect, the predicted $^{13}C/^{12}C$ ratio in the respired CO_2 of **HD**

171   microcosms was calculated from the values in the control microcosms **D**, **H**, and **B**, and

172   compared to the measured $^{13}CO_2/^{12}CO_2$ ratio in **HD** microcosms (see Supplementary Methods

173 for more details). The predicted $^{13}CO_2/^{12}CO_2$ ratio for **HD** (0.078 ± 0.003) did not differ

174 significantly (paired t-test, $p > 0.05$) from the measured value (0.073 ± 0.003). In **HD**

175 microcosms, 87.8 ± 6.3 % of respired CO_2 originated from zooplankton carcasses when

176 normalized to the background respiration of microcosms **B.**

177 **Interactions between microbial community and DOC quality**

178 The interactions between bacterial community composition and DOC quality in

179 microcosms **HD**, **H**, and **D** revealed specific patterns in bacterial substrate preferences (Fig.

180 4). The connections between bacterial genera and DOC qualities significantly differed

181 between microcosms **H** and **D** (Fig. 4a). Thus, bacteria positively interacting with DOC

182 concentration as well as humification and fluorescence indices are the ones thriving in the

183 presence of humic matter (Fig. 4a). On the contrary, bacteria negatively associated with these

184 parameters are favored by carcasses (Fig. 4a). Similarly, genera positively correlated with

185 DOC concentration, fluorescence index and A/T peak ratio (Fig. 4b, comparing microcosms

186 **HD** and **D**) seem to be favored by humic matter when *Daphnia* carcasses are available.

187 However, those genera negatively correlated with these parameters are suppressed by humic

188 matter in the presence of carcasses.

189 **Discussion**

190 The main objective of this study was to track the degradation of zooplankton

191 carcasses, as a so far largely neglected but common and labile carbon source (Tang *et al.*,

192 2014). Our study reveals selection of defined bacterial communities in the presence of

193 carcasses strongly related to the specific DOM quality released from carcasses. However,

194 carcass-induced availability of LOM and related shifts in bacterial community composition

195 did not result in significant changes in the turnover of the added ROM pool. Consequently,

196 our data do not support a "priming effect" (Bianchi *et al.*, 2011) of refractory humic matter

197 removal in the presence of relatively labile carbon from *Daphnia* carcasses. Nevertheless, the

198 bacterial community composition was greatly affected by the presence of carcass carbon.

199 Thus, our study adds new quantitative and qualitative data on bacterial carbon utilization

200 related to changes in the community composition induced by changes in substrate quality, i.e.

201 addition of zooplankton carcasses, and adds new insights in microbial-organic matter

202 interactions.

Bacterial community composition depending on carbon source

204 As outlined above, we did not measure any quantitative changes in organic matter

205 degradation between **HD** microcosms and the predicted values calculated based on the

206 parameters of single-carbon source microcosms **H** and **D**. Consequently, the presence of

207 LOM from *Daphnia* carcasses did not change the degradation of humic matter but rather

208 influenced the bacterial community.

209 According to our data on beta-diversity of bacterial assemblages (Fig. 2), the type of

210 treatment strongly affected the bacterial community composition in each microcosm. In

211 microcosms with no extra organic matter addition (**B**), the bacterial community remained

212 similar to the initial inoculum indicating that experimental changes in environmental

213 conditions did not modify the bacterial community composition drastically (Fig. 2). Overall,

214 the addition of carcass LOM was the main driver of the bacterial community composition in

215 the **D** and **HD** microcosms (Fig. 2). Bacterial taxa introduced into the microcosms with the

216 carcasses may also have an effect on bacterial community composition and richness.

