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1 **Leaf litter diversity and structure of microbial decomposer**  
2 **communities modulate litter decomposition in aquatic systems**

3

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**18 Abstract**

- 19 1. Leaf litter decomposition is a major ecosystem process that can link aquatic to  
20 terrestrial ecosystems by flows of nutrients. Biodiversity and ecosystem  
21 functioning research hypothesizes that the global loss of species leads to  
22 impaired decomposition rates and thus to slower recycling of nutrients.  
23 Especially in aquatic systems an understanding of diversity effects on litter  
24 decomposition is still incomplete.
- 25 2. Here we conducted an experiment to test two main factors associated with  
26 global species loss that might influence leaf litter decomposition. Firstly, we  
27 tested whether mixing different leaf species alters litter decomposition rates  
28 compared to decomposition of these species in monoculture. Secondly, we  
29 tested the effect of the size structure of a lotic decomposer community on  
30 decomposition rates.
- 31 3. Overall, leaf litter identity strongly affected decomposition rates, and the  
32 observed decomposition rates matched measures of metabolic activity and  
33 microbial abundances. While we found some evidence of a positive leaf litter  
34 diversity effect on decomposition, this effect was not coherent across all litter  
35 combinations and the effect was generally additive and not synergistic.
- 36 4. Microbial communities, with a reduced functional and trophic complexity,  
37 showed a small but significant overall reduction in decomposition rates  
38 compared to communities with the naturally complete functional and trophic  
39 complexity, highlighting the importance of a complete microbial community on  
40 ecosystem functioning.

41        5. Our results suggest that top-down diversity effects of the decomposer  
42            community on litter decomposition in aquatic systems are of comparable  
43            importance as bottom-up diversity effects of primary producers.

44    **Key words:** *Alnus glutinosa*, Biodiversity ecosystem functioning, *Fagus sylvatica*,  
45    microcosm experiment, *Populus nigra*, protists, *Quercus robur*.

46

## 47    **Introduction**

48            Litter decomposition is a major process in nutrient recycling and plays an  
49    important role in the functioning of ecosystems (Hättenschwiler, Tiunov & Scheu  
50    2005; Findlay 2012; Handa *et al.* 2014; García-Palacios *et al.* 2016; Bista *et al.* 2017).  
51    Plant detritus not only forms the vast majority of the dead organic matter pool in  
52    terrestrial systems, but is also an important source of energy in aquatic systems  
53    (Anderson & Sedell 1979). In aquatic systems, dead organic matter from plants can be  
54    generated *in situ* by aquatic vascular plants (i.e., autochthonous litter). However, *ex situ*  
55    (allochthonous) litter from tree leaves is often the more important source of organic  
56    matter (Fisher & Likens 1973; Gessner, Chauvet & Dobson 1999). Thereby, the  
57    surrounding terrestrial vegetation strongly affects both the composition and quantity of  
58    leaf litter input into aquatic systems (e.g., Hladysz *et al.* 2010; Hladysz *et al.* 2011), and  
59    such flows can even generate non-trivial linkages between ecosystems (Loreau,  
60    Mouquet & Holt 2003; Gravel *et al.* 2010; Harvey *et al.* 2016, 2017, Gounand *et al.*,  
61    2017).

62            Recent work demonstrated that the decomposition of litter in lotic aquatic  
63    systems can be modulated by various factors related to litter type, decomposer and  
64    detritivore community type and general abiotic conditions (e.g., Lecerf *et al.* 2007;  
65    Woodward *et al.* 2012; Bruder *et al.* 2014; Frainer *et al.* 2015; Collins *et al.* 2016;

66 Stocker *et al.* 2017). As all of these main drivers of litter decomposition are affected by  
67 various environmental changes (e.g., Boyero *et al.* 2011; Frossard *et al.* 2013; Hines *et*  
68 *al.* 2014), understanding their independent and interactive effects on leaf litter  
69 decomposition and nutrient turnover is of high interest in order to predict the  
70 consequences of changes on ecosystem functioning (Handa *et al.* 2014).

71         The study of how litter diversity affects decomposition has especially attracted  
72 interest in terrestrial systems, with some studies showing an accelerated decomposition  
73 rate when increasing litter diversity (Wardle, Bonner & Nicholson 1997; Cardinale *et*  
74 *al.* 2011), while others finding no or even a negative relationships (for meta-analyses,  
75 see Gartner & Cardon 2004; Srivastava *et al.* 2009). As mentioned, however, a  
76 significant portion of terrestrial litter decomposition is occurring in aquatic systems  
77 (Ball *et al.* 2010). Surprisingly, in aquatic ecosystems the focus has often been on  
78 effects of leaf litter quality, climate or the structure of the decomposer community (e.g.,  
79 Frossard *et al.* 2013; Frainer *et al.* 2015; García-Palacios *et al.* 2016; Hines, Reyes &  
80 Gessner 2016) on decomposition rates, rather than on effects of litter diversity *per se*  
81 (but see e.g., Gessner *et al.* 2004; Giller *et al.* 2004; Handa *et al.* 2014). Consequently,  
82 the specific effects of leaf litter diversity and identity and the decomposer community  
83 in aquatic systems are still not completely resolved and have been proposed to be to  
84 some degree system dependent (Hättenschwiler, Tiunov & Scheu 2005; Cardinale *et al.*  
85 2011; Lecerf *et al.* 2011). Furthermore, in aquatic ecosystems, leaf litter decomposition  
86 can be controlled both by bottom-up (litter diversity, see Gessner *et al.* 2004; Giller *et*  
87 *al.* 2004; Handa *et al.* 2014; García-Palacios *et al.* 2016) and top-down (Srivastava &  
88 Bell 2009; Srivastava *et al.* 2009) processes, and a synthesis of their relative role has  
89 not yet emerged (Giller *et al.* 2004).

