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1 **Title:** Plant litter diversity increases microbial abundance, fungal diversity, and carbon and
2 nitrogen cycling in a Mediterranean shrubland

3

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26 **Abstract**

27 The consequences of predicted climate change on ecosystem processes is difficult to
28 evaluate, because biodiversity is also susceptible to change resulting in complex interactions on
29 ecosystem functioning. With an experimental approach, we aimed to understand how plant
30 community diversity (through different plant litter mixtures) and climate change (through
31 decreased precipitation) may impact microbial abundance and diversity and affect C and N
32 cycling in a Mediterranean shrubland. Along a natural plant diversity gradient, we manipulated
33 the amount of precipitation and followed leaf litter decomposition during one year. We found
34 that multi-species litter mixtures had higher microbial abundance, lower bacterial diversity and
35 higher fungal diversity than predicted from single-species litter. In addition, C and N release
36 increased with increasing litter species richness. Microbial abundance and diversity were
37 positively, but weakly, correlated to the litter mixture effects on C and N release. Drier
38 conditions increased microbial diversity but had no effect on microbial abundance. The net
39 release of N from decomposing litter was lower with reduced precipitation irrespective of litter
40 species richness and composition, while that of C was higher or lower depending on litter
41 species composition. The relationships between microbial communities and litter mixture
42 effects on C and N release were altered under drier conditions. Our data provide clear evidence
43 that microbial decomposers and the processes they drive, respond to changing plant community
44 diversity and composition in a Mediterranean shrubland. We highlighted the importance of
45 *Quercus coccifera* that appears to be a key species in shaping microbial communities and
46 driving synergistic effects on C and N release more than the three other shrub species. Our study
47 also suggests that shifts in the plant community composition may have stronger impacts on
48 litter decomposition and nutrient cycling than relatively subtle changes in precipitation as
49 simulated in our study.

50

51 **Keywords**

52 Biodiversity-functioning relationship; Climate change; Mediterranean shrubland; Litter
53 decomposition; Microbial community; Relative mixture effect

54

55 **1. Introduction**

56 Decomposition of plant material is a key ecosystem function determining the carbon
57 and nitrogen cycles to a great extent (Cadish and Giller, 1997; Bardgett, 2005). In most of
58 natural ecosystems, litter material from different plant species decomposes together. Numerous
59 studies have shown that mixtures of litter from different plant species decompose at different
60 rates compared to what is expected from the component species (reviewed in Gartner and
61 Cardon, 2004; Hättenschwiler et al., 2005; Gessner et al., 2010). Among the mechanisms
62 underlying these litter diversity effects, complementary resource use by the decomposer
63 community may be particularly important for explaining synergistic litter mixing effects
64 (Hättenschwiler et al., 2005). However, it is difficult to quantify this mechanism
65 (Hättenschwiler et al., 2011), especially regarding the role of microbial decomposers. This
66 limits the understanding of how resource diversity interacts with decomposer community
67 effects on carbon and nitrogen cycling during decomposition. Indeed, soil microorganisms are
68 the major drivers of litter decomposition and nutrient mineralization (Bardgett, 2005; de Graaff
69 et al., 2010), but it is presently not well understood if and how litter mixture effects on
70 decomposition are related to shifts in the structure and composition of the microbial
71 decomposer community. Previous studies have provided some evidence that microbial biomass
72 and diversity respond positively to litter mixing (Blair et al., 1990; Bardgett and Shine, 1999;
73 Kominoski et al., 2007; Chapman et al., 2013), which could result from an increased diversity
74 of substrates and associated niches for microorganisms (Tilman et al., 1997; Hooper and
75 Vitousek, 1998; Hättenschwiler et al., 2011). Additionally, it has been shown that

76 decomposition rates increased with increasing microbial diversity, due to complementarity and
77 facilitation mechanisms that enhance microbial exploitation of organic matter (Robinson et al.,
78 1993; Setälä and McLean, 2004; Tiunov and Scheu, 2005), in accordance with theoretical
79 predictions (Loreau, 2001).

