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### ► To cite this version:

Mathieu Santonja, Quentin Foucault, Anaïs Rancon, Thierry Gauquelin, Catherine Fernandez, et al.. Contrasting responses of bacterial and fungal communities to plant litter diversity in a Mediterranean oak forest. *Soil Biology and Biochemistry*, 2018, 125, pp.27-36. 10.1016/j.soilbio.2018.06.020 . hal-01864314

**HAL Id: hal-01864314**

**<https://univ-rennes.hal.science/hal-01864314>**

Submitted on 24 Jan 2019

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1 **Title:** Contrasting responses of bacterial and fungal communities to plant litter diversity in a  
2 Mediterranean oak forest

3

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23

24 **Abstract**

25           Tree species diversity of forested ecosystems control the diversity of leaf litter inputs to  
26 the soil, with cascading effects on the microbial communities colonizing decomposing litter.  
27 However, the extent to which bacterial and fungal communities inhabiting the litter layer are  
28 affected by shifts in tree species diversity is not well understood. To investigate the role of litter  
29 species diversity, litter species identity and litter functional traits on bacterial and fungal  
30 communities of a typical Mediterranean oak forest, we set up a yearly field litterbag experiment  
31 that considered leaf litter mixtures of four abundant species: *Quercus pubescens*, *Acer*  
32 *monspessulanum*, *Cotinus coggygria* and *Pinus halepensis*. We found that both bacterial and  
33 fungal communities varied strongly during decomposition but showed distinct succession  
34 patterns. Both communities were also strongly influenced by litter species diversity, litter  
35 identity and litter functional traits. The intensity and the direction of these effects varied during  
36 decomposition. Litter diversity effects were mediated by litter species composition rather than  
37 litter species richness, highlighting the importance of litter species identity - and associated  
38 litter traits - as drivers of microbial communities. Both the “mass-ratio hypothesis”, measured  
39 through the community weighted mean (CWM) litter traits, and the “niche complementarity  
40 hypothesis”, measured through the functional dissimilarity (FD) of litter traits, contributed to  
41 litter diversity effects, with a greater relative importance of FD compared to CWM, and with  
42 an overall stronger impact on fungal than on bacterial communities. Interestingly, increasing  
43 FD was related to decreasing bacterial diversity, but increasing fungal diversity. Our findings  
44 provide clear evidence that any alteration of plant species diversity produces strong cascading  
45 effects on microbial communities inhabiting the litter layer in the studied Mediterranean oak  
46 forest.

47

48 **Keywords:** Bacteria; fungi; litter decomposition; litter traits; Mediterranean forest; plant-soil  
49 interaction

50

## 51 **1. Introduction**

52         Alterations of plant communities due to ongoing global change, including climate, land-  
53 use and resource availability changes, have already been observed and are presumed to continue  
54 (Vitousek et al., 1997; Walther et al., 2002; van der Knaap et al., 2018). Studies of the  
55 relationship between plant species diversity and ecosystem functioning have received growing  
56 attention in the last decade (Diaz and Cabido, 2001; Loreau et al., 2001). The majority of such  
57 studies investigated how plant diversity affects litter decomposition efficiency (Gartner and  
58 Cardon, 2004; Hättenschwiler et al., 2005), but only a few have examined how it affects the  
59 organisms running the process (Bardgett and Shine, 1999; Leloup et al., 2018). Soil  
60 microorganisms are indeed major drivers of litter decomposition and mineralization (Bardgett,  
61 2005; de Graff et al., 2010) and their responses to changes of leaf litter diversity should interact  
62 with the decomposition process efficiency.

63         Litter microbial communities are strongly influenced by the quantity and the quality of  
64 litter input from the plant community (Calderon et al., 2001; Lalor et al., 2007). Differences in  
65 litter quality among tree species have been reported to affect the abundance and composition of  
66 soil bacterial and fungal communities (Grayston et al., 1998; Aponte et al., 2013; Prescott and  
67 Grayston, 2013). Both nitrogen (N) and phosphorus (P) availabilities actually shape microbial  
68 decomposer communities as they are often limiting elements in the soil and play a central role  
69 in resource competition (Cleveland and Liptzin, 2007; Mooshammer et al., 2014). Other plant  
70 litter traits such as secondary metabolites are recognized to repress the biomass and activity of  
71 microbial communities (Fierer et al., 2001; Ushio et al., 2013; Chomel et al., 2014, 2016). For  
72 instance, Chomel et al. (2014) observed a negative correlation between phenolic concentrations  
73 and fungal biomass during litter decomposition in a Mediterranean pine forest, calling attention

74 to the inhibitory effect of phenolic compounds from pine leaf litter toward fungi ([Kainulainen](#)  
75 [et al., 2003](#); [Kraus et al., 2003](#); [Hättenschwiler et al., 2005](#)). Likewise, [Amaral and Knowles](#)  
76 [\(1998\)](#) reported that monoterpenes inhibited the activity and growth of certain soil microbial  
77 groups while stimulating others.

78         However, in most natural ecosystems, litter material from various plant species  
79 decomposes together. As key actors of litter decomposition, microbial communities are likely  
80 responding to the variation in species composition of the litter input that determines litter  
81 functional diversity. According to the “mass-ratio hypothesis” ([Grime, 1998](#)), ecosystem  
82 properties are strongly related to the relative input of each species, implying that functional  
83 traits of the dominant plant species mainly determine ecosystem function ([Garnier et al., 2004](#))  
84 and thus the litter trait control over microbial communities changes along community-weighted  
85 mean (CWM) trait values. Alternatively, the “niche complementarity hypothesis” argues that  
86 the functional diversity (FD) of trait-values promotes an ecosystem’s functioning ([Petchey and](#)  
87 [Gaston, 2006](#); [Diaz et al., 2007](#)), for instance, by improving the availability of complementary  
88 resources for microbial communities ([Wardle et al., 1997](#); [Barantal et al., 2014](#); [Handa et al.,](#)  
89 [2014](#)). These two mechanisms can operate simultaneously by affecting microbial communities  
90 as a result of plant (litter) composition change. However, our knowledge about the relative  
91 importance of these theories in controlling microbial decomposer communities is very limited.

92         The influence of tree species diversity on litter microbial communities has been studied  
93 in boreal, temperate and tropical ecosystems, while information from Mediterranean  
94 ecosystems is extremely scarce (e.g. [Shihan et al., 2017](#); [Santonja et al., 2017a, 2017b](#)).  
95 Mediterranean forests have a different species composition than temperate forests due to the  
96 Mediterranean climate characteristics that have shaped species distribution ([Quézel and Médail,](#)  
97 [2003](#)). Remarkable contrasts of temperature and humidity across seasons, and in particularly  
98 the summer drought period, correspond to particular leaf litter traits, including secondary

99 metabolites (Chomel et al., 2014; Chomel et al., 2016; Hashoum et al., 2017), that have the  
100 potential to influence microbial decomposer communities (Schimel et al., 2007; Williams and  
101 Rice, 2007; Brockett et al., 2012).

102 In this study we assessed the potential consequences of a forest composition shift on  
103 microbial communities associated with decomposing leaves, including both loss and gain of  
104 tree species. We conducted a full-factorial *in situ* decomposition experiment over a gradient of  
105 litter species diversity in a Mediterranean downy oak forest. Downy oak (*Quercus pubescens*  
106 Willd.) is broadly distributed from northern Spain to the Caucasus (Quézel and Médail, 2003),  
107 and is the dominant species structuring many forests of the northern parts of the Mediterranean  
108 basin. We used leaf litter from the three dominant woody plant species naturally present in the  
109 forest (*Q. pubescens*, *Acer monspessulanum* L. and *Cotinus coggygria* Scop.) and one pine  
110 species (*Pinus halepensis* Mill.), which may become more frequent in downy oak forest in the  
111 future in response to climate change (i.e. under warmer and drier environment; Gauchere et  
112 al., 2008; Bede-Fazekas et al., 2014). We aimed to determine the effects of (i) litter species  
113 richness, (ii) litter species composition, (iii) litter species identity and (iv) litter functional trait  
114 values on the dynamics of microbial communities, during one-year leaf litter decomposition.  
115 Using fingerprinting methods, we explored in parallel fungal and bacterial community  
116 dynamics at six time points, expecting to reveal differences across microbial decomposers  
117 groups that have distinct trophic niches (Boer et al., 2005; Buée et al., 2009; Lopez-Mondejar  
118 et al., 2015). We hypothesized that H1: microbial diversity and community structure associated  
119 to decomposed leaves or needles change with decomposition time, as the litter quality changes  
120 over the course of decomposition (Snajdr et al., 2011; Baldrian et al., 2012); H2: microbial  
121 diversity increases with the increase of litter species richness, by increasing resource diversity  
122 and microhabitat heterogeneity; H3: microbial community parameters (diversity and  
123 community structure) are also determined by the plant identity in the litter mixture, as

124 differences of litter quality among plant species would lead to distinct effects on microbial  
125 community parameters; H4: microbial diversity responds to “niche complementarity  
126 hypothesis” rather than to “mass-ratio hypothesis”, as litter mixtures with contrasting litter  
127 quality improve the availability of different resources and microhabitat for microorganisms. We  
128 also hypothesized that H5: the relationships between microbial decomposers and litter diversity  
129 decrease with decomposition time, as resource diversity changes during leaf litter  
130 decomposition, leading to homogenization of litter quality across litter mixtures.

131

## 132 **2. Materials and methods**

133

### 134 **2.1. Study site**

135 This study was conducted in the Oak Observatory at the OHP (O<sub>3</sub>HP) experimental site  
136 located in the research center “Observatoire de Haute Provence”, 60 km north of Marseille,  
137 South of France (43°56'115" N, 05°42'642" E). The site is 680 m above sea level, and presents  
138 a mean annual temperature of 11.9 °C and a mean annual precipitation of 830 mm (1967 - 2000  
139 period, WMO standard temperature and precipitation: 1960-2003 St Michel l'Observatoire /  
140 Meteo France 04192001; 2003-2010 Dauphine / Meteo France 04068001 - on and close to the  
141 OHP, respectively). Temperature and rainfall during the experiment showed a seasonal  
142 distribution characteristic of a Mediterranean climate, with maximum rainfall in June and  
143 October, maximum temperature in July and August, and a dry summer season lasting less than  
144 two months, typical of a supramediterranean bioclimatic stage ([Supplementary Fig. S1](#)).  
145 According to the French Référentiel Pédologique ([Baize and Girard, 1998](#)), the soil is a pierric  
146 calcosol (with S horizon between limestone rocks) or a calcarisol when limestone appears less  
147 than 25 cm deep. The pH is between 6.5 and 7.5 for the A horizon and 7.5 the for S horizon.

