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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., Hladyz *et al.* 2010; Hladyz *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

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

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

134 We used natural aquatic microbial decomposer communities of two different
135 structural complexities to test for possible interactive effects of the decomposer
136 community trophic structure with litter diversity. Natural microbial communities
137 originated from a small, dammed stream surrounded by deciduous forest near Pfäffikon
138 ZH, Switzerland (location: 47° 22' 27.1" North, 8° 48' 08.3" East) (see also Mächler &
139 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 μm). 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 μm).
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 μm 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 μm , 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 μm (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 27th
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 ± 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 O₂
198 levels. While we did not see a pronounced development of a photosynthetic biofilm, the
199 longer term dynamics in O₂ concentrations likely included a combination and
200 equilibrium between O₂ consumption during decomposition and O₂ production by
201 phototrophic organisms. We thus see the O₂ 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 & Christencesn 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 O₂ concentrations in the complete vs. size-fractionated communities
264 (Fig. 4 and table 3). The mixing of leaf litter generally resulted in intermediate O₂
265 concentrations compared to single leaf litter treatments (i.e., additive effects on O₂
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 O₂ 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 (expect 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

338 ecosystems, from lentic to lotic ecosystems, and also different leaf types/leaf species
339 and conditioning. Overall, recent studies in lotic systems, where decomposition by
340 fungi is found important (e.g., Gessner & Chauvet 1994; Hieber & Gessner 2002;
341 Dang, Chauvet & Gessner 2005; Gessner *et al.* 2007), fairly consistently report a lack
342 of a synergism (Ferreira, Encalada & Graça 2012; Bruder *et al.* 2014), suggesting that
343 leaf identity might be a more important factor than litter diversity in determining
344 decomposition rates. While we could not measure fungi themselves, but focused on the
345 free-living decomposer community present in the supernatant, our results are in high
346 concordance with these findings, and the observed additive effects of mixing leaf litter
347 could arise from two different mechanisms. Either the component species get degraded
348 at the same rate in mixtures as in monocultures, or mixing leaf litter affected the
349 decomposition of the two component leaf litter species in opposing directions, with the
350 sum of overall decomposition resulting in an overall additive effect. While *Alnus* leaves
351 decomposed differently depending on the co-occurring leaves (Fig. 3a,e), we found that
352 leaves of *Fagus*, *Populus* and *Quercus* did not decompose differently when mixed with
353 other species (Figs. 3b–d & 4f–h; tables A2 & S4 Supporting Information). Thus, we
354 found differences in decomposition of leaves in some combinations, while not in other
355 combinations. Constant decomposition rates of a focal species when mixed with other
356 species had also been previously observed (Ferreira, Encalada & Graça 2012; Bruder *et*
357 *al.* 2014). This would provide some support for the first mechanism, that leaf litter gets
358 degraded with a constant rate regardless of the presence of other species. Importantly,
359 however, these past studies focused on the effect of fungi on decomposing leaves,
360 while we could not measure fungi themselves. Thus, our results need to be interpreted
361 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 O₂ concentrations in the microcosms (Fig. 5) and
379 the observed O₂ 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 O₂ 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 O₂ 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 O₂, 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.

Leaf type	N content (mg N/g dry weight, mean±sd)	P content (mg P/g dry weight, mean±sd)	C:N atomic ratio (mean±sd)	C:P atomic ratio (mean±sd)	N:P atomic ratio (mean±sd)
<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

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
Community	1	4.89	98	75.06	5.98	0.016
Resource Type	10	91.93	99	79.95	11.25	< 0.0001
Interaction	10	4.37	88	70.70	0.53	0.86
NULL			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 Chi²-tests, which test for independency.

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

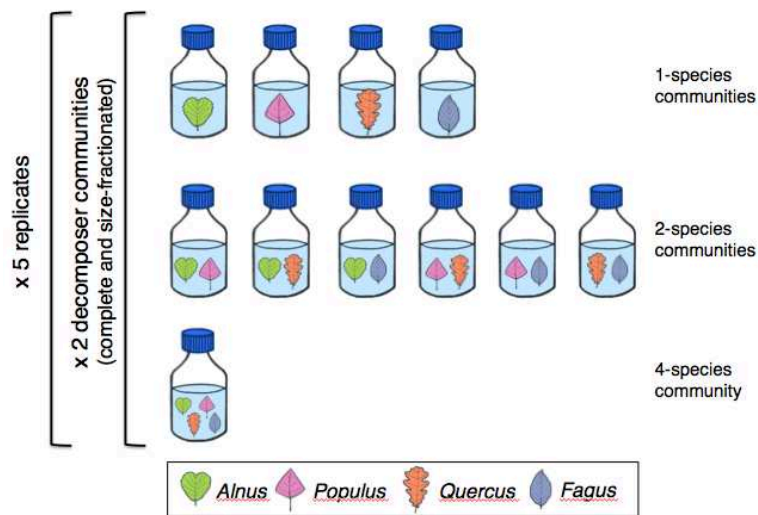
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685 Fig. 4: Average concentrations of dissolved oxygen (mean±se) across the whole
686 experiment. Each line represents oxygen concentrations from microcosms with the
687 single leaf litter species treatments as resource types (green = *Alnus*, blue = *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±se) across the whole experiment.
694 Panels show densities (cell counts ml⁻¹; a, b), living biomass (µg ml⁻¹; c, d) and median
695 cell size distribution (µm; 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).