90           Here, we studied how the diversity and identity of allochthonous leaf litter from  
91 common tree species and the size structure of a natural aquatic microbial decomposer  
92 community extracted from a lotic system (small, dammed forest stream; see Fig. S1 in  
93 Supporting information) affect litter decomposition in aquatic ecosystems. To achieve  
94 this goal, we used four leaf litter species (alder, beech, poplar and oak; Fig. 1) in  
95 experimental mono-, bi- and poly-cultures, and exposed them to decomposition by a  
96 natural aquatic microbial community and a microbial community of which we  
97 manipulated the size structure by excluding larger, potentially predatory, eukaryotic  
98 microbial organisms. We followed decomposition of leaves and tracked microbial  
99 activity (oxygen concentration) and community dynamics of free-living microbes  
100 (density and size structure of bacteria and protists) to functionally link the structure of  
101 the microbial decomposer community and leaf litter diversity to the process of litter  
102 decomposition. Our approach explicitly allowed us to address both bottom-up diversity  
103 effects of leaf litter as well as top-down diversity effects of decomposer organisms on  
104 decomposition.

105

## 106 **Methods**

### 107 *General experimental set-up*

108 We tested the effects of leaf litter quality and diversity and the structural complexity of  
109 the decomposer community on litter decomposition in a microcosm laboratory  
110 experiment. We used leaf litter from four tree species common and native to Central  
111 Europe that display a range of litter quality: black alder (*Alnus glutinosa*), European  
112 beech (*Fagus sylvatica*), black poplar (*Populus nigra*) and pedunculate oak (*Quercus*  
113 *robur*); in the following we refer to these four species using their genus name. We  
114 selected these species as *Alnus* and *Populus* are considered to be good quality

resources, while *Quercus* and *Fagus* are known to be generally of lower quality (see for example Hladysz *et al.* 2009; Frainer *et al.* 2015). We used naturally senesced, air-dried leaves. Previous to the experiment, the leaves from all four species were mixed together and leached in river water for 24 hours so that water-soluble and possibly inhibitory compounds in the leaves (e.g., tannins) could leach out. We then cut leaf discs ( $\varnothing = 2.5$  cm) from all leaf species and dried them for 60 hours in a drying oven. The leaf discs were then individually weighed. We used a subset of leaves from the same batch as used in the experiment and analysed them for carbon, nitrogen, and phosphorus content using established protocols (phosphorus: San++ automated wet chemistry analyzer, Skalar Analytical B.V., Breda, Netherlands; nitrogen and carbon: Flash 2000 Elemental Analyzer coupled with Delta V Advantage IRMS, both manufactured by ThermoFisher Scientific, Bremen, Germany). The values reported from these measurements in table 1 are the same as also reported in Little & Altermatt (in review).

In each microcosm we placed a total of four leaf discs of different species combinations: microcosms contained either a single leaf litter species (i.e., four leaf discs of either *Alnus*, *Fagus*, *Populus* or *Quercus* respectively), mixtures of two leaf litter species (i.e., two leaf discs of two leaf species, in all possible pairwise combinations) or leaf discs of all four species (i.e., one leaf disc from each species), resulting in 11 different leaf litter treatments (Fig. 1).

We used natural aquatic microbial decomposer communities of two different structural complexities to test for possible interactive effects of the decomposer community trophic structure with litter diversity. Natural microbial communities originated from a small, dammed stream surrounded by deciduous forest near Pfäffikon ZH, Switzerland (location: 47° 22' 27.1" North, 8° 48' 08.3" East) (see also Mächler & Altermatt 2012). We sampled the water including the microbial communities near the

140 inflow (Fig. S1 in Supporting Information), such that our study looks at water and  
141 microbial decomposers that are characteristic of a lotic system. Twenty liters of water  
142 was sampled in October 2015 and filtered on site to remove large aquatic organisms  
143 such as macroinvertebrates or vertebrate larvae (mesh size 250  $\mu\text{m}$ ). The filtered water  
144 contained the natural microbial decomposer community consisting of bacteria, fungi  
145 and protists, and henceforth is referred to as the “complete decomposer community”  
146 (“CDC”). To obtain a size-fractionated community (“SFC”) with a reduced functional  
147 and trophic complexity (i.e., exclusion of large organisms such as predatory rotifers or  
148 ciliates), we filtered half of the water through a much finer filter (mesh size 11  $\mu\text{m}$ ).  
149 Many of these microbial organisms are rather flexible in their body structure (e.g.,  
150 amoeba which can change their shape very plastically and have substantial intraspecific  
151 variability in size, see Giometto *et al.*, 2013), and thus the 11  $\mu\text{m}$  filter is not a clear-cut  
152 threshold: some organisms may pass when small, but grow bigger thereafter, or some  
153 organisms are much longer than 11  $\mu\text{m}$ , but very slender, and can thus still pass.  
154 Overall, however, the filtering significantly reduced the abundance and occurrence of  
155 organisms larger than 10  $\mu\text{m}$  (linear mixed effect model,  $p < 0.001$ ), thus proving the  
156 effectiveness of the filtering.

157       While focusing here on bacteria and protists, we recognize the important role of  
158 fungi for decomposition processes in lotic systems (e.g., Gessner & Chauvet 1994;  
159 Hieber & Gessner 2002; Dang, Chauvet & Gessner 2005; Gessner *et al.* 2007). To  
160 ensure that microbial (i.e. also fungal) colonization of leaves could occur, all leaves  
161 were conditioned in one vessel filled with stream water for 24 h. Furthermore,  
162 microbial communities, including fungal spores, came in through the water sampled  
163 from the dammed forest stream and used for the experiment. We could, however, not  
164 measure fungal components in the leaf biomass for logistic and technical reasons.

165 Importantly, however, our goal was to study the effect of leaf litter identity and  
166 decomposer community size structure, but not community identity of the latter.

167 All microcosms were filled with 100 mL of the corresponding decomposer  
168 community (CDC versus SFC), with five replicates per treatment combination,  
169 resulting in a total of 110 microcosms (Fig. 1). Microcosms were filled with the  
170 different resource types (leaves) and the corresponding decomposer community on 27<sup>th</sup>  
171 October 2015 and leaf litter was subsequently incubated in these aquatic microcosms  
172 for a decomposition period of 72 days. The experiment took place in a climate room  
173 with a constant temperature of  $18 \pm 1$  °C and a day/night-cycle of 12 h light and 12 h  
174 darkness. All handling and work was conducted using standard microbiology  
175 procedures, including sterile handling procedures and autoclaving all material (such as  
176 pipettes, glassware etc.) previous to its use. Cultures were regularly screened visually  
177 with a stereomicroscope (Leica M205 C, Leica Microsystems, Heerbrugg, Switzerland)  
178 at a 10 to 160-fold magnification, using dark-field illumination. Further general  
179 handling and laboratory procedures for such aquatic microcosms are described in detail  
180 in Altermatt *et al.* (2015).