148 According to the WRB classification ([IUSS Working group WRB, 2006](#)) the soil can be  
149 classified as a mollic leptosol.

150 The study site is a Mediterranean natural old-growth oak forest belonging to the site  
151 Natura 2000 “FR9302008 Vachères”, in which 53 different plant species were identified. This  
152 forest already existed in the late 18th century ([Hilaire et al., 2012](#)) and was managed for  
153 centuries by coppicing. *Q. pubescens* (Downy oak: 75% coverage) and *A. monspessulanum*  
154 (Montpellier maple: 25% coverage) are the two dominant tree species, with understory  
155 vegetation dominated by *C. coggygia* (smoke tree: 30% coverage). *P. halepensis* (Aleppo pine)  
156 is present very close to the downy oak forest but only in open environments.

157

## 158 **2.2. Experimental set-up**

159 This experiment used litter of four species: *A. monspessulanum*, *C. coggygia*, *P.*  
160 *halepensis* and *Q. pubescens* that will be named *Acer*, *Cotinus*, *Pinus* and *Quercus* hereafter.  
161 Freshly abscised leaves of the four species were collected over the whole period of maximum  
162 litter fall from October to November using litter traps suspended under the relevant species.  
163 Leaves were dried at ambient temperature and stored until the beginning of the experiment.

164 Leaf litter decomposition was studied for 320 days using the litterbag method ([Swift et](#)  
165 [al., 1979](#)). Litterbags measuring 20 cm × 20 cm with a mesh size of 4 mm were filled with 10  
166 g of dry leaf litter and placed in the field in January. Fifteen species mixtures were made with  
167 an equal partitioning on a dry mass basis between species: a single-mixture litter for each  
168 species (four types), all possible two-species mixture combinations (six types), all possible  
169 three-species mixtures (four types) and a four-species mixture. There were thus 15 treatments,  
170 comprising four levels of species richness: 1, 2, 3 and 4 species. A total of 360 litterbags (15  
171 types × 6 sampling dates × 4 replicates) were used for the experiment. We used a nested  
172 experimental design with four blocks of 100 m<sup>2</sup> (spaced of 10 m) where replicates were



173 randomly scattered and spaced of 1 m in each block. We retrieved four replicates of each  
174 treatment (i.e. one per block) every 50 (all the sampling dates) or 70 days (the last sampling  
175 date, because violent rain events prevented sampling).

176 After removal, litterbags were immediately sealed in plastic bags to prevent loss of litter  
177 material, and transported to the laboratory. Leaves were separated according to species, which  
178 was possible even with small fragments of litter owing to marked morphological differences  
179 among species. To prevent contamination of litter by soil, we wiped each leaf thoroughly before  
180 analysis. Samples were freeze-dried (Lyovac GT2®), weighed and ground to powder.

181

### 182 **2.3. Litter traits**

183 Four litter samples of each species were taken to determine the initial characteristics of  
184 the litter ([Supplementary Table S1](#)). Carbon (C) and nitrogen (N) content of the litters were  
185 determined by thermal combustion on a Flash EA 1112 series C/N elemental analyzer (Thermo  
186 Scientific, USA).

187 Phosphorus (P) was extracted with 20 ml of nitric acid from remaining dry ash after  
188 combustion of 0.5 g of subsamples at 500 °C for 5 h in a muffle furnace. The pH was adjusted  
189 to 8.5 with a 40% NaOH solution; 1 ml of each sample, 0.2 ml of mixed reagent (emetic tartar  
190 and ammonium molybdate solution), 0.04 ml of ascorbic acid and 0.76 ml of distilled water  
191 were placed directly in a spectrophotometer microcuvette. After 150 min, the reaction was  
192 completed, and phosphorus concentration was measured at 780 nm with a UV/Vis  
193 spectrophotometer (Thermo Scientific, USA).

194 Total phenolic concentrations were measured colorimetrically by the method of  
195 [Peñuelas et al. \(1996\)](#) using gallic acid as a standard. A 0.25 g litter sample was dissolved in 20  
196 ml of a 70% aqueous methanol solution, shaken for 1 h and then filtered (0.45 µm filter); 0.25  
197 ml of filtered extract was mixed with 0.25 ml Folin-Ciocalteu reagent ([Folin and Denis, 1915](#)),

198 0.5 ml of saturated aqueous Na<sub>2</sub>CO<sub>3</sub> (to stabilize the color reaction) and 4 ml of distilled water.  
199 After 60 min, the reaction was completed, and concentration of phenolics was measured at 765  
200 nm on a UV/Vis spectrophotometer (Thermo Scientific, U.S.A.).

201 Terpenes were extracted from 0.5 g of litter sample with 5 ml of dichloromethane, and  
202 50 µl of an internal standard (dodecane) was added. The samples were filtered and then  
203 analyzed by gas-chromatography/mass-spectrometry (GC-MS) on an Agilent HP6890 system  
204 equipped with an MSD5973 Network mass detector, an ALS7673 automatic injector and an  
205 HP5-MS apolar column (30 m × 0.25 mm × 0.25 µm; J&W Agilent Technologies, Crawford  
206 Scientific, Lanarkshire, Scotland, UK). Retention indices were determined relative to injected  
207 Wisconsin Diesel Range Hydrocarbons (Interchim, Montluçon, France) and confirmed by  
208 comparison against expected literature values (Adams, 2007). Finally, the total terpene  
209 concentration was measured as the sum of each of the individual terpene compounds.

210 To assess the “mass-ratio hypothesis”, we calculated the community-weighted mean  
211 traits (CWM) of the litter mixtures as the average trait values of the litter mixtures following  
212 Garnier et al. (2004) as  $Trait_{CWM} = \sum_{i=1}^n p_i \times trait_i$  where  $p_i$  is the relative abundance for  
213 species  $i$  and  $trait_i$  is the trait value for species  $i$ . To assess the “niche complementarity  
214 hypothesis”, we calculated functional dissimilarity (FD) of litter mixtures according to Rao’s  
215 quadratic entropy (Botta Dukát, 2005) for each litter mixture as:  $Trait_{FD} = \sum_{i=1}^n \sum_{j=1}^n p_i p_j * d_{ij}$   
216 where  $p_i$  and  $p_j$  are the relative abundance for shrub species  $i$  and  $j$  in the litter mixture, and  $d_{ij}$   
217 the Euclidian distance between species  $i$  and  $j$  for the trait considered. Because the measured  
218 traits differ in their units, we used normalized values (using a z-scored standardization so as to  
219 get a mean of zero and a standard deviation of one) to calculate functional dissimilarity.  
220 According to the “mass-ratio hypothesis”, the highest scores of CWM are reached for species  
221 within the mixture that exhibit the highest trait values. On the other hand, according to the  
222 “niche complementarity hypothesis”, the highest scores of FD are reached for very dissimilar

223 litter species within the mixture. Since we predicted that microbial diversity responds to the  
224 “niche complementarity hypothesis” rather than to the “mass-ratio hypothesis” (i.e. research  
225 hypothesis H4), we expect that the increase in microbial diversity to be better correlated to the  
226 increase in FD values than to the increase in CWM values.

227

#### 228 ***2.4. Bacterial and fungal communities***

229 Extraction of microbial cells from litter was achieved by washing 100 mg of freeze-  
230 dried ground litter material with 1.5 ml of sterile saline solution (0.9 % NaCl; w/v). The  
231 suspension was mixed by vortexing for 5 s and then centrifuged for a few seconds in order to  
232 pellet the leaf residues. Microorganisms were recovered from the supernatant following transfer  
233 to a new microtube and being centrifuged (16000 g, 30 min) to form a microbial pellet that was  
234 frozen at -20°C until DNA extraction. Total DNA was extracted from each microbial sample  
235 by using the PowerSoil-htp 96 wells DNA isolation kit (MoBio, Laboratories, Inc.), adjusting  
236 the protocol to the use of a vacuum manifold. DNA samples were stored at -20°C until analysis.

237 We used Automated Ribosomal Intergenic Spacer Analysis (ARISA) to characterize  
238 bacterial communities ([Ranjard et al., 2001](#)), and terminal Restriction Fragment Length  
239 Polymorphism (tRFLP) to characterize fungal communities ([Liu et al., 1997](#)). The bacterial  
240 16S-23S rDNA Intergenic Spacer (ITS) was amplified using the primers [6fam]-s-d-Bact-1522-  
241 b-S-20 (5'-TGCGGCTGGATCCCCTCCTT-3') and L-D-Bact-132-a-A-18 (5'-  
242 CCGGGTTTCCCCATTCGG-3'). The fungal 18S-5,8S ITS1 region was amplified using the  
243 primers [HEX]-ITS1-F (5'-CTTGGTCATTTAGAGGAAGTAA-3'; [Gardes and Bruns, 1993](#))  
244 and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'; [White et al., 1990](#)). Both PCR reactions  
245 were performed in 20 µl mixtures containing 200 µM dNTPs, 5 % (v:v) DMSO, 0.5 µM of each  
246 primer and 0.25 units GoTaq DNA-polymerase with the corresponding 5X PCR buffer  
247 (Promega). After initial denaturation at 94°C for 5 minutes, we ran 35 cycles of: denaturation

248 at 94°C for 1 minute, followed by annealing at 55°C for 1 minute, elongation at 72°C for 1  
249 minute and a final elongation at 72°C for 10 minutes. PCR products of the fungal ITS were  
250 digested by adding 5 U of *Hinf*I restriction enzyme and 2 µl of the corresponding restriction  
251 buffer (Fermentas). Following verification of the ARISA and tRFLP products by using 2%  
252 NuSieve-agarose gel electrophoresis, these products were diluted with sterile distilled water  
253 (1/20), and analyzed with a capillary sequencer ABI 3730 (Applied Biosystem). One microliter  
254 of the diluted sample was mixed with 0.8 µl of GeneScan-2500 ROX-labelled size standard and  
255 8 µl of deionized formamide; denaturation was completed at 95°C for 5 min before capillary  
256 electrophoresis in the POP-7 polymer, during 3 h with 7.5 kV run-voltage. The output series of  
257 peak-sizes corresponding to bacterial or fungal operating taxonomic units (OTUs) were  
258 analyzed by using the GeneMapper® v4.1 program (Applied Biosystem). Size standard peaks  
259 were defined individually, parameters of the internal AFLP-method were set up in order to  
260 detect peak-sizes in the 300-1200 bp range, with bin-windows set up to one bp and lower  
261 detection limits of peak-heights fixed individually at values ranging from 1 to 100 Raw  
262 Fluorescent Units (RFUs).