80 Abundance, community structure, and activity of soil microorganisms are strongly
81 controlled by water availability (Angel et al., 2010; Schimel et al., 2007; Williams and Rice,
82 2007; Cregger et al., 2012; Kaisermann et al., 2013). Thus, climate change-related
83 modifications in the precipitation regime are likely to affect the microbial decomposer
84 community, with potential consequences on decomposition dynamics, carbon cycling and
85 nutrient availability for plants (Hobbie, 1996; Aerts, 1997; Knapp et al., 2008). In
86 Mediterranean ecosystems, water availability is the most important environmental constraint
87 for decomposition, with biological processes being strongly regulated by the seasonally
88 contrasting climate condition such as summer drought and episodic drying/rewetting cycles
89 (Sardans and Peñuelas, 2013). Regional climate models predict an increase in both temperature
90 and drought conditions in the Mediterranean region in the future (Giorgi and Lionello, 2008;
91 Polade et al., 2014). These changes are expected to result in increased frequency, intensity and
92 duration of drought, especially during the summer (Dubrovsky et al., 2014). Increased drought
93 should lead to more limiting conditions for soil microorganisms and may provoke shifts in the
94 microbial community composition (Pesaro et al., 2004; Schimel et al., 2007). Such changes of
95 microbial communities are also likely affecting the decomposition process. However, the
96 relationship between climate change, microbial community composition and decomposition is
97 not well understood.

98 In this study, we examined litter decomposition in a field experiment in which we
99 manipulated the amount of precipitation along a natural plant diversity gradient in a
100 Mediterranean shrubland. We specifically investigated the decomposition of leaf litter mixtures

101 along with their associated microbial communities compared to their respective single litter
102 species treatments with or without reduced rainfall during one year. We hypothesized a positive
103 effect of litter species diversity on microbial communities (i.e. increase of abundance and
104 diversity) and on litter decomposition (i.e. increase of carbon and nitrogen release). We
105 hypothesized a negative effect of drier conditions on microbial communities (i.e. decrease of
106 abundance and diversity) and on litter decomposition (i.e. decrease in carbon and nitrogen
107 release), as soil moisture has been reported as the most limiting environmental factor in
108 Mediterranean ecosystems. Furthermore, we explored the potential relationships between
109 microbial communities and carbon and nitrogen release. We hypothesized that the frequently
110 observed non-additive litter mixture effects on litter decomposition are correlated to the
111 abundance and diversity of fungal and bacterial decomposers. In a last hypothesis, we predicted
112 that drier conditions would attenuate the non-additive litter mixture effects on litter
113 decomposition as well as the potential relationships between microbial communities and non-
114 additive litter mixture effects.

115

116 **2. Materials and Methods**

117

118 **2.1. Study site**

119 The study site was located in the Massif de l'Etoile near Marseille, France (43° 22' N,
120 5°25' E) at 275 m above sea level (see Montès et al. (2008) for a detailed description of the
121 study site). The mean annual precipitation is 552 mm and the mean annual temperature is 14.6
122 °C (mean values over the period 2002-2012 averaged across the two meteorological stations in
123 Marignane (43°26'N, 5°12'E) and Marseille (43°15'N, 5°22'E) closest to our study site). The
124 soil is classified as shallow rendzina developed over limestone bedrock with with 66 % of
125 stones in the top 50 cm, a mean pH of 7.9, a mean C:N ratio of 18 and a mean CEC of 36.8

126 cmol.kg⁻¹ (means from 92 different soil samples taken in the study plots; see Shihan et al. (2017)
127 for further details).