181

182 *Response variables*

183 Our primary response variable was leaf biomass loss (as a proxy for  
184 decomposition rates). Oxygen concentration and the composition and structure of  
185 bacteria and protist communities were used as complementary response variables  
186 underlying drivers of decomposition/decomposer activity.

187 To measure leaf biomass loss, we removed the leaf discs after 72 days of  
188 incubation and carefully cleaned them from the biofilm under running tap water. We

189 then dried the leaf discs at 60 °C for 60 hours and measured the final dry mass of all  
190 individual leaf discs.

191         We measured dissolved oxygen concentrations in the microcosms every two  
192 days during the first four weeks of the experiment and thereafter for organizational  
193 reasons twice a week for the remaining six weeks with an optical oxygen meter  
194 (PreSens Fibox 4 Optical Oxygen Meter). Oxygen concentration is often negatively  
195 correlated with microbial activity, and can in parts be used as a proxy of it (Briand *et*  
196 *al.* 2004). Importantly however, in our case there were also likely photosynthetic  
197 organisms present, such that microbial activity could to some degree also increase O<sub>2</sub>  
198 levels. While we did not see a pronounced development of a photosynthetic biofilm, the  
199 longer term dynamics in O<sub>2</sub> concentrations likely included a combination and  
200 equilibrium between O<sub>2</sub> consumption during decomposition and O<sub>2</sub> production by  
201 phototrophic organisms. We thus see the O<sub>2</sub> measurements reflecting microbial  
202 activities in a broader sense.

203         We measured density and cell size distributions of free-living protists and other  
204 microorganisms (e.g., rotifers) with a diameter >5 µm in the decomposer communities  
205 with a Cell Counter and Analyzer System (CASY) model TTC (Roche Diagnostics  
206 GmbH) at weekly intervals during the experiment (Mächler & Altermatt 2012;  
207 Altermatt *et al.* 2015). We took 0.5 mL samples and diluted them 1:20 with the isotonic  
208 buffer solution CASYTon®. Cell counts were performed with a 150 µm capillary, and  
209 individual cell counts and cell size measurements were used to estimate the total  
210 biomass of decomposers in the microcosms (Giometto *et al.* 2013; Altermatt *et al.*  
211 2015).

212         Finally, we measured abundance of bacteria with a BD Accuri™ C6 flow  
213 cytometer (Becton-Dickinson) during the experiment at roughly one-week intervals.

214 Samples were diluted with filtered Evian® according to expected densities within the  
215 microcosms, stained with 20 µl of the fluorescent dye SYBR® Green and incubated for  
216 13 minutes at 37 °C. The measurements were made from 50 µL samples and a  
217 threshold value of 800 on FL1-H (green fluorescence level). We used well-established  
218 gating settings to distinguish between background noise and bacterial counts (Altermatt  
219 *et al.* 2015).

220

#### 221 *Data Analysis*

222 We used the *R* software version 3.3.2 (R Development Core Team 2016) for all  
223 statistical analyses. We calculated the proportion of the final leaf litter dry weight  
224 compared to the initial leaf litter dry weight as the decomposition rate (odds ratio). We  
225 used generalized linear models (GLMs) with quasi-binomial link functions to examine  
226 the influence of our predictor variables, resource type and decomposer community  
227 type, on leaf mass loss. To disentangle the effects of the different resource types we  
228 conducted *post-hoc* multiple linear pairwise Tukey-test comparisons using the R-  
229 package ‘multcomp’ (Hothorn *et al.* 2016).

230 For the proximate response variables, we used linear mixed effect models in the  
231 R-package ‘lmerTest’ (Kuznetsova, Brockhoff & Christensen 2015) to test the effects  
232 of leaf litter diversity and consumer community on oxygen concentrations, total cell  
233 counts, living biomass, median organism size and bacterial densities in the community.  
234 The resource type and the decomposer community were used as fixed effects whereas  
235 time was used as a random effect.

236

#### 237 **Results**

238 Leaf litter decomposition differed significantly between litter types and combinations  
239 thereof, and between the two decomposer community types (Fig. 2 and table 2). There  
240 was no interaction between leaf litter treatment and decomposer community structure.  
241 In all treatments, *Populus* and *Alnus* leaves were more strongly decomposed than  
242 *Fagus* and *Quercus* leaves, and most of these differences were significant or marginally  
243 significant (decomposition *Populus* > *Fagus*,  $p < 0.001$ ; decomposition *Populus* >  
244 *Quercus*,  $P < 0.001$ ; decomposition *Populus* > *Alnus*,  $p = 0.03$ ; decomposition *Alnus* >  
245 *Fagus*,  $p = 0.08$ ; decomposition *Alnus* > *Quercus*,  $p = 0.07$ ; decomposition *Fagus* ~  
246 *Quercus*,  $p = 0.97$ ; Figs. 2 & 3, complete statistical details are given in table S1 in  
247 Supporting Information). Size-fractionated communities showed a small but significant  
248 reduction in decomposition rates compared to complete communities, which included  
249 higher trophic levels and larger organisms (Fig. 2, table 2). Overall, the most common  
250 effect of mixing different leaf types on decomposition rates was additive, but we also  
251 found some synergistic effects (the expected value is the mean of the two species'  
252 values in monoculture and denoted by the red line in Fig. 2; the observed value,  
253 indicated by the bar, is in some cases higher than the expected value; see tables A2 &  
254 A4 for full overview of statistical results). When looking at decomposition rates of each  
255 leaf litter species individually, we found no differences in decomposition for leaves of  
256 *Fagus*, *Populus* or *Quercus* when decomposed alone compared to in mixture with other  
257 species (all  $p > 0.05$ ; Fig. 3b–d & 3f–h; tables S2, S3 & S4 in Supporting Information).  
258 In stark contrast, *Alnus* leaves decomposed at significantly higher rates when mixed  
259 with other leaf species ( $p < 0.0002$ ; Fig. 3a & 3e, table S5 in Supporting Information).  
260 Oxygen concentrations showed pronounced temporal dynamics with a drastic  
261 decrease in the first five days, and a subsequent increase to a stable value after about 30  
262 days. We found highly significant effects of leaf litter type on  $O_2$  concentration and

263 significantly lower O<sub>2</sub> concentrations in the complete vs. size-fractionated communities  
264 (Fig. 4 and table 3). The mixing of leaf litter generally resulted in intermediate O<sub>2</sub>  
265 concentrations compared to single leaf litter treatments (i.e., additive effects on O<sub>2</sub>  
266 concentration, Fig. S3 to S8 in Supporting Information).