263

## 264 **2.5. Statistical analyses**

265 Statistical analyses were performed with the PRIMER-E software (version 6.1, Primer-  
266 E Ltd, Plymouth, United Kingdom) for multivariate analyses, and with the R software (version  
267 3.3.1, The R Foundation for Statistical Computing, Vienna, Austria) for univariate analyses.

268 Bacterial and fungal OTU (Operational Taxonomic Unit) - abundance matrices based  
269 on ARISA and tRFLP fingerprints were analyzed with PRIMER-E. The “DIVERSE” routine  
270 was ran to obtain the Shannon’s indices of diversity ( $H' = -\sum p_i \times \log_{10}p_i$ ), where  $p_i$  is the  
271 proportion of the total abundance arising from the  $i^{th}$  species. The abundance value of each  
272 OTU was standardized by total abundance of the sample and then log-transformed ( $\text{Log } X+1$ )

273 before generating Bray-Curtis similarity matrices with minimum transformed-value as a  
274 dummy variable. To visualize distance between samples, we used Principal Coordinate  
275 Ordination (PCO) plots. We assessed whether microbial community structure differed  
276 according to the time of decomposition, the litter diversity and the presence of plant species in  
277 the litter mixtures by using analysis of similarity (ANOSIM) routine and multivariate analysis  
278 of variance (PERMANOVA) that was set up with 9999 permutations and unrestricted  
279 permutation of raw data method. The dispersion of the microbial communities shown by the  
280 PCO was studied using the PERMDISP routine. We determined bacterial and fungal  
281 communities' turnover rates during litter decomposition ([Supplementary Table S2](#);  
282 [Supplementary Fig. S2](#)) for each litter mixture treatment, as the linear regression estimate of  
283 Bray-Curtis dissimilarity changes, between samples collected after 50 days decomposition and  
284 all successive collection dates (i.e. following 100, 150, 200, 250 and 320 days decomposition).

285 We used a general linear model approach to test for the effects of litter diversity  
286 (separated in i) litter species composition, ii) litter species richness, iii) litter species identity  
287 and iv) litter functional traits) on bacterial and fungal diversity. Due to the large number of  
288 potentially important predictors, we carried out three distinct statistical models. The first model  
289 tested the impact of litter diversity (decomposed in litter species richness and litter species  
290 composition), time of decomposition (expressed as harvest date) and block on bacterial and  
291 fungal diversity (expressed as the Shannon-Wiener index). The second model tested the impact  
292 of litter species identity (i.e. the presence/ absence of a particular species), time of  
293 decomposition and block on bacterial and fungal diversity.

294 For a more detailed understanding of how the diversity of leaf litter affected microbial  
295 diversity, we evaluated the effects of mean traits ( $Trait_{CWM}$ ) and functional trait dissimilarities  
296 ( $Trait_{FD}$ ) of the 8 measured litter traits ([Supplementary Table S1](#)). A principal component  
297 analyses (PCA), based on these 8 litter traits across litter mixtures, allowed to define the two

298 first components of each PCA (i.e. CWM1 and CWM2, and FD1 and FD2) as characteristic  
299 vectors of CWM and FD, respectively. We then performed a third model in order to decipher  
300 the relative contributions of CWM1, CWM2, FD1, FD2, time of decomposition and block on  
301 bacterial and fungal diversity.

302 Finally, we performed regressions analyses to test for the relationships between litter  
303 functional traits ( $Trait_{CWM}$  and  $Trait_{FD}$ ) and bacterial and fungal community turnover rates  
304 ([Supplementary Table S2](#)).

305

### 306 **3. Results**

307

#### 308 ***3.1. Microbial community dynamics during decomposition***

309 Overall, 746 bacterial and 448 fungal OTUs were detected in the litter during this  
310 experiment. Time of litter decomposition explained the largest proportion of the variation in  
311 microbial diversity ([Tables 1-3](#)). Bacterial diversity increased at the beginning of litter  
312 decomposition and remained constant from 150 days to the end of the experiment ([Fig. 1a](#)).  
313 Fungal diversity increased during decomposition and reached its maximum value after 320 days  
314 of litter decomposition ([Fig. 1d](#)). Microbial community dissimilarities varied importantly  
315 during time of litter decomposition (PERMANOVA, bacterial community: Pseudo- $F = 15.5$ ,  $P$   
316  $< 0.001$ ; fungal community: Pseudo- $F = 19.1$ ,  $P < 0.001$ ). Both bacterial and fungal  
317 communities changed during the decomposition experiment ([Supplementary Fig. S3](#)). While  
318 the dissimilarity among fungal communities remained steady during decomposition  
319 ([Supplementary Fig. S3b](#)), the dissimilarity among bacterial communities decreased leading to  
320 the homogenization of bacterial community across mixtures over time ([Supplementary Fig.](#)  
321 [S3a](#)).

322

### 323 **3.2. Litter diversity effects on microbial communities**

324 Litter species composition accounted for a higher portion of the overall variance of  
325 microbial diversity than litter species richness (Table 1; Supplementary Table S3). Bacterial  
326 diversity was higher in the three- and four-species litter mixtures compared to the single and  
327 two-species litter mixtures (Fig. 1b). Fungal diversity was the lowest in single-species litter  
328 mixtures; it increased with increasing species richness and reached its maximum value in the  
329 three- and four-species litter mixtures (Fig. 1e). Microbial diversity differed among the 15 litter  
330 species combinations (Figs. 1c and 1f). However, the litter mixture effects on microbial  
331 diversity, including both litter species composition and litter species richness, varied during the  
332 course of the decomposition (Litter species richness  $\times$  Time and Litter species composition  $\times$   
333 Time interactions, Table 1). Specifically, bacterial diversity was partially affected by litter  
334 species richness (i.e. after 100, 200 and 320 days of decomposition; Supplementary Table S3;  
335 Figs. 2b, 2d and 2f), while fungal diversity was affected by litter species richness during all the  
336 decomposition process, but not after 320 days of decomposition (Supplementary Table S3; Fig.  
337 2l). Litter species composition affected microbial diversity throughout the decomposition  
338 periods, except for bacterial diversity after 150 days (Supplementary Table S3).

339 Litter species richness influenced the fungal community dissimilarity by increasing the  
340 resemblance of community in multi-species litter mixtures compared to single-species litter  
341 (PERMANOVA, Pseudo- $F = 2.67$ ,  $P < 0.01$ ). On the contrary, litter species richness had no  
342 effect on the bacterial community dissimilarity (PERMANOVA, Pseudo- $F = 1.08$ ,  $P = 0.2403$ ).  
343 Litter species composition strongly influenced fungal communities, but had reduced effects on  
344 bacterial communities (Supplementary Fig. S4b).

345

### 346 **3.3. Litter identity effects on microbial communities**

347 Measuring the response of microbial diversity to the presence of each plant species in  
348 the mixtures revealed the major effects of *Quercus*, that increased both bacterial and fungal  
349 diversities all along the decomposition process and the effect of *Pinus* that increased  
350 specifically fungal diversity at most sampling time. Except for *Quercus*, the effects of the three  
351 other plant species on microbial diversity strongly depended on decomposition time (Table  
352 2). The presence of *Acer* enhanced bacterial diversity after 100 and 320 days of decomposition  
353 (Time  $\times$  *Acer* presence interaction, Table 2; Fig. 3a). *Cotinus* decreased both bacterial and  
354 fungal diversities after 50 days of decomposition and, on the opposite, positively affected both  
355 bacterial and fungal diversities at later decomposition stages (Time  $\times$  *Cotinus* presence  
356 interaction, Table 2; Figs. 3b and 3f). The presence of *Pinus* enhanced fungal diversity until  
357 250 days of decomposition, whereas *Pinus* decreased both bacterial and fungal diversities after  
358 320 days of decomposition (Time  $\times$  *Pinus* presence interaction, Table 2; Figs. 3c and 3g).  
359 Presence of *Quercus* enhanced both bacterial and fungal diversities throughout the  
360 decomposition process (Table 2; Figs. 3d and 3h).

361 Microbial community dissimilarities also differed according to the identity of the plant  
362 species in the litter mixture (Supplementary Fig. S5). The bacterial community dissimilarity  
363 was affected by the presence of *Acer* (ANOSIM,  $R = 0.16$ ,  $P < 0.001$ ; Supplementary Fig. S5a)  
364 and *Pinus* (ANOSIM,  $R = 0.13$ ,  $P < 0.001$ ; Supplementary Fig. S5e), but not by the presence  
365 of *Cotinus* or *Quercus*. The fungal community dissimilarity was mainly affected by the presence  
366 of *Pinus* (ANOSIM,  $R = 0.32$ ,  $P < 0.001$ ; Supplementary Fig. S5f) and to a lower extent by  
367 *Acer* (ANOSIM,  $R = 0.18$ ,  $P < 0.001$ ; Supplementary Fig. S5b). Interestingly, the influence of  
368 *Quercus* on fungal community dissimilarity was covered by the influence of *Pinus* (ANOSIM,  
369  $R = 0.16$ ,  $P < 0.001$ ; Supplementary Fig. S5h). In other words, the presence of *Quercus* had a  
370 different effect on litter mixtures either containing or excluding *Pinus*.