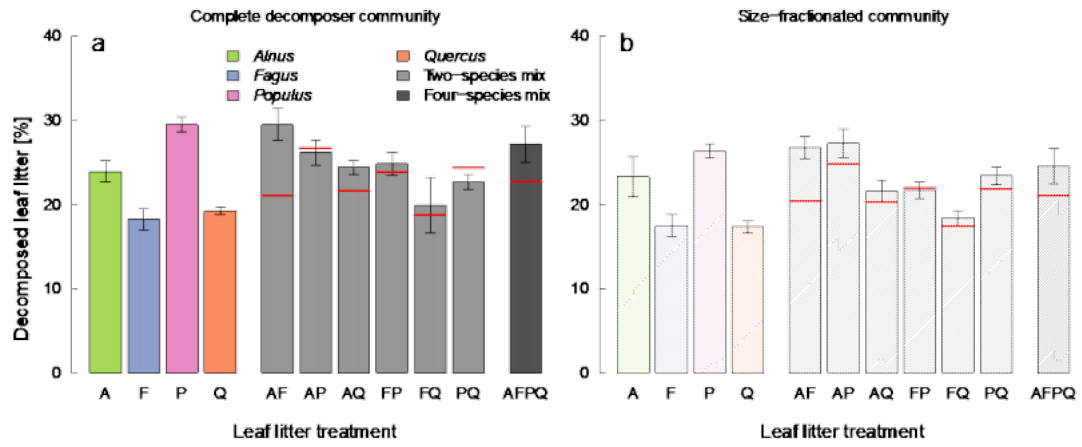
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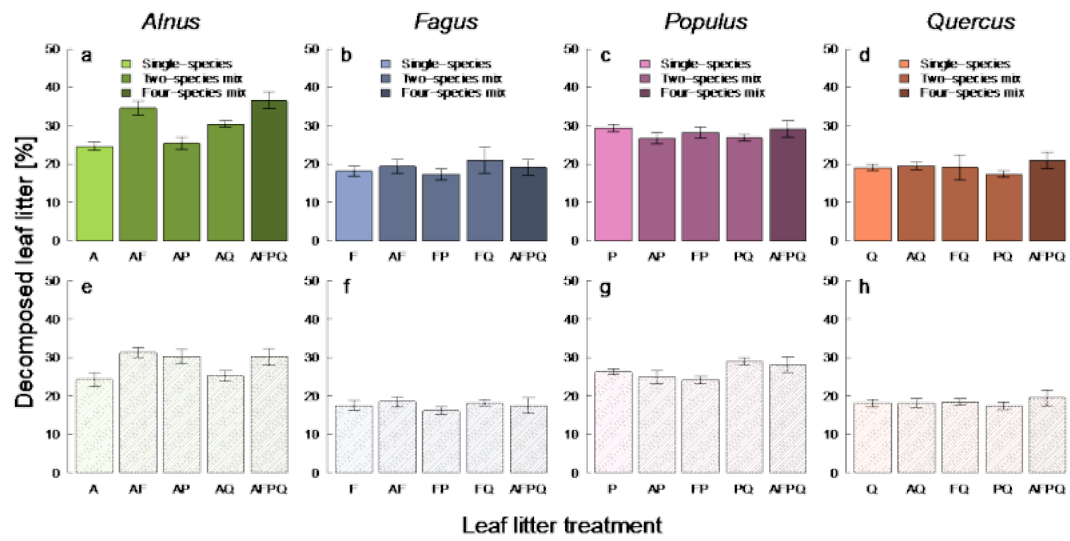
701 **Figure 1**