217 However, according to literature data, zooplankton carcasses are not primarily decomposed by

218 their native-associated bacterial communities, but rather by ambient bacteria (Bickel and

219 Tang, 2010). This notion is also reflected by the fact that bacteria richness is the lowest in the

220 **HD** and **D** treatments. Thus, in natural waters with a high amount of dead zooplankton,

221 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

271   present study about half of all sequences in the **HD** and **D** microcosms belonged to a single

272   OTU (OTU 1, *Pseudomonas sp.*). In a similar study of Blanchet et al. (2017), the bacterial

273   diversity was not affected by amino acid additions, possibly because free amino acids are

274   simple compounds which can be consumed simultaneously by many members of the

275   community (Trusova et al., 2012). Thus, this pattern can be best explained by the fact that

276   zooplankton carcasses provide microbial habitats and complex, yet labile carbon sources

277   shifting the overall bacterial community towards a less diverse, more uneven, and more

278   copiotrophic community.

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

280   An abrupt increase of LOM availability following mass zooplankton mortality

281   commonly observed in natural waters (reviewed by Tang et al. 2014), leads to a substantial

282   input of both DOC and POC. In agreement with a previous study (Tang *et al.*, 2006) *Daphnia*

283   carcasses lost their mostly labile internal tissues rapidly, whereas the chitin-based carapace

284   was more resistant to dissolution and microbial decomposition (Fig S2).

285   However, in our study the leached fraction was rapidly consumed by numerous

286   ambient bacteria (Table 1), and did not increase the DOC concentration significantly in the

287   microcosms with zooplankton carcasses (**D** and **HD**). This statement is supported by our

288   observation that adding 1.334 ± 0.038 mg C L^{-1} with *Daphnia* carcasses resulted in only 0.055

289   ± 0.011 and 0.065 ± 0.010 mg C L^{-1} of carcasses-derived DOC in **D** and **HD** microcosms,

290   respectively (Fig. 3).

291   On the other hand, the chitin-based structure of the carapace was only partially

292   degraded and also used a surface for attachment. At the end of the experiment, 0.270 ± 0.014

293   and 0.258 ± 0.007 mg C L^{-1} originating from *Daphnia* carcasses (19-20% of the initial

294   quantity) remained in the POC fraction of the **D** and **HD** microcosms, respectively (Fig. 3),

295   mainly represented by the remaining carapace and the bacterial biomass (Fig. S2). This is

296 further confirmed by the finding that bacterial taxa degrading chitin were greatly favored in

297 the presence of carcasses at the end of the incubation (Fig. 4). The difference between non-

298 carcass-derived POC in microcosms **D** and **HD** (0.059 ± 0.003 and 0.096 ± 0.003 mg L^{-1},

299 respectively; Fig. 3) presumably occur due to humic matter aggregation converting DOC into

300 POC. This assumption is supported by a two-fold POC increase in the **H** microcosms

301 compared to the initial value (Table 1).

302 In our experiment we used 40 *Daphnia* carcasses per liter, a high but still natural value

303 (Dubovskaya *et al.*, 2003). Most of the organic carbon originating from carcasses was

304 respired by the bacterial community within the two weeks of incubation. Therefore, in natural

305 systems, the considerable amount of LOM released by zooplankton carcasses (Tang *et al.*,

306 2014), can directly affect the functioning of the ecosystem by accelerating microbial carbon

307 turnover and respiratory carbon losses to the atmosphere at short time scales. The more

308 recalcitrant part of the carapace may persist for a longer time, and eventually escapes the

309 water column to be further processed in the sediments (Tang *et al.*, 2014), being also

310 important for carbon sequestration. Consequently, the balance between microbial degradation

311 of zooplankton carcasses and organic matter storage in sediments has a great influence on the

312 aquatic carbon cycle.

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

314 Humic matter was chosen as a recalcitrant carbon source for its ubiquity in aquatic

315 ecosystems and as an important part of the carbon pool in the global carbon cycle. Humic

316 matter can represent up to 80% of the total DOM in freshwaters (Rocker *et al.*, 2012a).

317 Although humic matter is considered as recalcitrant, it can at least partially be decomposed by

318 bacteria (Hutalle-Schmelzer *et al.,* 2010; Rocker *et al.*, 2012a; Kisand *et al.*, 2013).