267 Leaf litter type also significantly influenced microbial cell counts (eukaryotic  
268 and prokaryotic) and total microbial biomass (Fig. 5 and table 3). As expected, filtering  
269 communities initially with a 11 µm filter removed and significantly reduced organisms  
270 >10 µm in SFC compared to CDC ( $p < 0.01$ ). The removal of the larger organisms  
271 resulted in a marginally significantly lower median organism size in the size-  
272 fractionated community compared to the whole microbial community (table 3). Median  
273 size increased in all treatments consistently over time. Surprisingly, decreasing  
274 structural (i.e., size) complexity of the communities did not significantly affect  
275 proximate microbial community structures over time (Fig. 5), even though the ultimate  
276 effects on decomposition were detectable and significant (see above). Initially,  
277 microbial abundance increased in microcosms containing leaves of *Populus* or *Alnus*  
278 (in both microbial community types) and of *Quercus* (only in the SFC; Fig. 5a/b). After  
279 this initial peak, abundances decreased and stabilized to a constant value after 30 days.  
280 The abundance of microbes in microcosms containing *Fagus* was low during the whole  
281 decomposition process. Mixing leaf litter mostly resulted in intermediate values of cell  
282 counts (additive effects of leaf mixture, data not shown). Biomass of the microbial  
283 community at the end of the experiment was highest in microcosms containing  
284 *Quercus*, followed by *Alnus*, *Populus* and *Fagus*. Similarly, the median of organisms'  
285 cell size distribution steadily and significantly increased over time in the decomposer  
286 communities (Fig. 5e/f), although without a significant difference between the leaf litter  
287 treatments (table 3).

288           In contrast to these overall microbial community shifts, bacterial densities  
289 significantly declined over time in all treatment combinations (Fig. S2 Supporting  
290 Information), with significant differences between leaf litter treatments but no  
291 significant effect of initial community structure (table 3). There was no consistent  
292 influence of mixing leaf litter on bacterial abundances, but often they were intermediate  
293 compared to the single leaf-litter treatments (additive effects of leaf mixture, data not  
294 shown).

295

## 296 **Discussion**

297 We found that leaf litter identity strongly influenced litter decomposition rates, but that  
298 rates were also modulated by the structural composition of the free-living decomposer  
299 community. Consistent with previous work in stream systems, mixing leaf litter  
300 generally exhibited an additive rather than a synergistic effect on decomposition (e.g.,  
301 Kominoski *et al.* 2007). Additionally, we found that manipulating the size structure of  
302 the decomposer community has a direct influence on decomposition rates and on  
303 biological processes (microbial activity as measured by O<sub>2</sub> concentration), while some  
304 of the proximate measures of community structure were not significantly affected.  
305 Specifically, a complete decomposer community showed faster decomposition  
306 compared to the sized-fractionated decomposer community. The size-fractionated  
307 communities were not only lacking larger organisms due to the filtering (size threshold  
308 of the filtration was about 10–15 µm), but the whole community overall consisted of  
309 marginally significantly smaller organisms. The removal of larger organisms likely  
310 resulted also in a removal of trophically higher microbes, such as predatory rotifers or  
311 ciliates, or other specific functional types of organisms. The predominant absence of  
312 synergistic litter diversity effect on free-living aquatic decomposition rates may render

313 interpretations and extrapolations of decomposition rates more predictable, as the  
314 majority of effects was additive.  
315

316 *Leaf Litter Decomposition*

317 Leaf litter identity and associated traits are a crucial factor affecting rates of litter  
318 decomposition in aquatic systems (Webster & Benfield 1986; Lecerf *et al.* 2007;  
319 Gessner *et al.* 2010; Bruder *et al.* 2014). Thereby, both the content and ratio of C, N  
320 and P as well as lignin are important determinants of leaf litter decomposition.  
321 Generally, the higher the N-content (or the N content relative to the C content), the  
322 better leaves can be decomposed. Our observed decomposition rates are in good  
323 accordance to the measured C:N ratios (table 1), and the P- and N-content of the leaves:  
324 C:N ratio was *Quercus* ~ *Fagus* > *Populus* > *Alnus*, which matched (except for *Populus*  
325 and *Alnus* reversed in most cases) the decomposition rates. In analogy, the more lignin  
326 a leaf contains, the slower its decomposition (Hladyz *et al.* 2009; Schindler & Gessner  
327 2009; Frainer *et al.* 2015). Our findings of decomposition rates are consistent when  
328 comparing them to lignin contents of our leaf species derived from literature data:  
329 *Fagus* and *Quercus*, which are generally having highest lignin contents (e.g., Hladyz *et*  
330 *al.* 2009; Frainer *et al.* 2015), were decomposed the slowest. In contrast, *Populus* with a  
331 generally low lignin content (e.g., Frainer *et al.* 2015) was decomposed the fastest.  
332 *Alnus* has intermediate, but rather variable lignin contents (e.g., Hladyz *et al.* 2009;  
333 Frainer *et al.* 2015) and—depending on the decomposer community structure—were  
334 decomposed either as well as *Populus* or as slowly as *Fagus* and *Quercus*.