702



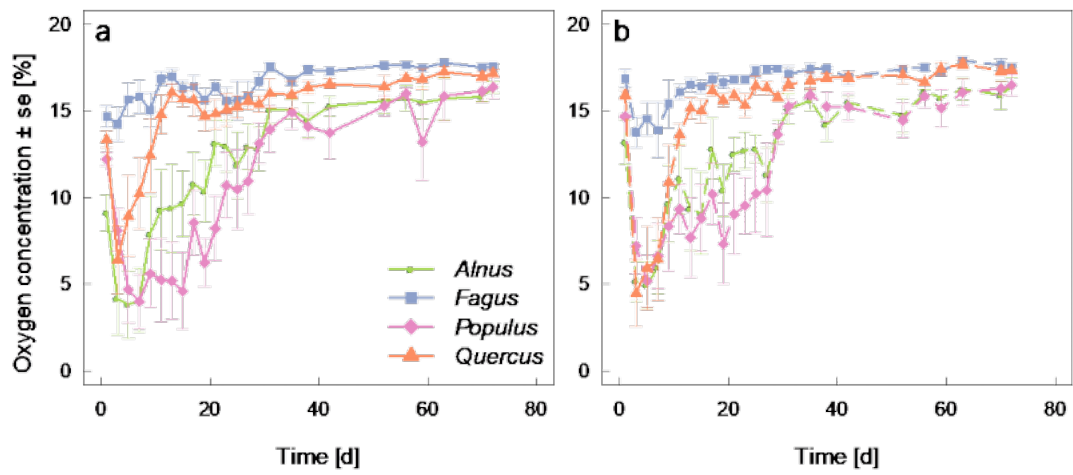
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704 **Figure 2**



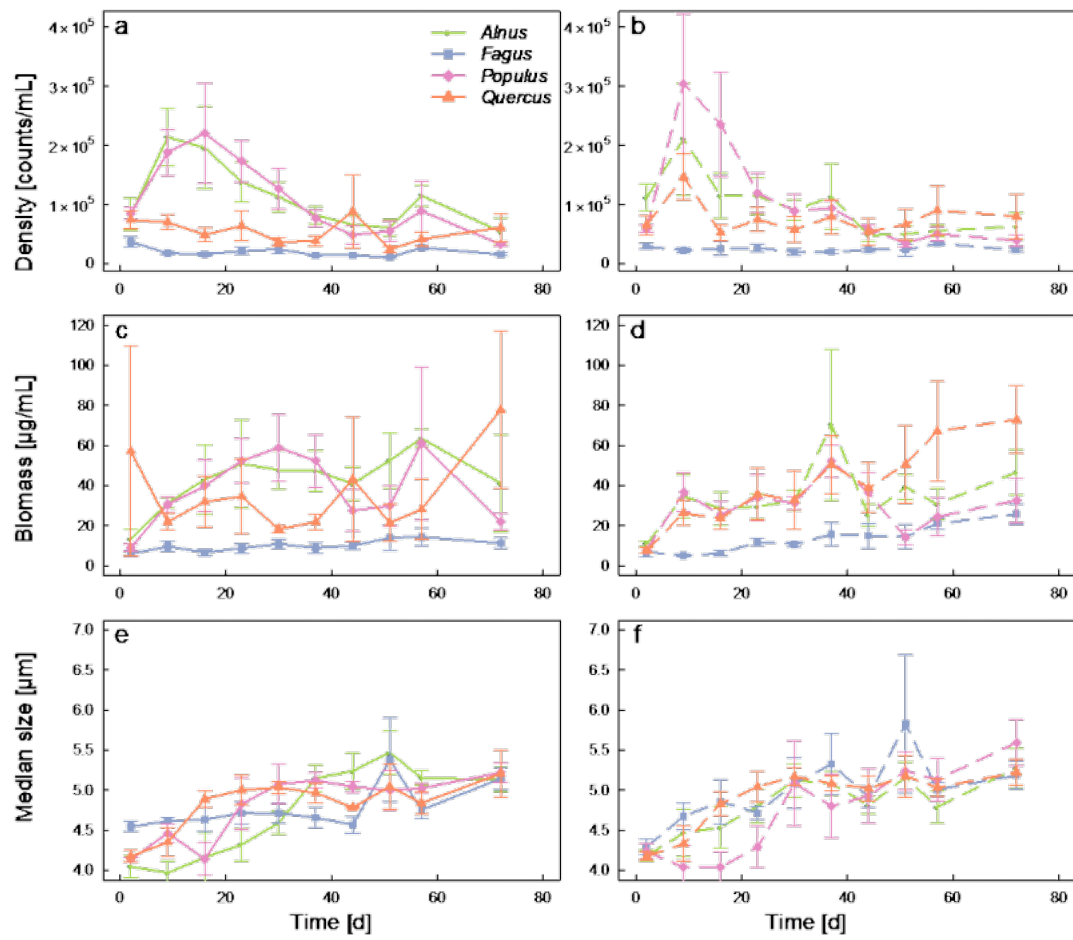
705

706 **Figure 3**



707

708 **Figure 4**



709

710 **Figure 5**

711