319 Furthermore, the degradation of humic acids by marine and estuarine bacterial communities

320 seems to be favored by specific environmental conditions, e.g. along a salinity gradient

321 (Rocker *et al.*, 2012a; Rocker *et al.*, 2012b; Kisand *et al.*, 2013). Consequently, recalcitrance

322 and lability of organic matter are not *per se* intrinsic chemical characteristics (Schmidt *et al.*,

323 2011), and may only account for specific environmental settings (Bianchi *et al.*, 2015).

324   In our experiment, we incubated *Daphnia* carcasses and humic matter in different

325 combinations to test for a priming effect of bacterial degradation of ROM induced by the

326 addition of carcass LOM. The addition of a mixture of natural humic matter and *Daphnia*

327 carcasses resulted in the degradation of organic matter and an isotopic ratio of the respired

328 CO_2 similar to what we predicted based on our linear addition model with humic matter or

329 carcasses as the sole carbon source. Moreover, based on DOM characterization (Table 1), the

330 natural humic matter from Lake Grosse Fuchskuhle was only little degraded by bacteria,

331 irrespective of LOM addition via *Daphnia* carcasses. Consequently, and in agreement with

332 previous studies (Bengtsson *et al.*, 2014; Catalán *et al.*, 2015; Dorado-García *et al.*, 2015), we

333 could not detect any quantitative changes in bacterial ROM degradation when using carcass

334 LOM as a potential primer (Table 1).

335   Recently, a number of studies have investigated the prevalence of a priming effect in

336 aquatic ecosystems (e.g., van Nugteren *et al.*, 2009; Guenet *et al.*, 2013; Kuehn *et al.*, 2014;

337 Steen *et al.*, 2015). Various types of ecosystems (marine, lentic, lotic) and habitats (pelagic,

338 hyporheic, sediments) have been tested, as well as different sources of LOM (carbohydrates,

339 algae leachate, gastropod mucus, etc.) and ROM (terrestrial plant tissues, lignocellulose,

340 humic matter, etc.) have been used. Although some authors have found support for ROM

341 priming by more labile organic matter, mainly of algal origin (van Nugteren *et al.*, 2009;

342 Guenet *et al.*, 2013; Hotchkiss *et al.*, 2014; Bianchi *et al.*, 2015; Gontikaki *et al.*, 2015),

343 others did not reveal any evidence for a positive priming effect (Bengtsson *et al.*, 2014;

344 Catalán *et al.*, 2015; Dorado-García *et al.*, 2015; Blanchet *et al.*, 2017), or even found a

345 negative priming effect (Gontikaki *et al.*, 2013) with ROM being decomposed slower in the

346 presence of a labile carbon source. Thus, it appears that the absence or presence of the

347   priming effect may strongly depend on specific environmental or experimental conditions,

348   which may also explain the absence of humic matter degradation in our HD treatments using

349   *Daphnia* carcasses LOM as the primer.

Limitations and applications of the study

351   The degradation of ROM (such as humic matter) is a combination of two main

352   processes: microbial and photochemical decomposition (Amado *et al.*, 2015). The most

353   efficient humic matter microbial degraders in aquatic systems are fungi (Grinhut *et al.*, 2007).

354   Generally, fungi have a higher capacity then bacteria to synthesize the extracellular oxidative

355   enzymes involved in ROM degradation and thus more readily and successfully initiate humic

356   matter degradation (Rojas-Jimenez *et al.*, 2017). In contrast, bacteria join the process later as

357   degraders of humic matter metabolites (Grossart and Rojas-Jimenez, 2016; Rojas-Jimenez *et*

358   *al.*, 2017). Due to our pre-filtration step to avoid the presence of protozoan grazers and large

359   phytoplankton as has been frequently done in similar incubation experiments (Fonte *et al.*,

360   2013; Guenet *et al.*, 2013; Attermeyer *et al.*, 2014, 2015; Blanchet *et al.*, 2015), fungi, which

361   could potentially constitute an important component of the aquatic priming effect, were

362   removed.