335       So far, various effects of leaf litter diversity on decomposition rates were found,  
336 including additive (Srivastava *et al.* 2009; Frainer *et al.* 2015) and synergistic effects  
337 (Lecerf *et al.* 2011; Handa *et al.* 2014). Importantly, these studies cover different

ecosystems, from lentic to lotic ecosystems, and also different leaf types/leaf species and conditioning. Overall, recent studies in lotic systems, where decomposition by fungi is found important (e.g., Gessner & Chauvet 1994; Hieber & Gessner 2002; Dang, Chauvet & Gessner 2005; Gessner *et al.* 2007), fairly consistently report a lack of a synergism (Ferreira, Encalada & Graça 2012; Bruder *et al.* 2014), suggesting that leaf identity might be a more important factor than litter diversity in determining decomposition rates. While we could not measure fungi themselves, but focused on the free-living decomposer community present in the supernatant, our results are in high concordance with these findings, and the observed additive effects of mixing leaf litter could arise from two different mechanisms. Either the component species get degraded at the same rate in mixtures as in monocultures, or mixing leaf litter affected the decomposition of the two component leaf litter species in opposing directions, with the sum of overall decomposition resulting in an overall additive effect. While *Alnus* leaves decomposed differently depending on the co-occurring leaves (Fig. 3a,e), we found that leaves of *Fagus*, *Populus* and *Quercus* did not decompose differently when mixed with other species (Figs. 3b–d & 4f–h; tables A2 & S4 Supporting Information). Thus, we found differences in decomposition of leaves in some combinations, while not in other combinations. Constant decomposition rates of a focal species when mixed with other species had also been previously observed (Ferreira, Encalada & Graça 2012; Bruder *et al.* 2014). This would provide some support for the first mechanism, that leaf litter gets degraded with a constant rate regardless of the presence of other species. Importantly, however, these past studies focused on the effect of fungi on decomposing leaves, while we could not measure fungi themselves. Thus, our results need to be interpreted with some care when being compared to these other studies.

362 As mentioned above, we also found strong exceptions to this overall additive  
363 effect of mixing leaf litter species (Fig. 4). When mixing *Fagus* or *Quercus* with *Alnus*  
364 leaves, we observed higher overall decomposition than the expected average of the two  
365 component species (Fig. 2, AF AQ and AFPQ treatments; tables A1 & A3 Supporting  
366 Information). In our experiment we observed these non-additive effects only when  
367 mixing a low quality leaf litter (i.e., *Fagus* and *Quercus* with a low nitrogen content;  
368 table 1) with a high quality leaf litter (especially *Alnus* with a high nitrogen content;  
369 table 1) (see also Vos *et al.* 2013). In addition, *Fagus* also had the lowest phosphorus  
370 content (table 1) and is generally reported to have a high lignin content (Frainer *et al.*  
371 2015), making it the most dissimilar leaf quality type relative to *Alnus*. As a possible  
372 consequence, the diversity effect was most pronounced when mixing *Alnus* with *Fagus*,  
373 indicating that dissimilarities in leaf litter qualities are clearly a prerequisite for  
374 accelerated decomposition rates. While not explicitly studied (and not addressable with  
375 our study design), this could indicate some support of a functional diversity effect.

376

#### 377 *Proximate effects on microbial and bacterial communities*

378 Leaf litter identity strongly influenced O<sub>2</sub> concentrations in the microcosms (Fig. 5) and  
379 the observed O<sub>2</sub> concentrations during the early phase of the experiment closely  
380 matched the inverse of overall decomposition rates. The strong temporal fluctuations  
381 with an initial decrease in O<sub>2</sub> concentrations, and a subsequent increase and then steady  
382 state could be explained by a combination of depletion of nutrients (Dilly & Munch  
383 1996) resulting in lower decomposer activities during the latter half of the experiment  
384 (and O<sub>2</sub> diffusing into the medium), the potential formation of a photosynthetically  
385 active biofilm, in which microbial activity was not only consuming but also producing  
386 O<sub>2</sub>, or the presence of leachates and inhibitory compounds during the initial phase and

387 an associated community turn-over during the experiment from fungi to bacteria  
388 dominance. Initial colonization and decomposition of the leaves results in a rapid  
389 decomposition of the more labile compounds, while more recalcitrant compounds can  
390 only be accessed later on.

391       Microbial cell counts, representing the number of free-living eukaryotic  
392 organisms such as protists, showed as expected the inverse pattern to oxygen  
393 concentrations (Fig. 5): an initial increase of organisms could be detected, but then the  
394 number of organisms decreased. Bacterial densities also declined over time (Fig. S2  
395 Supporting Information). This is consistent with an initial high availability of nutrients  
396 but subsequent depletion. Surprisingly, however, the total biomass increased steadily  
397 over time (Fig. 5), paralleled by an increase in the median cell size of the community  
398 over time (Fig. 5). This suggests a shift in the community structure towards fewer  
399 larger organisms.

400       In the complete decomposer community, larger, possibly bacterivorous, protists  
401 were likely present, which are expected to substantially reduce bacteria abundances. As  
402 a consequence, we expected lower decomposition rates. However, we found the  
403 opposite result. This counterintuitive increase in decomposition rates in the presence of  
404 larger bacterivorous/predatory protists has also been seen in other studies (Barsdate &  
405 Prenski 1974, Ribblett *et al.* 2005), and has been explained by a high turnover of  
406 bacteria leading to a better physical state of the bacterial community consequently  
407 enhancing decomposition. We see three mutually non-exclusive explanations. First, it  
408 could be a top-down effect of the larger microorganisms (“meiofauna”) on the smaller  
409 decomposers. However, in our case bacterial densities did not vary with the structure of  
410 the decomposer community (CDC vs. SFC), arguing against this positive effect of  
411 grazing. Second, the meiofauna itself may not only consist of predators, but also

412 include some decomposers. Thus, the meiofauna would to some level increase  
413 predation but also increase decomposition. In that case, the complete decomposer  
414 community would actually also include a potentially higher diversity of leaf consumers.  
415 Finally, it could also indicate a distinct enzymatic capacity towards more recalcitrant  
416 compounds. A meta-analysis indeed provided evidence for a *per se* positive  
417 relationship between consumer diversity (decomposer community) and decomposition  
418 rates (Srivastava *et al.* 2009). Such a diversity effect at the decomposer level can result  
419 from several mechanisms. First, facilitation among microorganisms can occur during  
420 the process of litter decomposition (De Boer *et al.* 2005). Additionally, complementary  
421 resource use can ensue (Gessner *et al.* 2010), resulting in the break-down of a wider  
422 range of leaf litter components. The latter mechanism though can only occur if species  
423 are functionally diverse. Our experiment showed a pronounced positive effect of  
424 trophic complexity in microbial communities on leaf litter decomposition rates (see  
425 also Handa *et al.* 2014). Whether this is a consequence of species richness or functional  
426 diversity is challenging to unravel, because by reducing the functional diversity via  
427 size-fractioning the community, we simultaneously reduced species richness. Overall,  
428 our results underpin that the trophic complexity of a decomposer community (e.g., see  
429 also Stocker *et al.* 2017), also at the microbial level, is crucial for the functioning of the  
430 litter decomposition process.