371



### 372 **3.4. Control of microbial communities by mass ratio or niche complementarity hypothesis**

373 Principal component analysis of CWM traits showed that the first PCA axis (CWM1),  
374 explaining 66.3% of traits variation, was determined mainly by high C and terpen  
375 concentrations and low phenolic concentration (Fig. 4a). The second PCA axis (CWM2),  
376 explaining 26.4% variation, was related mainly to high values of C:N and C:P ratios, and low  
377 values of N and P concentrations (Fig. 4a). Principal component analysis of FD traits showed  
378 that the first PCA axis (FD1), explaining 45% of trait dissimilarity variation, was related mainly  
379 to increasing dissimilarities of phenolic concentration, and of C:P and N:P ratios (Fig. 4b). The  
380 second axis (FD2), explaining 30.5% variation, was related mainly to increasing dissimilarity  
381 of terpen and C concentrations, and of C:N ratio (Fig. 4b).

382 When evaluating jointly the effects of CWM and FD traits, we found that bacterial  
383 diversity was mostly controlled by time of decomposition, FD2 and the interaction FD2  $\times$  time  
384 of decomposition (Table 3). Increasing FD2 scores (i.e. decreasing functional dissimilarity of  
385 terpen, C and C:N ratios) were related to higher bacterial diversity index after 200, 250, and  
386 320 days of decomposition, as indicated by the FD2  $\times$  time of decomposition interaction (Table  
387 3; Fig. 5) and by the negative relationship between the bacterial communities turnover rate and  
388 FD2 (Table 4).

389 In contrast to bacterial diversity, both CWM and FD traits strongly controlled fungal  
390 diversity (Table 3). Fungal diversity was significantly affected by time of decomposition,  
391 CWM1, FD2 as well as the interactions CWM1  $\times$  time of decomposition, CWM2  $\times$  time of  
392 decomposition and FD2  $\times$  time of decomposition (Table 3). Increasing CWM1 scores  
393 (correlated mainly to decreasing phenolic concentration) were related to higher fungal diversity,  
394 except after 320 days of decomposition (CWM1  $\times$  time of decomposition interaction, Table 3).  
395 Increasing CWM2 scores (mainly correlated to increasing values of C:N and C:P ratios) were  
396 related to higher fungal diversity after 50 and 100 days of decomposition (CWM2  $\times$  time of

397 decomposition interaction, [Table 3](#)). The relationship between FD2 and fungal diversity  
398 opposed to that observed with bacterial diversity: increasing FD2 scores (i.e. correlated mainly  
399 to decreasing C, terpen and C:N dissimilarities) were related to lower fungal diversity, except  
400 after 320 days of decomposition (FD2  $\times$  time of decomposition interaction, [Table 3](#); [Fig. 5](#)).

401 The relationships between the dynamic of fungal diversity during litter decomposition  
402 and both CWM1 and FD2 values were confirmed by similar relationships with fungal  
403 communities' turnover rate (i.e. negative correlation of fCTR with CWM1 and positive  
404 correlation of fCTR with FD2; [Table 4](#)).

405

#### 406 **4. Discussion**

407 In line with our first hypothesis, microbial community's changes were important across  
408 decomposition time. While the fingerprinting methods used are likely to detect DNA of living  
409 microorganisms and relic DNA that is not fully degraded during the experiment ([Carini et al.,](#)  
410 [2017](#)), our result is consistent with previous studies on the seasonal variation of microbial  
411 communities, reporting that variations in soil moisture, temperature or leaf litter chemistry  
412 during decomposition were most likely causes of changes in microbial communities ([Snajdr et](#)  
413 [al., 2011](#); [Voriskova et al., 2014](#); [Lopez-Mondejar et al., 2015](#); [Purahong et al., 2015](#)). The  
414 bacterial community showed a faster dynamic and reached a higher diversity than the fungal  
415 community, which confirms previous studies ([Baldrian et al., 2012](#); [Urbanova et al., 2015](#);  
416 [Santonja et al., 2017a](#)). Interestingly, we observed that the community heterogeneity among  
417 litter mixtures remained constant along the decomposition process for fungi, while it gradually  
418 disappeared for bacteria (Supplementary Fig. S3). This finding supports the idea that the rapid  
419 establishment of bacterial diversity is driven by the availability of simple compounds at early  
420 decomposition stages ([Moorhead and Sinsabaugh, 2006](#)); then, simple compounds decrease in  
421 quantity to the advantage of recalcitrant compounds, which triggers selective changes in the

422 bacterial decomposer community. Comparatively, the fungal decomposer community showed  
423 an apparently lower diversity that gradually established to its maximum value at later  
424 decomposition stages. These results suggest that diversification of the fungal decomposer  
425 community at advanced litter decomposition stages relies on competition for recalcitrant  
426 compounds utilization and to late colonization of the remaining leaf tissue (Moorhead and  
427 Sinsabaugh, 2006).

428 In agreement with our second hypothesis, microbial diversity increased with litter  
429 species richness. Previous studies have provided evidence that microbial biomass and diversity  
430 respond positively to litter mixing (Blair et al., 1990; Bardgett and Shine, 1999; Chapman and  
431 Newman, 2010; Santonja et al., 2017a), with differences in litter properties causing resource  
432 diversification and habitat heterogeneity for microbial decomposers (Tilman et al., 1997;  
433 Hooper and Vitousek, 1998; Hättenschwiler et al., 2011). Unlike Santonja et al. (2017a) who  
434 reported that only fungal diversity increased after one year of litter decomposition in a  
435 Mediterranean oak shrubland, here we observed that both bacterial and fungal diversities  
436 increased during the process in the studied Mediterranean oak forest. We also clearly  
437 demonstrated that the litter diversity effects are mediated by litter species composition rather  
438 than litter species richness, highlighting the importance of litter species identity in litter  
439 mixtures as a driver of microbial community diversity. We partially confirmed our hypothesis  
440 that microbial responses to litter species diversity (both composition and richness) decrease  
441 with decomposition time. Because it is generally assumed that initial differences in litter quality  
442 among different plant species converge to similar quality during litter decomposition (e.g.  
443 Moore et al., 2005; Preston et al., 2009), we expected to observe greater litter diversity control  
444 of microbial communities during the early stages of decomposition. Interestingly, litter  
445 diversity effects varied during the experiment but were not restricted to the early decomposition  
446 stages.

447 In line with our third hypothesis, microbial community proxies were sensitive to the  
448 litter species identity, as the presence of the four plant species affected - positively or negatively  
449 - microbial diversities. Previous studies also highlighted the importance of tree species identity  
450 as drivers of microbial communities, rather than changes in tree species richness (Scheibe et  
451 al., 2015; Urbanova et al., 2015). As for the litter diversity effect, the litter identity effect on  
452 microbial communities was irregular across decomposition times. Interestingly, the positive  
453 effects of *Q. pubescens* on both bacterial and fungal diversities remained constant during all the  
454 decomposition process. Since *Q. pubescens* is the dominant tree in the studied Mediterranean  
455 oak forest, microorganisms could be well adapted to colonize and degrade this litter type. *A.*  
456 *monspessulanum* enhanced sporadic bacterial diversity, but showed a noticeable effect on  
457 microbial community dissimilarities, suggesting an important contribution on bacterial  
458 community composition. This effect is probably related to high contents of N and P  
459 (Supplementary Table S1) that are generally limiting nutrients in the soil and would be mostly  
460 favorable to heterotrophic bacterial decomposers (Cleveland and Liptzin 2007; Mooshammer  
461 et al., 2014). *C. coggygria* expressed negative effects on both bacterial and fungal diversities  
462 only at the earliest decomposition stage, which then reverted to a positive effect at later  
463 decomposition stages. This could be attributed to its strong initial phenolic content  
464 (Supplementary Table S1) that was shown to affect microbial colonization of leaf litter (Chomel  
465 et al., 2014) and is later on rapidly lixiviated (73% phenolic content loss after 100 days of  
466 decomposition; Santonja et al., 2015). As the alien species of the study, *P. halepensis* is the  
467 only coniferous species among the four studied woody-plant and is characterized by the highest  
468 terpene content. Its presence in the litter mixtures noticeably enhanced fungal diversity until  
469 later decomposition stages (although this effect decreased during the experiment; Fig. 3g) and  
470 also showed striking effects on fungal community dissimilarities. These results suggest that  
471 terpenes favor fungal decomposer diversity. Interestingly the effect of *Q. pubescens*

472 superimposed to the effects of *P. halepensis* leading to four distinct fungal communities  
473 (Supplementary Fig. S5h). Overall, the fungal community composition showed essentially  
474 more susceptibility to the identity of plant species present in the leaf litter mixtures than the  
475 bacterial community composition (Supplementary Fig. S4), stressing the tight and specialist  
476 relationship between plant species and fungal decomposer community, compared to the more  
477 relaxed and generalist relationship with bacterial decomposer community (Moorhead and  
478 Sinsabaugh, 2006).

479 In line with our fourth hypothesis, both mass-ratio (measured through community  
480 weighted mean (CWM) of litter traits) and niche complementarity (measured through  
481 functional dissimilarity (FD) of litter traits) hypotheses contributed to the litter diversity effects  
482 on microbial decomposer communities, with a greater relative importance of the niche  
483 complementary compared to the mass ratio hypothesis. According to the niche complementarity  
484 hypothesis, litter mixtures with contrasting litter quality improve the availability of different  
485 resources for decomposers (Tilman et al., 1997; Hooper and Vitousek, 1998; Schimel and  
486 Hättenschwiler, 2007). But, surprisingly, increasing functional dissimilarity of litter traits -  
487 hence resource availability, was unfavorable to the bacterial community turnover rate and  
488 diversity while conversely, it was beneficial to the fungal community turnover rate and diversity  
489 in the studied Mediterranean oak forest. In contrast to bacterial diversity, the first and second  
490 components of the CWM PCA were both associated to fungal diversity, highlighting the  
491 contribution of the traits mass ratio as additional drivers of fungal diversity. Decreasing mean  
492 phenolic concentration of litter mixtures (i.e. CWM1) was related to higher turnover rate of the  
493 fungal community, affecting fungal diversity until 250 days of decomposition. This suggests  
494 that fungal communities are more sensitive to phenolics than bacterial communities.  
495 Interestingly, increasing mean C:N ratio value of litter mixtures (i.e. CWM2) was related to  
496 higher fungal diversity after 50 and 100 days of decomposition. The C:N ratio of bacterial

497 biomass is expected to vary between 3 and 6 while the C:N ratio of fungal biomass is expected  
498 to vary between 5 and 15 (McGill et al., 1981). Subsequently, fungal communities are expected  
499 to have lower N requirement than bacteria (Güsewell and Gessner, 2009), and thus, being less  
500 N limited, they would be able to colonize and develop on poor-quality litter at early  
501 decomposition stages.