363   Photochemical degradation can break down/oxidise recalcitrant DOM compounds,

364   such as fulvic and humic substances, into more labile molecules (Spencer *et al.*, 2009;

365   Stubbins *et al.*, 2010). It is likely that in natural systems photochemical and microbial

366   degradation work synergistically and thus contribute to the priming effect. On the other hand,

367   UV radiation can also affect biodegradability of LOM in the presence of humic matter

368   (Tranvik and Kokalj, 1998). Our study was conducted in the dark as in other priming effect

369   studies (e.g. Bianchi *et al.,* 2015; Catalán *et al.*, 2015) to avoid photosynthesis which could

370   have obstructed the detection of differences in carbon oxidation between treatments and

371   controls. Thus, it is clear that photodegradation of humic substances was not taken into

372 account in the present study and might have reduced our possibilities to measure a positive

373 priming effect. However, our study is directly applicable to the situation when the

374 zooplankton carcasses are sinking into the pelagic zone at depths with very low light

375 penetration.

376 The incubation temperature in our experiment was relatively high (20°C) and not

377 typical for deep waters. Nevertheless, if temperature might have a strong influence on

378 bacterial community composition and its activity (Adams *et al.*, 2010), a lower temperature

379 only slows down the degradation process but not the biodegradability of carcasses. Therefore,

380 in natural systems, the potential of zooplankton carcasses to release a consequential amount of

381 carbon in the atmosphere and to select for specific bacterial community might happen over

382 longer time scales than the ones observed in our experiment.

383 Another factor that can affect DOM degradation is the oxygen concentration, which

384 usually decreases sharply with depth in the pelagic zone of humic lakes. In our experimental

385 microcosms we maintained 100% oxygen saturation due to mixing with headspace air.

386 However, Tang *et al.* (2006) observed only a small difference between microbial communities

387 decomposing carcasses in anaerobic vs. aerobic conditions, presumably because zooplankton

388 carcasses represent anoxic microenvironments even when oxygen is abundant in the

389 surrounding waters (Glud *et al.*, 2015).

390 Although the experimental set-up might have its limitations, our findings are relevant

391 to natural environments such as the metalimnion of humic lakes and other deep water layers

392 where the maximum percentage of dead zooplankton is found (Dubovskaya *et al.*, 2018).

393 **Conclusions**

394 We observed a pronounced change in bacterial community composition in microcosms

395 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).

419   One set of microcosms (**HD**) was amended with humic matter and ^{13}C-labeled

420   *Daphnia* carcasses (Fig. S1). The detailed description of the amendments including their

421   preparation is available in the Supplementary Methods. Carcasses control microcosms (**D**)

422   were amended solely with ^{13}C-labeled *Daphnia* carcasses in the same quantity and from the

423   same batch of *Daphnia* as in **HD** microcosms. Humic matter control microcosms (**H**) were

424   amended with humic matter only in the same quantity as in **HD** microcosms (Fig. S1).

425   Further, blank microcosms (**B**) with no added organic matter were included to determine

426   whether the bacterial community was capable of growing without the extra addition of

427   organic carbon (Fig. S1). Each experimental treatment was conducted in five replicates.

Bacterial counts and community composition

429   Microbial abundances were determined after filtration of 5 mL of water through

430   Nucleopore track-etched membrane filters with a pore size of 0.2 μm (Whatman, UK). Then,

431   samples were stained with 4',6-diamidino-2-phenylindole (DAPI) to monitor cell numbers

432   using an imaging system linked to a Leica epifluorescence microscope. Pictures were taken

433   from 30-50 fields and abundances were determined using the CellC software (Tampere

434   University of Technology, Finland, https://sites.google.com/site/cellcsoftware/).

435   For DNA extraction, 150 mL of water was filtered through 0.22 μm GVWP filters

436   (Millipore, Germany) and total DNA was extracted according to a modified protocol