431

#### 432 *Conclusion*

433 We found that leaf litter identity and quality significantly and strongly influence  
434 decomposition rates. Only in the case of *Alnus* and *Fagus*, mixing leaf litter species  
435 resulted in synergistic effects in decomposition rates. For the other species  
436 combinations, the effects were additive. This suggests that the diversity of primary

437 producers is not as important in the process of litter decomposition as in other  
438 ecosystem functions, such as primary production. Importantly, decomposition rates  
439 were higher in microbial decomposer communities that were not size-fractionated  
440 compared to microbial decomposer communities in which medium to large-sized  
441 microbes were initially removed, even though many of our metrics characterizing these  
442 communities (e.g., size structure, abundance etc.) were surprisingly similar throughout  
443 the experiment. This finding implies that trophic diversity and functional traits of the  
444 decomposer community are important for litter decomposition and subsequent nutrient  
445 cycling. Overall, top-down effects due to loss of species or functional groups in the  
446 decomposer community may be as important as bottom-up effects via leaf litter (i.e.,  
447 resource) diversity highlighting the sensitivity of decomposition processes to future  
448 environmental changes.

449

#### 450 **Author Contributions**

451 All authors planned and designed the study; FS conducted the experiment and collected  
452 the data; FS and FA analyzed the data. FS and FA led the writing of the manuscript. All  
453 authors contributed critically to the drafts and gave final approval for publication.

454

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462

463 **Data accessibility**

464 All data from the study will be archived on Dryad upon acceptance of the manuscript.

465

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639

#### 640 **SUPPORTING INFORMATION**

641 Additional supporting information may be found in the online version of this article.

642

643 **Tables**

644 Table 1: Leaf litter composition (Nitrogen N, Phosphorus P, Carbon to Nitrogen C:N,  
 645 Carbon to Phosphorus C:P and Nitrogen to Phosphorus N:P ratios) of the leaf litter  
 646 species used in the experiments.

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<b>Leaf type</b>	<b>N content (mg N/g dry weight, mean±sd)</b>	<b>P content (mg P/g dry weight, mean±sd)</b>	<b>C:N atomic ratio (mean±sd)</b>	<b>C:P atomic ratio (mean±sd)</b>	<b>N:P atomic ratio (mean±sd)</b>
<i>Alnus</i>	23.94±4.63	0.799±0.156	20.90±4.68	1386.19±326.53	66.59±7.71
<i>Fagus</i>	7.24±2.37	0.373±0.023	69.22±16.63	2798.77±196.72	43.09±14.63
<i>Populus</i>	10.99±4.34	0.725±0.091	43.98±12.37	1363.56±160.31	34.22±14.97
<i>Quercus</i>	6.58±0.89	0.467±0.113	73.85±11.30	2380.66±608.91	32.08±5.68

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647

648

649 Table 2: GLM on the effect of the decomposer community and the type of resource  
 650 (leaf litter type/combination) on litter decomposition.

Source	Df	Deviance	Resid. Df	Resid. Dev	F-value	P-value
<b>Community</b>	1	4.89	98	75.06	5.98	0.016
<b>Resource Type</b>	10	91.93	99	79.95	11.25	< 0.0001
<b>Interaction</b>	10	4.37	88	70.70	0.53	0.86
<b>NULL</b>			109	171.89		

651

652

653 Table 3: Summary of linear mixed models used to test for effects of the decomposer  
 654 community, the resource type and their interaction on several response variables.  
 655 Dissolved oxygen concentration, density of protists, microbial biomass, median cell  
 656 size and bacterial density were used as response variables. Fixed effects were tested  
 657 with F-tests, which test for differences in means, whereas random effects were tested  
 658 with Chi<sup>2</sup>-tests, which test for independency.

<b>Response Variable</b>	<b>Source</b>	<b>Df</b>	<b>Den Df</b>	<b>F-/<math>\chi^2</math>-value</b>	<b>P-value</b>
<b>Oxygen Concentration</b>	<b>Community</b>	1	84	6.29	0.014
	<b>Resource Type</b>	10	84	17.61	< 0.0001
	<b>Interaction</b>	10	84	0.51	0.88
	<b>Day</b>	-	-	2079.3	< 0.0001
<b>Density</b>	<b>Community</b>	1	84	0.004	0.95
	<b>Resource Type</b>	10	84	6.05	< 0.0001
	<b>Interaction</b>	10	84	0.27	0.99
	<b>Day</b>	-	-	134.25	< 0.0001
<b>Biomass</b>	<b>Community</b>	1	84	0.07	0.79
	<b>Resource Type</b>	10	84	6.95	< 0.0001
	<b>Interaction</b>	10	84	0.67	0.75
	<b>Day</b>	-	-	29.4	< 0.0001
<b>Median size</b>	<b>Community</b>	1	84	2.99	0.09
	<b>Resource Type</b>	10	84	1.32	0.23
	<b>Interaction</b>	10	84	0.55	0.85
	<b>Day</b>	-	-	434.22	< 0.0001
<b>Bacterial Density</b>	<b>Community</b>	1	84	2.63	0.11
	<b>Resource Type</b>	10	84	3.80	0.0003
	<b>Interaction</b>	10	84	0.57	0.84
	<b>Day</b>	-	-	548.3	< 0.0001

659

## 660 **Figure legends**

661 Fig. 1. Experimental setup. We had 11 communities of different leaf litter diversities  
 662 (*Alnus*, *Fagus*, *Populus* and *Quercus* leaves as single species, all possible 2-species and  
 663 the 4-species combinations) that were exposed to complete and size-fractionated  
 664 decomposer communities, each combination replicated five times.