502

## 503 **5. Conclusion**

504 Our results from a field experiment clearly demonstrated that potential shifts in plant  
505 species diversity and associated litter traits may have strong cascading impacts on microbial  
506 decomposer communities in a Mediterranean oak forest. Both bacterial and fungal communities  
507 were influenced by litter species diversity, litter identity and litter functional traits. However,  
508 the intensity and the direction of these effects varied strongly during decomposition. Litter  
509 diversity effects were mediated by litter species composition rather than litter species richness,  
510 highlighting the importance of litter species identity (and associated litter traits) as driver of  
511 microbial communities. We also underscored the importance of *Quercus pubescens* (i.e. the  
512 dominant tree species of the studied oak forest) and *Pinus halepensis* (i.e. an alien tree species  
513 that may become more frequent in the studied oak forest in response to climate change) in  
514 structuring microbial communities. Both community weighted mean (CWM) litter traits (mass-  
515 ratio hypothesis) and functional dissimilarity (FD) of litter traits (niche complementarity  
516 hypothesis) contributed to litter diversity effects, with a greater relative importance of FD  
517 compared to CWM, and with an overall stronger impact on fungal compared to bacterial  
518 communities. Collectively, these findings also suggest that different aspects of litter diversity  
519 control microbial communities during litter decomposition.

520

## 521 **Acknowledgement**

522 We thank Sylvie Dupouyet, Jean-Philippe Orts and Ilja Reiter for their contribution to  
523 the set-up of the experiment and the field work, Caroline Lecareux, Alexia Pailler and Germain  
524 Bounou for their assistance to the chemical and microbial analyses, and the staff of the  
525 “Plateforme Génomique du Génopole de Toulouse Midi-Pyrénées” for technical assistance  
526 with the sequencing facilities. Funding was provided by the Centre National de la Recherche  
527 Scientifique (CNRS) through the EC2CO-BIOEFFECT action and the Agence Nationale pour la  
528 Recherche (ANR) through the project SecPriMe<sup>2</sup> (ANR-12-BSV7-0016-01). We also thank the  
529 French Region PACA and Europe for PhD grant attributed to Mathieu Santonja. Finally, we  
530 gratefully acknowledge the program MISTRALS (Mediterranean Integrated STudies at  
531 Regional And Local Scales), particularly the axe BioDivMeX, and the LabEx OT-Med (no  
532 ANR-11-LABX-0061) funded by the «Investissements d’Avenir» program of the French  
533 National Research Agency through the A\*MIDEX project (no ANR-11-IDEX-0001-02).

534

535 **Conflict of interest:** The authors declare that they have no conflict of interest.

536

## 537 **References**

538 Adams, R.P., 2007. Identification of essential oil components by gas chromatography / mass  
539 spectrometry, 4th ed. Allured Publishing Corporation, Carol Stream.

540 Amaral, J.A., Knowles, R., 1998. Inhibition of methane consumption in forest soils by  
541 monoterpenes. *Journal of Chemical Ecology* 24, 723-734.

542 Aponte, C., Garcia, L.V., Maranon, T., 2013. Tree species effects on nutrient cycling and soil  
543 biota: a feedback mechanism favouring species coexistence. *Forest Ecology and Management*  
544 309, 36-46.

545 Baize, D., Girard, M.C., 1998. A sound reference base for soils: the « référentiel pédologique ».  
546 INRA, Paris.

547 Baldrian, P., Kolarik, M., Stursova, M., Kopecky, J., Valaskova, V., Vetrovsky, T., Zifcakova,  
548 L., Snajdr, J., Ridl, J., Vlcek, C., Voriskova, J., 2012. Active and total microbial communities  
549 in forest soil are largely different and highly stratified during decomposition. *ISME Journal*,  
550 6, 248.

551 Barantal, S., Schimann, H., Fromin, N., Hättenschwiler, S., 2014. C, N and P fertilization in an  
552 Amazonian rainforest supports stoichiometric dissimilarity as a driver of litter diversity effects  
553 on decomposition. *Proceedings of the Royal Society of London Series B Biological Sciences*,  
554 281, 20141682.

555 Bardgett, R.D., 2005. *The biology of soil: a community and ecosystem approach*. Oxford  
556 University Press, Oxford.

557 Bardgett, R.D., Shine, A., 1999. Linkages between plant litter diversity, soil microbial biomass  
558 and ecosystem function in temperate grasslands. *Soil Biology and Biochemistry* 31, 317-321.

559 Bede-Fazekas, A., Horvath, L., Kocsis, M., 2014. Impact of climate change on the potential  
560 distribution of Mediterranean pines. *Quarterly Journal of the Hungarian Meteorological*  
561 *Service* 118, 41-52.

562 Blair, J.M., Parmelee, R.W., Beare, M.H., 1990. Decay-rates, nitrogen fluxes, and decomposer  
563 communities of single-species and mixed-species foliar litter. *Ecology* 71, 1976-1985.

564 Boer, W., Folma, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: impact  
565 of fungi on soil bacterial niche development. *FEMS Microbiology Reviews* 29, 795-811.

566 Botta Dukát, Z., 2005. Rao's quadratic entropy as a measure of functional diversity based on  
567 multiple traits. *Journal of Vegetation Science*, 16, 533-540.

568 Brockett, B.F.T., Prescott, C.E., Grayston, S.J., 2012. Soil moisture is the major factor  
569 influencing microbial community structure and enzyme activities across seven biogeoclimatic  
570 zones in western Canada. *Soil Biology and Biochemistry* 44, 9-20.



571 Buée, M., De Boer, W., Martin, F., Van Overbeek, L., Jurkevitch, E., 2009. The rhizosphere  
572 zoo: an overview of plant-associated communities of microorganisms, including phages,  
573 bacteria, archaea, and fungi, and of some of their structuring factors. *Plant and Soil* 321, 189-  
574 212.

575 Calderon, F.J., Jackson, L.E., Scow, K.M., Rolston, D.E., 2001. Short-term dynamics of  
576 nitrogen, microbial activity, and phospholipid fatty acids after tillage. *Soil Science Society*  
577 *American Journal* 65, 118-126.

578 Carini, P., Marsden, P.J., Leff, J.W., Morgan, E.E., Strickland, M.S., Fierer, N., 2017. Relic  
579 DNA is abundant in soil and obscures estimates of soil microbial diversity. *Nature*  
580 *Microbiology* 2, 16242.

581 Chapman, S.K., Newman, G.S., 2010. Biodiversity at the plant-soil interface microbial  
582 abundance and community structure respond to litter mixing. *Oecologia* 162, 763-769.

583 Chomel, M., Fernandez, C., Bousquet-Mélou, A., Gers, C., Monnier, Y., Santonja, M.,  
584 Gauquelin, T., Gros, R., Lecareux, C., Baldy, V., 2014. Secondary metabolites of *Pinus*  
585 *halepensis* alter decomposer organisms and litter decomposition during afforestation of  
586 abandoned agricultural zones. *Journal of Ecology* 102, 411-424.

587 Chomel, M., Guittonny-Larchevêque, M., Fernandez, C., Gallet, C., DesRochers, A., Paré, D.,  
588 Jackson, B.G., Baldy, V., 2016. Plant secondary metabolites: a key driver of litter  
589 decomposition and soil nutrient cycling. *Journal of Ecology* 104, 1527-1541.

590 Cleveland, C.C., Liptzin, D., 2007. C:N:P stoichiometry in soil: is there a “Redfield ratio” for  
591 the microbial biomass? *Biogeochemistry* 85, 235-252.

592 de Graaff, M.A., Classen, A.T., Castro, H.F., Schadt, C.W., 2010. Labile soil carbon inputs  
593 mediate the soil microbial community composition and plant residue decomposition rates.  
594 *New Phytologist* 188, 1055-1064.

595 Diaz, S., Cabido, M., 2001. Vive la difference: plant functional diversity matters to ecosystem  
596 processes. *Trends in Ecology and Evolution* 16, 649-655.

597 Diaz, S., Lavorel, S., De Bello, F., Quetier, F., Grigulis, K., Robson, T.M., 2007. Incorporating  
598 plant functional diversity effects in ecosystem service assessments. *Proceedings of the*  
599 *National Academy of Sciences of the USA* 104, 20684-20689.

600 Fierer, N., Schimel, J.P., Cates, R.G., Zou, J., 2001. Influence of balsam poplar tannin fractions  
601 on carbon and nitrogen dynamics in Alaskan taiga floodplain soils. *Soil Biology and*  
602 *Biochemistry* 33, 1827-1839.

603 Folin, O., Denis, W., 1915. A colorimetric method for the determination of phenols (and phenol  
604 derivates) in urine. *Journal of Biological Chemistry* 22, 305-308.

605 Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes-  
606 application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2, 113-118.

607 Garnier, E., Cortez, J., Billès, G., Navas, M.L., Roumet, C., Debussche, M., Laurent, G.,  
608 Blanchard, A., Aubry, D., Bellmann, A., Neill, C., Toussaint, J.P., 2004. Plant functional  
609 markers capture ecosystem properties during secondary succession. *Ecology* 85, 2630-2637.

610 Gartner, T.B., Cardon, Z.G., 2004. Decomposition dynamics in mixed-species leaf litter. *Oikos*  
611 104, 230-246.

612 Gauchere, C., Guiot, J., Misson, L., 2008. Changes of the potential distribution area of French  
613 Mediterranean forests under global warming. *Biogeosciences* 5, 1493-1504.