437   described by Nercessian *et al.* (2005) (see Supplementary Methods for details). DNA

438   concentrations were determined with a Quantus fluorometer (Promega, USA), following the

439   manufacturer's instructions. PCR, library preparation and sequencing was done by LGC

440   Genomics (Berlin, Germany). Briefly, the V3-V4 region was amplified using primers 341F-

441   785R (Klindworth *et al.*, 2013), followed by library preparation (2x300 bp) and sequencing

442   on a MiSeq Illumina platform. Sequences were quality checked and analyzed using Mothur

443   v1.37.6 (Schloss *et al.*, 2009), see Supplementary Methods for the detailed workflow. The

444   sequence data was deposited in Genbank under the following accession number:

445   PRJNA418906.

Organic carbon concentration and composition

447   Directly after sampling, water was passed through pre-combusted (4 h, 450°C) GF75

448   filters (Advantec, nominal pore size of 0.3 μm). Particulate organic carbon (POC) collected

449   on the filters was measured with an Eltra SC 800 (Eltra, Germany). One subsample of filtrate

450   was processed directly to measure the DOC concentration with a TOC-V$_{CPH}$ (Shimadzu,

451   Kyoto, Japan) as well as the phosphate concentration using a FIAstar 5000 (Foss, USA). Total

452   organic carbon (TOC) concentration was estimated by summing up the DOC and POC

453   concentrations.

454   Another subsample of filtrate was stored at 4°C for three weeks prior analysis with

455   liquid chromatography - organic carbon detection – organic nitrogen detection (LC-OCD-

456   OND, DOC Labor, Germany). The LC-OCD-OND allows distinguishing between HMWS,

457   LMWS, and humic substances (Huber *et al.*, 2011). Nevertheless, its sensitivity is not

458   sufficient for analyzing samples with low DOC concentration (<0.2 mg L^{-1}). That was the

459   case for the D and B microcosms which were therefore not analyzed by LC-OCD-OND.

Spectral characteristics of DOM

461   DOM optical characteristics were obtained with a UV-Vis spectrophotometer (Hitachi

462   U2900, Germany) and a spectrofluorometer (Hitachi F7000, Germany). Absorbance spectra

463   were recorded from 190 to 800 nm with an increment of 1 nm and used to compute the

464   specific ultraviolet absorbance at 254 nm (SUVA$_{254}$) and absorption spectral slopes (Weishaar

465   *et al.*, 2003; Helms *et al.*, 2008). SUVA$_{254}$ is an indicator of aromaticity and chemical

466   reactivity while the absorption slopes are used as proxies for DOM molecular weight.

467   Excitation emission matrices (EEMs) were generated with excitation wavelengths

468   ranging from 220 to 450 nm and emission wavelengths ranging from 230 to 600 nm, both

469   with 5 nm increments. EEMs were corrected with a MilliQ water sample and for inner filter

470   effect using the absorbance-based method (Christmann *et al.*, 1980; Murphy *et al.*, 2013).

471   Then, fluorescence, humification and freshness indices, as well as specific fluorescence

472   intensity at various peaks were calculated as described by Hansen *et al.* (2016).

473   **Carbon stable isotope ratio**

474   Stable isotope analysis of the respired CO_2 provides information on carbon substrates

475   metabolized by the microbial community (Fabian *et al.*, 2017). The ratio of $^{13}C/^{12}C$ in the

476   dissolved CO_2 was measured directly in each microcosm by a membrane-inlet mass-

477   spectrometer dissolved gas analyzer (HiCube pumping station, Pfeiffer Vacuum and Bay

478   Instruments membrane, USA), controlled by Quickdata software. The sampling capillary was

479   inserted through the PTFE-coated silicone septa prior to opening microcosms in order to

480   avoid gas leakages. Concentrations of $^{12}CO_2$ and $^{13}CO_2$ were obtained from the ion currents

481   sequentially recorded at mass to charge (m/z) ratios of 44 and 45, respectively.