665

666 Fig. 2: Decomposed leaf litter (mean $\pm$ se percentage of initial total litter dry biomass) of  
 667 different litter types and their combinations at the end of the experiment (day 72).  
 668 Colors indicate single species leaf litter treatments (green = *Alnus*, blue = *Fagus*, pink =  
 669 *Populus*, orange = *Quercus*), light grey is used for all possible pairwise combinations of  
 670 the leaf litter species, and dark grey indicates the four-species leaf litter combination;  
 671 all treatments are also labelled by the species name first-letter abbreviation. The  
 672 horizontal red lines give expected additive values (mean across the respective single  
 673 species treatments). Two different decomposer communities were used: (a) a natural,  
 674 complete decomposer community (filled bars) and (b) a size-fractionated decomposer  
 675 community (dashed bars).

676

677 Fig. 3: Decomposed leaf litter (mean $\pm$ se percentage of initial litter dry biomass) of  
 678 different litter types at the end of the experiment (day 72). For each of the four leaf  
 679 litter species (*Alnus*, *Fagus*, *Populus*, and *Quercus*), their biomass loss is given either  
 680 when they were in single-species microcosms, in two-species combinations or in the  
 681 four-species combination. The decomposer community was either a complete  
 682 decomposer community (solid bars; a–d) or a size-fractionated decomposer community  
 683 (dashed bars; e–h).

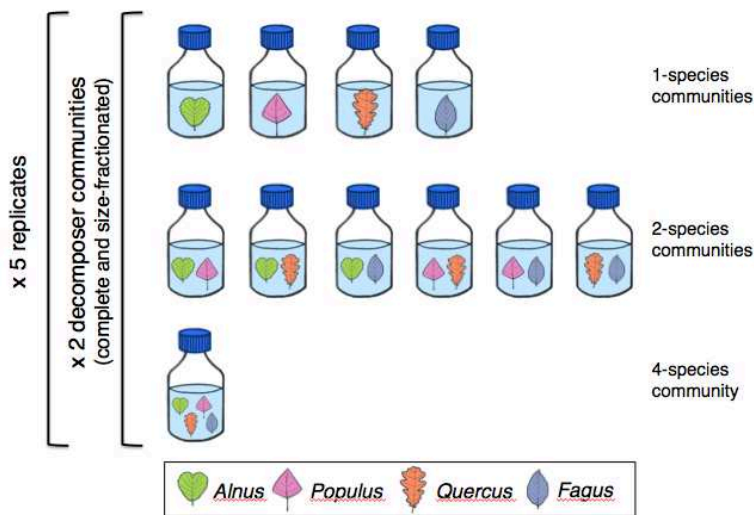
684

685 Fig. 4: Average concentrations of dissolved oxygen (mean $\pm$ se) across the whole  
686 experiment. Each line represents oxygen concentrations from microcosms with the  
687 single leaf litter species treatments as resource types (green = *Alnus*, blue = *Fagus*, pink  
688 = *Populus*, orange = *Quercus*). Solid lines indicate complete microbial decomposer  
689 communities (a) and dashed lines represent size-fractionated decomposer communities  
690 (b).

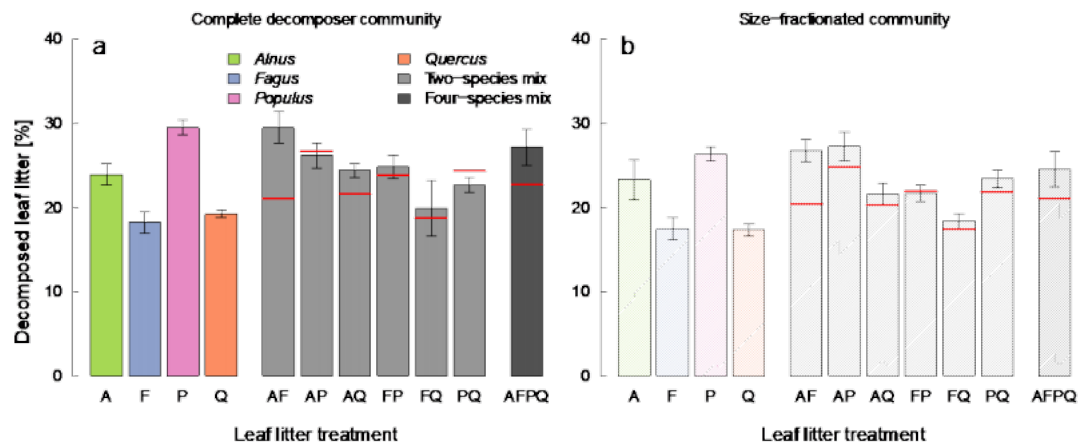
691

692 Fig. 5: Temporal variation of decomposer community metrics (CASY cell counter data  
693 of mostly eukaryotic microbial communities; mean $\pm$ se) across the whole experiment.  
694 Panels show densities (cell counts ml<sup>-1</sup>; a, b), living biomass ( $\mu$ g ml<sup>-1</sup>; c, d) and median  
695 cell size distribution ( $\mu$ m; e, f). Each line represents values from microcosms with the  
696 different single leaf litter species treatments (green = *Alnus*, blue = *Fagus*, pink =  
697 *Populus*, orange = *Quercus*). Solid lines indicate complete decomposer communities (a,  
698 c, e) and dashed lines represent size-fractionated communities (b, d, f).