614 Grayston, S.J., Wang, S.Q., Campbell, C.D., Edwards, A.C., 1998. Selective influence of plant  
615 species on microbial diversity in the rhizosphere. *Soil Biology and Biochemistry* 30, 369-378.

616 Grime, J.P., 1998. Benefits of plant diversity to ecosystems: immediate, filter and founder  
617 effects. *Journal of Ecology* 86, 902-910.

618 Güsewell, S., Gessner, M.O., 2009. N:P ratios influence litter decomposition and colonization  
619 by fungi and bacteria in microcosms. *Functional Ecology* 23, 211-219.

620 Handa IT, Aerts R, Berendse F, Berg MP, Bruder A, Butenschoen O, ... Hättenschwiler S.  
621 2014. Consequences of biodiversity loss for litter decomposition across biomes. *Nature*  
622 509:218-221.

623 Hashoum, H., Santonja, M., Gauquelin, T., Saatkamp, A., Gavinet, J., Greff, S., Lecareux, C.,  
624 Fernandez, C., Bousquet-Mélou, A., 2017. Biotic interactions in a Mediterranean oak forest:  
625 role of allelopathy along phenological development of woody species. *European Journal of*  
626 *Forest Research* 136, 699-710.

627 Hättenschwiler, S., Tiunov, A.V., Scheu S., 2005. Biodiversity and litter decomposition in  
628 terrestrial ecosystems. *Annual Review of Ecology, Evolution, and Systematics* 36, 191-218.

629 Hättenschwiler, S., Fromin, N., Barantal S., 2011. Functional diversity of terrestrial microbial  
630 decomposers and their substrates. *Comptes Rendus Biologies* 334, 393-402.

631 Hilaire C., Orts J.P., Boer M., Gauquelin T. 2012. Le domaine de l'Observatoire de Haute-  
632 Provence (OHP): hermas et chênaie pubescente, du XVIIIe siècle à nos jours. *Courrier*  
633 *scientifique du Parc naturel régional du Luberon et de la Réserve de biosphère Luberon-Lure*  
634 11, 8-21.

635 Hooper, D.U., Vitousek, P.M., 1998. Effects of plant composition and diversity on nutrient  
636 cycling. *Ecological Monographs* 68, 121-149.

637 IUSS Working Group WRB, 2006. World reference base for soil resources 2006 - World Soil  
638 Resources Reports No. 103. FAO, Rome.

639 Kainulainen, P., Holopainen, T., Holopainen, J.K., 2003. Decomposition of secondary  
640 compounds from needle litter of Scots pine grown under elevated CO<sub>2</sub> and O<sub>3</sub>. *Global Change*  
641 *Biology* 9, 295-304.

642 Kraus, T.E., Dahlgren, R.A., Zasoski, R.J., 2003. Tannins in nutrient dynamics of forest  
643 ecosystems-a review. *Plant and Soil* 256, 41-66.

644 Lalor, B.M., Cookson, W.R, Murphy, D.V., 2007. Comparison of two methods that assess soil  
645 community level physiological profiles in a forest ecosystem. *Soil Biology and Biochemistry*  
646 39, 454-462.

647 Leloup, J., Baude, M., Nunan, N., Meriguet, J., Dajoz, I., Le Roux, X., Raynaud, X., 2018.  
648 Unravelling the effects of plant species diversity and aboveground litter input on soil bacterial  
649 communities. *Geoderma* 317, 1-7.

650 Liu, W.T., Marsh, T.L., Heng, H.C., Forney, L.J., 1997. Characterization of microbial diversity  
651 by determining terminal restriction fragment length polymorphisms of genes encoding 16S  
652 rRNA. *Applied and Environmental Microbiology* 63, 4516-4522.

653 Lopez-Mondejar, R., Voriskova, J., Vetrovsky, T., Baldrian, P., 2015. The bacterial community  
654 inhabiting temperate deciduous forests is vertically stratified and undergoes seasonal  
655 dynamics. *Soil Biology and Biochemistry* 87, 43-50.

656 Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., Hooper, D.U.,  
657 Huston, M.A., Raffaelli, D., Schmid, B., Tilman, D., Wardle, D.A., 2001. Biodiversity and  
658 ecosystem functioning: Current knowledge and future challenges. *Science* 294, 804-808.

659 McGill, W.B., Hunt, H.W., Woodmansee, R.G., Reuss, J.O., 1981. Phoenix, a model of the  
660 dynamics of carbon and nitrogen in grassland soils. *Ecological Bulletins* 33:49-115.

661 Moore TR, Trofymow AJ, Siltanen M, Prescott C, CIDET Working Group. 2005. Litter  
662 decomposition and C, N and P dynamics in upland forest and peatland sites, central Canada.  
663 *Canadian Journal of Forest Research* 35:133-142.

664 Moorhead, D.L., Sinsabaugh, R.L., 2006. A theoretical model of litter decay and microbial  
665 interaction. *Ecological Monographs* 76, 151-174.

666 Mooshammer, M., Wanek, W., Zechmeister-Boltenstern, S., Richter, A., 2014. Stoichiometric  
667 imbalances between terrestrial decomposer communities and their resources: mechanisms and  
668 implications of microbial adaptations to their resources. *Frontiers in Microbiology* 5, 22.

669 Peñuelas, J., Estiarte, M., Kimball, B.A., Idso, S.B., Pinter, P.J., Wall, G.W., Garcia, R.L.,  
670 Hansaker, D.J., LaMorte, R.L., Hendrix, D.L., 1996. Variety of responses of plant phenolic  
671 concentration to CO<sub>2</sub> enrichment. *Journal of Experimental Botany* 47, 1463-1467.

672 Petchey, O.L., Gaston, K.J., 2006. Functional diversity: back to basics and looking forward.  
673 *Ecology Letters* 9, 741-758.

674 Prescott, C.E., Grayston, S.J., 2013. Tree species influence on microbial communities in litter  
675 and soil: Current knowledge and research needs. *Forest Ecology and Management* 309, 19-  
676 27.

677 Preston CM, Nault JR, Trofymow A, Smyth C, CIDET Working Group. 2009. Chemical  
678 changes during 6 years of decomposition of 11 litters in some Canadian forest sites. Part 1.  
679 Elemental composition, tannins, phenolics, and proximate fractions. *Ecosystems* 12:1053-  
680 1077.

681 Purahong, W., Kapturska, D., Pecyna, M.J., Jariyavidyanont, K., Kaunzner, J., Juncheed, K.,  
682 Uengwetwanit, T., Rudloff, R., Schulz, E., Hofrichter, M., Schloter, M., Kruger, D., Buscot,  
683 F., 2015. Effects of forest management practices in temperate beech forests on bacterial and  
684 fungal communities involved in leaf litter degradation. *Microbial Ecology* 69, 905-913.

685 Quézel, P., Médail, F., 2003. *Ecologie et biogéographie des forêts du bassin Méditerranéen*.  
686 Elsevier, Paris.

687 Ranjard, L., Poly, F., Lata, J.C., Mougel, C., Thioulouse, J., Nazaret, S., 2001. Characterization  
688 of bacterial and fungal soil communities by automated ribosomal intergenic spacer analysis  
689 fingerprints: biological and methodological variability. *Applied Environmental Microbiology*  
690 67, 4479-4487.

691 Santonja, M., Baldy, V., Fernandez, C., Balesdent, J., Gauquelin, T., 2015. Potential shift in  
692 plant communities with climate change in a Mediterranean Oak forest: consequence on

693 nutrients and secondary metabolites release during litter decomposition. *Ecosystems* 18, 1253-  
694 1268.

695 Santonja, M., Rancon, A., Fromin, N., Baldy, V., Hättenschwiler, S., Fernandez, C., Montes,  
696 N., Mirleau, P., 2017a. Plant litter diversity increases microbial diversity and carbon and  
697 nitrogen cycling in a Mediterranean shrubland. *Soil Biology and Biochemistry* 111, 124-134.

698 Santonja M., Fernandez C., Gers C., Proffit M., Gauquelin T., Reiter I., Cramer W., Baldy V.,  
699 2017b. Plant litter mixture partly mitigates the negative effects of extended drought on soil  
700 biota and litter decomposition in a Mediterranean oak forest. *Journal of Ecology* 105, 801-  
701 815.

702 Scheibe, A., Steffens, C., Seven, J., Jacob, A., Hertel, D., Leuschner, C., Gleixner, G., 2015.  
703 Effects of tree identity dominate over tree diversity on the soil microbial community structure.  
704 *Soil Biology and Biochemistry* 81, 219-227.

705 Schimel, J.P., Hättenschwiler, S., 2007. Nitrogen transfer between decomposing leaves of  
706 different N status. *Soil Biology and Biochemistry* 39, 1428-1436.

707 Schimel, J., Balsler, T.C., Wallenstein, M.D., 2007. Microbial stress-response physiology and  
708 its implications for ecosystem function. *Ecology* 88, 1386-1394.

709 Shihan, A., Hättenschwiler, S., Milcu, A., Joly, F.X., Santonja, M., Fromin, N., 2017. Changes  
710 in soil microbial substrate utilization in response to altered litter diversity and precipitation in  
711 a Mediterranean shrubland. *Biology and Fertility of Soils* 53, 171-185.

712 Snajdr, J., Cajthaml, T., Valaskova, V., Merhautova, V., Petrankova, M., Spetz, P., Leppanen,  
713 K., Baldrian, P., 2011. Transformation of *Quercus petraea* litter: successive changes in litter  
714 chemistry are reflected in differential enzyme activity and changes in the microbial  
715 community composition. *FEMS Microbiology Ecology* 75, 291-303.

716 Swift, M.J., Heal, O.W., Anderson, J.M., 1979. *Decomposition in terrestrial ecosystems.*  
717 University of California press, Berkeley.

718 Tilman, D., Lehman, C.L., Thomson, K.T., 1997. Plant diversity and ecosystem productivity:  
719 Theoretical considerations. *Proceedings of the National Academy of Sciences of the United*  
720 *States of America* 94, 1857-1861.