482   For the carbon stable isotope ratio in POC and DOC, 100 ml of water was passed

483   through GF75 filters (Advantec, USA) and both the flow-through and the filter (all five

484   replicates were pooled onto one filter to have enough material for subsequent analyzes) were

485   collected and freeze-dried. Moreover, we dried 1 mg of acid-killed *Daphnia* and

486   approximately 1 mg of extracted humic matter for carbon stable isotope ratio analysis. The

487   samples were analyzed with an Elemental Analyzer (Thermo Flash EA 2000), coupled to a

488   continuous-flow isotope ratio mass spectrometer (Thermo Finnigan Delta V) via an open split

489   interface (Thermo Finnigan Conflow IV) in the IRMS Laboratory of the Leibniz Institute for

490   Baltic Sea Research Warnemünde (Germany), and with a PDZ Europa ANCA-GSL elemental

491   analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd.,

492   Cheshire, UK) at the UC Davis Stable Isotope Facility (USA).

Decomposition calculations

494   To test for a possible priming effect, we used an addition model as in Hannides and

495   Aller (2016). Initial and final total organic carbon concentrations in the humic control, i.e.

496   TOC_i (H) and TOC_f (H), respectively, in the carcass control, i.e. TOC_i (D) and TOC_f (D), and

497   in the blank, i.e. TOC_i (B) and TOC_f (B), respectively, were used to predict the degradation of

498   total organic carbon (ΔTOC) in the **HD** microcosm. Thereby, we assumed that the

499   degradation rates of carbon sources observed in the control microcosms are conserved when

500   humic matter and carcasses are incubated together. The predicted ΔTOC(H+D) was compared

501   with the measured ΔTOC(HD) to detect any enhanced degradation of organic carbon caused

502   by a potential "priming effect". Similarly, data on CO_2 concentration and isotope ratio

503   ($^{13}CO_2/^{12}CO_2$) in the microcosms was used to calculate respiration of recalcitrant and labile

504   carbon pools. We assumed that if a priming effect is present, there should be a difference in

505   the observed isotope ratio between measured and predicted CO_2, based on the sum of control

506   values.

507   The carcass carbon fraction (F_c) in DOC, POC and CO_2 was calculated via a stable

508   isotope mixing model (Hopkins and Ferguson, 2012). We also applied Keeling plot analyses

509   of dissolved CO_2 (Pataki *et al.*, 2003) to estimate the $\delta^{13}C$ of the carbon source respired in the

510   respective microcosms. Descriptive calculation formulas are available in the Supplementary

511   Methods.

Statistical analyses

513   For alpha-diversity calculations of bacterial communities, individual samples were

514   rarefied to the lowest number of reads in a sample (10080), with 10 iterations per sample

515   using QIIME (Caporaso *et al.*, 2010). Shannon biodiversity index was calculated to estimate

516   richness and evenness of the microcosm communities. Beta-diversity of bacterial

517   communities was calculated by using the Bray-Curtis similarity coefficients (Bray and Curtis,

518   1957) using QIIME.

519   All analyses described below were run via R version 3.3.3 (R Development Core

520   Team, 2006). R package *vegan* (vegan: Community Ecology Package, 2017, https://CRAN.R-

521   project.org/package=vegan) was used to carry out principal coordinate analysis (PCoA) and to

522   calculate ANOSIM and SIMPER (Clarke, 1993).

523   Bacterial community composition was linked to DOC characteristics which

524   significantly differentiated microcosms. We used the least absolute shrinkage and selection

525   operator (LASSO) to select genera that were influenced by a variation in DOM composition

526   (Traving *et al.*, 2016). Before designing the LASSO models, we summed the abundances of

527   OTUs belonging to the same genus and performed a centered log-ratio transformation (Gloor

528   and Reid, 2016) of the absolute abundances of all genera. To visualize the outcome, we

529   depicted the selected genera in two different networks using Cytoscape software (Shannon *et*

530   *al.*, 2003).