699

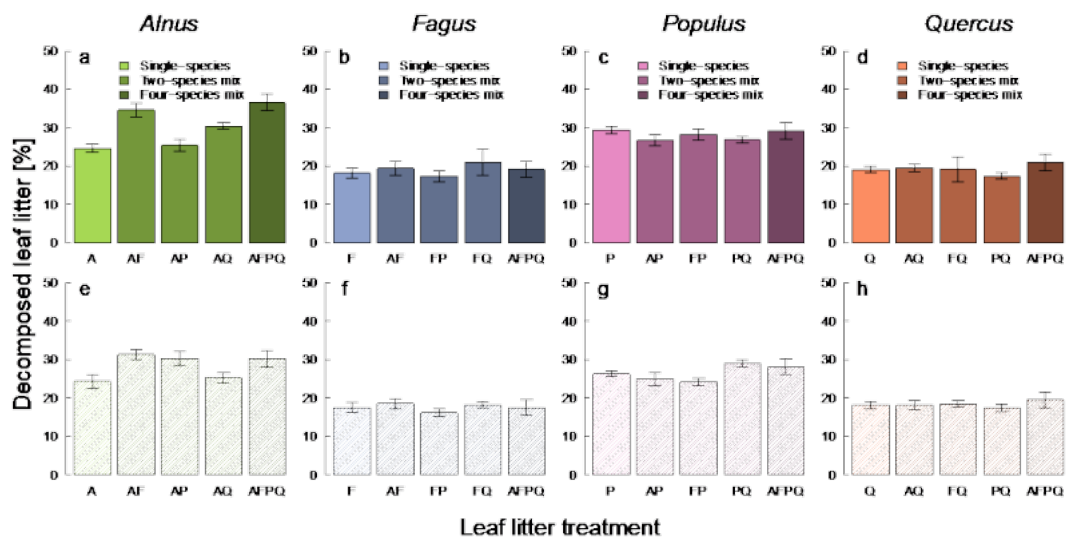


**Figure 1**



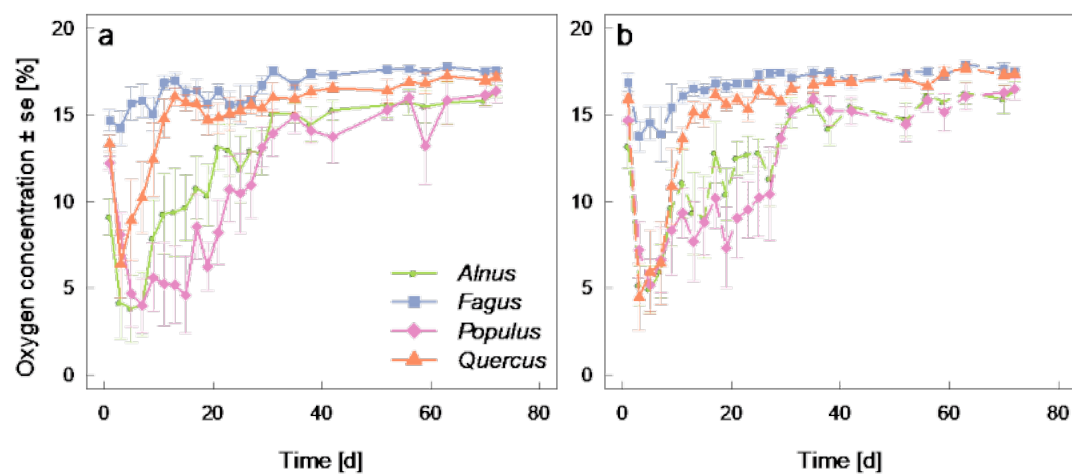
703

704 **Figure 2**



705

706 **Figure 3**



707

708 **Figure 4**

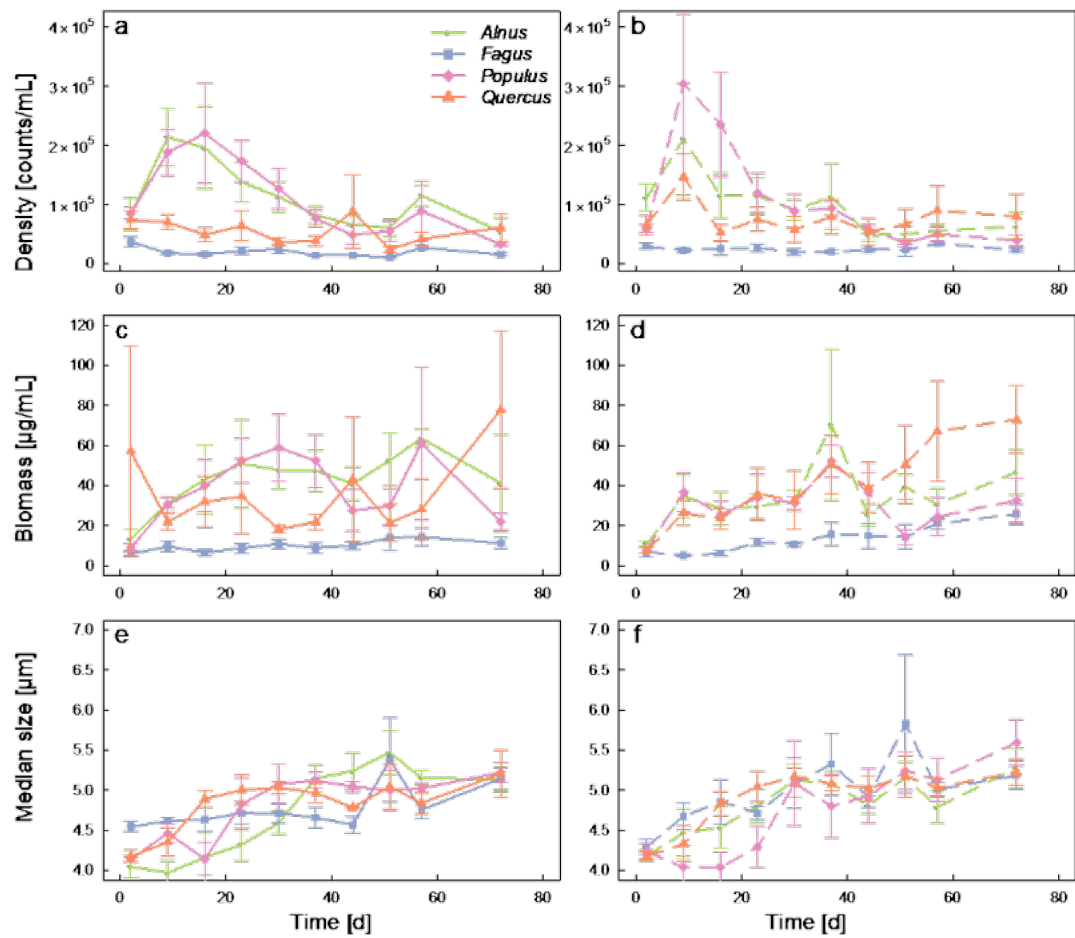


Figure 5