721 Urbanova, M., Snajdr, J., Baldrian, P., 2015. Composition of fungal and bacterial communities  
722 in forest litter and soil is largely determined by dominant trees. *Soil Biology and*  
723 *Biochemistry* 84, 53-64.

724 Ushio, M., Balsler, T.C., Kitayama, K., 2013. Effects of condensed tannins in conifer leaves on  
725 the composition and activity of the soil microbial community in a tropical montane forest.  
726 *Plant and Soil* 365, 157-170.

727 van der Knaap, Y.A., Bakker, M.M., Alam, S.J., Witte, J.P.M., Aerts, R., van Ek, R., van  
728 Bodegom, P.M., 2018. Projected vegetation changes are amplified by the combination of  
729 climate change, socio-economic changes and hydrological climate adaptation measures. *Land*  
730 *Use Policy* 72, 547-562.

731 Vitousek, P.M., Mooney, H.A., Lubchenco, J., Melillo, J.M., 1997. Human Domination of  
732 Earth's Ecosystems. *Science* 277, 494-499.

733 Voriskova, J., Brabcova, V., Cajthaml, T., Baldrian, P., 2014. Seasonal dynamics of fungal  
734 communities in a temperate oak forest soil. *New Phytologist* 201, 269-278.

735 Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin,  
736 J.M., Hoegh-Guldberg, O., Bairlein, F., 2002. Ecological responses to recent climate change.  
737 *Nature* 416, 389-395.

738 Wardle, D.A., Bonner, K.I., Nicholson, K.S., 1997. Biodiversity and plant litter: Experimental  
739 evidence which does not support the view that enhanced species richness improves ecosystem  
740 function. *Oikos* 79, 247-258.

741 White, T.J., Bruns, T.D., Lee, S.B., Taylor, J.W., 1990. Amplification and direct sequencing of  
742 fungal ribosomal RNA genes for phylogenetics. In: M.A. Innis, M.A., Gelfand, D.H., Sninsky,

743 J.J., White, T.J. (Eds.), PCR protocols: a guide to methods and applications. Academic Press,  
744 New York, pp. 315-322.

745 Williams, M.A., Rice, C.W., 2007. Seven years of enhanced water availability influences the  
746 physiological, structural, and functional attributes of a soil microbial community. *Applied Soil*  
747 *Ecology* 35, 535-545.

748



749 **TABLES**

750

751 **Table 1** Effects of litter diversity (decomposed in species richness and species composition),  
 752 time of litter decomposition and block on Shannon diversity index for bacterial and fungal  
 753 decomposer communities. d.f. = degrees of freedom, %SS = percentage of sums of squares. *F*-  
 754 values and associated *P*-values (with the respective symbols \* for  $P < 0.05$ , \*\* for  $P < 0.01$ ,  
 755 and \*\*\* for  $P < 0.001$ ) are indicated

756

	Bacterial diversity			Fungal diversity		
	d.f.	%SS	<i>F</i> -value	d.f.	%SS	<i>F</i> -value
Richness	3	1.5	<b>3.9**</b>	3	5.9	<b>30.6***</b>
Composition	11	8.8	<b>6.5***</b>	11	8.8	<b>12.4***</b>
Time	5	33.2	<b>53.5***</b>	5	44.5	<b>138.1***</b>
Block	3	0.6	1.6	3	0.1	0.3
Richness × Time	15	4.9	<b>2.6**</b>	15	3.0	<b>3.1***</b>
Composition × Time	55	20.4	<b>3.0***</b>	55	22.3	<b>6.3***</b>
Residuals	247	30.7		239	15.4	

757

758

759 **Table 2** Effects of the presence of each litter species, time of litter decomposition and block on  
760 Shannon diversity index for bacterial and fungal decomposer communities. d.f. = degrees of  
761 freedom, %SS = percentage of sums of squares. *F*-values and associated *P*-values (with the  
762 respective symbols \* for  $P < 0.05$ , \*\* for  $P < 0.01$ , and \*\*\* for  $P < 0.001$ ) are indicated  
763

	Bacterial diversity			Fungal diversity		
	d.f.	%SS	<i>F</i> -value	d.f.	%SS	<i>F</i> -value
Acer	1	0.6	3.4	1	0.0	0.0
Cotinus	1	0.3	1.8	1	0.0	0.1
Pinus	1	1.2	<b>7.4**</b>	1	11.9	<b>119.6***</b>
Quercus	1	3.2	<b>19.1***</b>	1	1.5	<b>15.0***</b>
Time	5	32.8	<b>38.7***</b>	5	44.5	<b>89.6***</b>
Block	3	0.5	1.0	3	0.1	0.2
Acer × Time	5	2.5	<b>2.9*</b>	5	0.9	1.8
Cotinus × Time	5	2.4	<b>2.8*</b>	5	2.5	<b>5.0***</b>
Pinus × Time	5	2.7	<b>3.2**</b>	5	8.2	<b>16.4***</b>
Quercus × Time	5	1.7	2.0	5	0.8	1.6
Residuals	307	52.0		299	29.7	

764

765

766 **Table 3** Effects of community weighted mean traits (CWM), functional trait dissimilarities  
767 (FD), time of litter decomposition and block on Shannon diversity index for bacterial and fungal  
768 decomposer communities. CWM1 and CWM2, and FD1 and FD2 represent the two first  
769 components of the PCAs conducted using the CWM or the FD values in Fig. 4. d.f. = degrees  
770 of freedom, %SS = percentage of sums of squares. *F*-values and associated *P*-values (with the  
771 respective symbols \* for  $P < 0.05$ , \*\* for  $P < 0.01$ , and \*\*\* for  $P < 0.001$ ) are indicated

772

	Bacterial diversity			Fungal diversity		
	d.f.	%SS	<i>F</i> -value	d.f.	%SS	<i>F</i> -value
CWM1	1	0.3	1.4	1	5.1	<b>47.5***</b>
CWM2	1	0.0	0.2	1	0.0	0.4
FD1	1	0.3	1.5	1	0.0	0.3
FD2	1	2.2	<b>9.8**</b>	1	11.3	<b>105.5***</b>
Time	5	43.6	<b>39.6***</b>	5	50.6	<b>94.3***</b>
Block	3	0.1	0.6	3	0.0	0.2
CWM1 × Time	5	2.1	1.9	5	2.7	<b>5.1***</b>
CWM2 × Time	5	1.1	1.0	5	1.5	<b>2.8*</b>
FD1 × Time	5	0.2	0.2	5	0.2	0.4
FD2 × Time	5	3.1	<b>2.8*</b>	5	6.4	<b>11.9***</b>
Residuals	213	46.9		206	22.1	

773

774

775 **Table 4** Effects of community weighted mean traits (CWM) and functional trait dissimilarities  
776 (FD) on bacterial and fungi community turnover rates during leaf litter decomposition. Adjusted  
777  $R^2$  values in simple linear regressions and associated  $P$ -values (with the respective symbols \*  
778 for  $P < 0.05$  and \*\* for  $P < 0.01$ ) are indicated. The sign of the regression coefficient is indicated  
779 in brackets. bCTR= bacterial community turnover rate; fCTR= fungal community turnover rate;  
780 ns= non-significant relationship

781

	bCTR			fCTR		
CWM1	0.01	(+)	ns	0.38	(+)	*
CWM2	0.02	(-)	ns	0	(+)	ns
FD1	0.25	(+)	ns	0.01	(+)	ns
FD2	0.53	(+)	**	0.42	(-)	*

782

783

784 **FIGURE LEGEND**

785

786 **Fig. 1** Bacterial (a, b, c) and fungal (d, e, f) diversity (expressed as Shannon diversity index  $H'$ )  
787 according to time of decomposition (a, d), litter species richness (b, e) and litter species  
788 composition (c, f). Values are means  $\pm$  SE (n = 60 for each decomposition time; n = 96, 144,  
789 96 and 24 for 1-, 2-, 3-, and 4-species litter mixtures, respectively; n = 24 for each litter species  
790 composition). Different letters denote significant differences between treatments with  
791 a<b<c<d<e<f<g. A = *Acer*, C = *Cotinus*, P = *Pinus*, Q = *Quercus*. Combinations of capital  
792 letters correspond to combinations of plant species in litter mixtures

793

794 **Fig. 2** Effects of litter species richness on bacterial (a, b, c, d, e, f) and fungal (g, h, i, j, k, l)  
795 diversity (expressed as Shannon diversity index  $H'$ ) according to time of decomposition. Values  
796 are means  $\pm$  SE (n = 16, 24, 16 and 4 for 1-, 2-, 3-, and 4-species litter mixtures at each  
797 decomposition time, respectively). Different letters denote significant differences between  
798 treatments with a<b<c

799

800 **Fig. 3** Bacterial (a, b, c, d) and fungal (e, f, g, h) diversity (expressed as Shannon diversity index  
801  $H'$ ) according to the presence of *Acer* (a, e), *Cotinus* (b, f), *Pinus* (c, g) and *Quercus* (d, h).  
802 Values are means  $\pm$  SE (n = 32 and 28 for species presence and absence at each decomposition  
803 time, respectively). Presence = grey bar and Absence = white bar. Microbial diversity  
804 significantly different according to the presence/absence of litter species is indicated with the  
805 respective symbols \* for  $P < 0.05$ , \*\* for  $P < 0.01$ , and \*\*\* for  $P < 0.001$

806

807 **Fig. 4** Principal component analysis (PCA) of community-weighted mean traits (a) and  
808 functional trait dissimilarity (b) based on the litter traits of the 11 multiple-species litter

809 mixtures. Variance explained by each principal component and associated eigenvalues are  
810 shown in brackets. A = *Acer*, C = *Cotinus*, P = *Pinus*, Q = *Quercus*. Combinations of capital  
811 letters correspond to combinations of plant species in litter mixtures