531   All differences between treatments were tested using a one-way analysis of variance

532   (ANOVA) and post-hoc Tukey tests. Pairwise comparisons between initial and final

533   parameters of microcosms and between two treatments were done using Student's t-test and

534   Wilcoxon rank sum test. Normality was checked by Shapiro–Wilks tests when necessary.

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810   **Table and Figure legends**

811   **Table 1.** Chemical and optical parameters of experimental microcosms varying in

812   carbon sources: B_i – blank initial, B_f – blank final, H_i – humic matter initial, H_f – humic

813   matter final, D_i – carcasses initial, D_f – carcasses final, HD_i – humic matter + carcasses initial,

814   HD_f – humic matter + carcasses final. Parameters in D_i and HD_i were not measured directly

815   but calculated from parameters in B_i and H_i. Values are given as means of five replicates ±

816   standard errors, except for the initial DNA concentration values obtained from a single

817   measurement. nd = not determined.

818   **Table 2.** Alpha-diversity of bacterial communities in the microcosms with additions of

819   humic matter (**H**), *Daphnia* carcasses (**D**), humic matter and carcasses together (**HD**), and

820   blank with no carbon sources (**B**). Values are given as means of five replicates ± standard

821   errors, except for the initial inoculate (**I**) obtained from a single measurement, and **B**

822   microcosms (four replicates due to too low DNA content in one sample).

823   **Table 3**. Abundances (in %) of the quantitatively prominent OTUs contributing the

824   most to the dissimilarity among the inoculum sample (**I**) and the end points of the microcosms

825   with additions of humic matter (**H**), *Daphnia* carcasses (**D**), humic matter and carcasses

826   together (**HD**), and blank with no carbon sources (**B**). Values are given as means of five

827   replicates ± standard errors, except for the inoculum sample obtained from a single

828   measurement, and **B** microcosms (four replicates due to low DNA content of two samples

829   which were pooled).

830   **Fig. 1** Relative abundance of major bacterial phyla and classes of Proteobacteria in the

831   initial sample (**I**) and the end points of microcosms with additions of humic matter (**H**),

832   *Daphnia* carcasses (**D**), humic matter and carcasses together (**HD**), and blank with no carbon

833   sources (**B**).

834   **Fig. 2** Principal coordinate analysis of the initial sample (I) and the end points of

835   microcosms with additions of humic matter (**H**), *Daphnia* carcasses (**D**), humic matter and

836   carcasses together (**HD**), and blank with no carbon sources (**B**), based on Bray-Curtis

837   community similarity, calculated as relative abundance of operational taxonomic units

838   (OTUs).

839   **Fig. 3** Carbon fractions originated from *Daphnia* carcasses at the end of the

840   experiment in pools of dissolved (DOC) and particulate organic carbon (POC) as well as in

841 dissolved CO_2 in microcosms with humic matter and *Daphnia* carcasses (**HD**) and carcasses

842 only (**D**). The area size of diagrams is relative to carbon concentration.

843   **Fig. 4** Network of bacterial genera connected with major DOM parameters,

844 significantly different in pairs of microcosms: a – microcosms **H** – humic matter, and **D** –

845 carcasses; b – microcosms **HD** – humic matter + carcasses, and **D** – carcasses. Nodes that

846 interact positively are connected by solid black edges, nodes connected by dashed edges have

847 negative interactions. Legend: DOC – concentration of DOC; HIX – humification index, FIX

848 – fluorescence index, fresh – freshness index, SUVA254 – Specific UV absorbance at 254

849 nm, A/T – ratio between the specific fluorescence at peak A (excitation 240-260 nm/emission

850 400-500 nm, UVC humic-like fluorescent component) and peak T (excitation 270-285

851 nm/emission 340-380 nm, tryptophan-like fluorescent component).