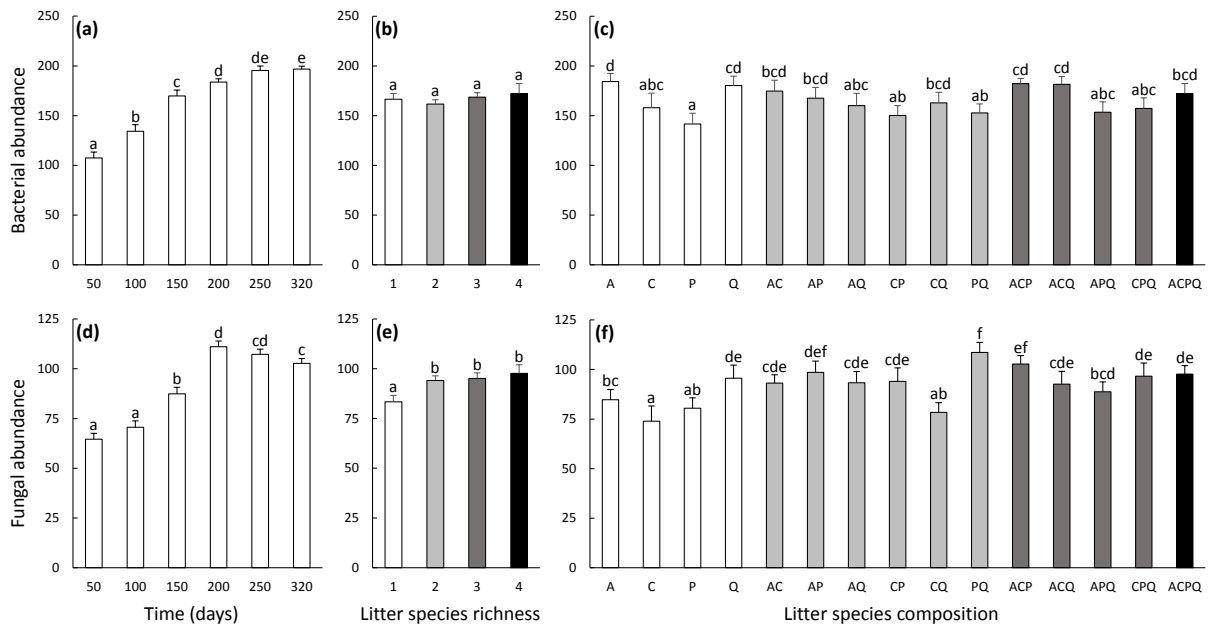
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813 **Fig. 5** Bacterial (white symbol) and fungal (grey symbol) diversity (expressed as Shannon  
814 diversity index  $H'$ ) as a function of functional trait dissimilarities (FD2 values; Fig. 4). Each  
815 symbol represents the mean value ( $n = 4$ ) of each of the 11 multi-species litter mixtures.  
816 Significant linear relationships are indicated with dotted (bacteria) or solid (fungi) lines,  
817 adjusted  $R^2$  and associated  $P$ -values (\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ). BD = Bacterial  
818 diversity; FD = Fungal diversity

819

820 **Fig. 1**

821

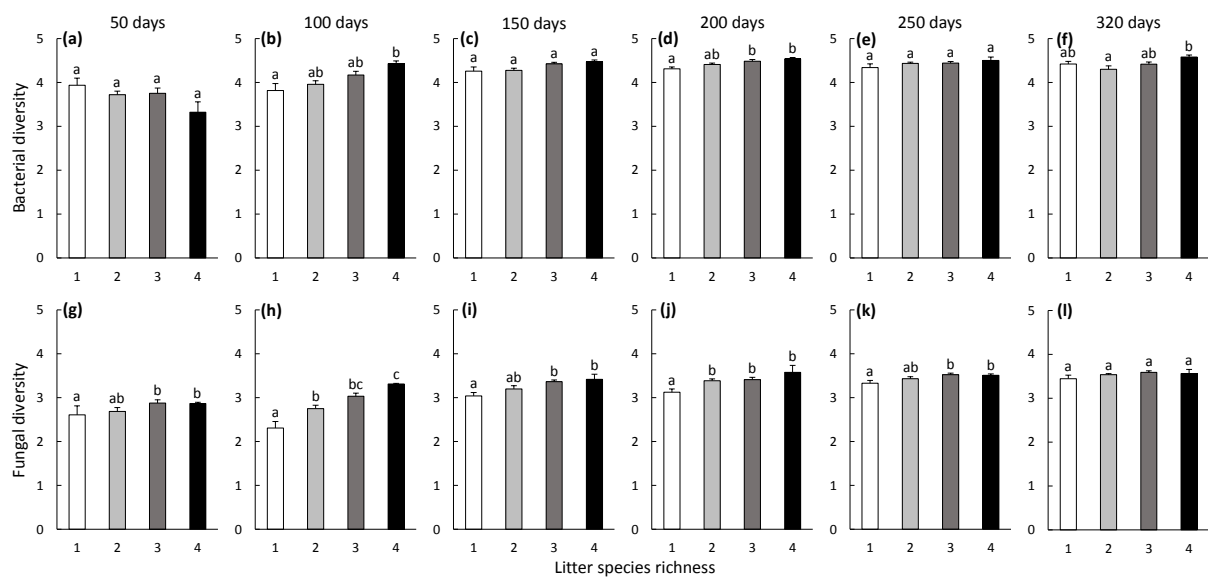


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824 **Fig. 2**

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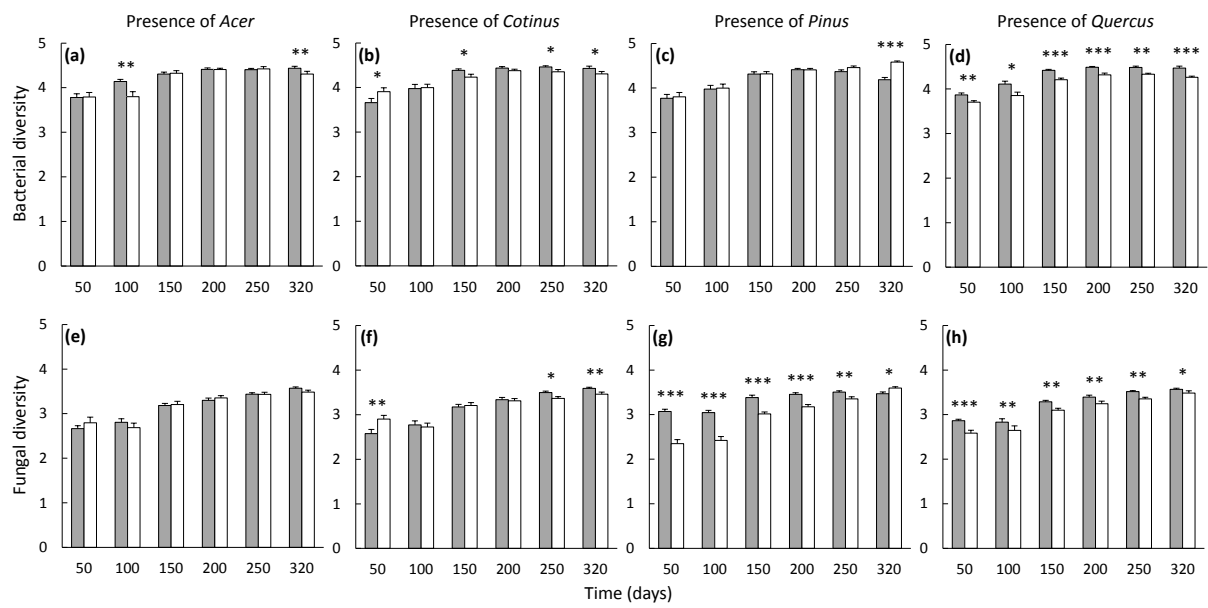
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828 **Fig. 3**

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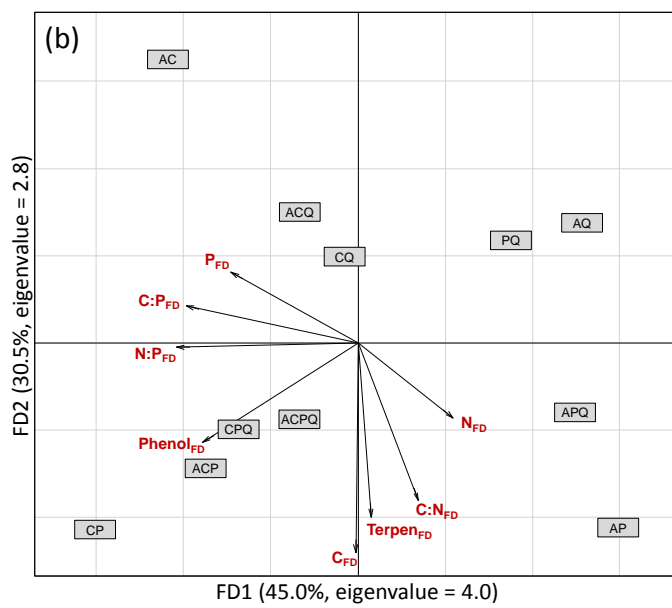
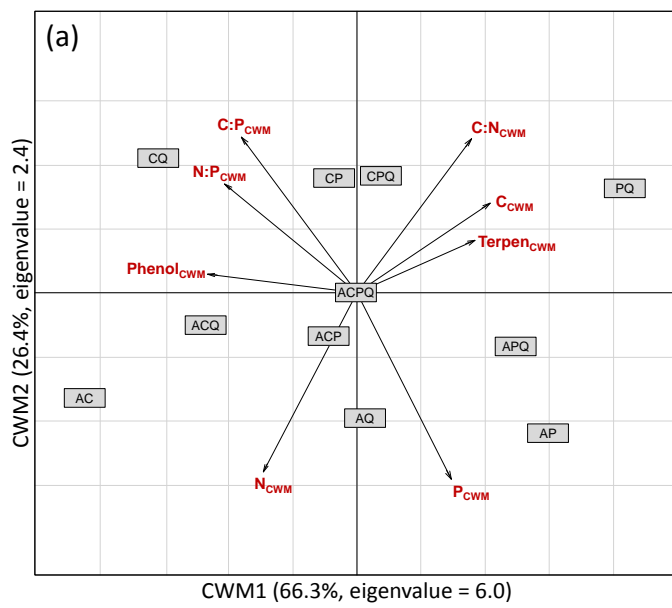


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832 **Fig. 4**

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