128 The vegetation is a woody shrub-dominated “garrigue”, with shrub heights ranging
129 between 0.2 and 1.4 m (Montès et al., 2008) and a heterogeneous cover ranging between 25%
130 and 95%. Four woody shrub species dominate the community: *Quercus coccifera* L. (*Quercus*,
131 with an average cover across all plots of 36 %), *Cistus albidus* L. (*Cistus*, 18 %), *Ulex*
132 *parviflorus* Pourr. (*Ulex*, 10 %), and *Rosmarinus officinalis* L. (*Rosmarinus*, 9 %).

133

134 **2.2. Experimental setup**

135 The study site is characterized by a natural small-scale mosaic of assemblages of distinct
136 compositions of the four dominating woody shrub species (i.e. *Quercus*, *Cistus*, *Rosmarinus*
137 and *Ulex*) that allowed establishing a series of plots varying in species richness of these four
138 species. Ninety-two 4 × 4 m plots were selected based on plant community composition in order
139 to include all 15 possible combinations of the four dominant shrub species (i.e. 4 single-species
140 litter, 6 two-species mixtures, 4 three-species mixtures, and one four-species mixture). The
141 distance between plots varied from 1 to 30 m (Fig. S1). Each of the 15 different plant
142 combinations was replicated six times, except for the four-species mixture that was replicated
143 eight times. All plots were equipped with a 4 m × 4 m solid aluminum frame, held 2 m above
144 the ground by aluminum posts at the outer border of the 16 m² plot area and fixed to the ground
145 with reinforcing bars. Stainless steel gutters were mounted on top of the aluminum frame.
146 Contrary to the rain exclusion plots, the gutters in the control plots were mounted upside down
147 in order to let the precipitation fall on the vegetation. A supplementary PVC gutter and a pipe
148 mounted at the border of the frame allowed to evacuate the rainwater away from the plots. Over
149 the 6 replicates for each plant combinations (8 for the four-species mixture), 3 replicates were
150 randomly assigned to control plots and the other 3 to rain exclusion plots (4 of each for the four-

151 species mixture). The experimental rain exclusion was set up in October 2011. The exact
152 amount of precipitation excluded was estimated using (i) rain gauges installed at ground level
153 underneath the gutters in both control and rain-excluded plots and (ii) TDR100 probes
154 (Campbell Scientific Inc., Logan, Utah) installed in seven control and eight rain-excluded plots
155 at 10 cm soil depth and by (iii) determining the gravimetric humidity in the soil sampled in
156 control and excluded plots. Compared to control plots, the rain exclusion plots received on
157 average $12 \pm 2\%$ less rainfall. This exclusion resulted in an average lower soil humidity of -6.5
158 % (that could reach between -13 and -24 % during rain events) at 10 cm soil depth between
159 control and rain exclusion plots (determined by permanently installed TDR probes; Fig. S2).

160 We collected freshly fallen leaf litter from all four woody shrub species during the
161 period of maximum leaf litterfall between June and July 2011 with litter traps suspended
162 underneath the canopy at about 15 cm from the ground. Leaf litter was regularly retrieved from
163 litter traps every two days, immediately sorted into species, air-dried at room temperature and
164 stored dry until the beginning of the experiment.

165 In order to mimick natural conditions during litter decomposition, leaf litter mixtures
166 were placed in specifically designed field mesocosms, consisting of “open-bottom” PVC
167 cylinders (5 cm tall) covered with 1 mm mesh net on top and with windows on the side covered
168 with 10 mm mesh net, similar to those used in Barantal et al. (2011). We constructed these field
169 mesocosms in order to allow direct contact of the litter with the soil surface and full access of
170 the soil fauna without flattening the litter material as is typically the case with traditional
171 litterbags. Mesocosms were filled with a total of six grams of air-dried leaf litter reflecting the
172 species composition of the respective plot (i.e. 4 single-species litters and 11 different litter
173 mixtures), with equal proportions of the different litter species in the mixtures. In December
174 2011, a total of 368 cylinders (15 litter treatments \times 2 precipitation treatments \times 3 plots (4 plots
175 for 4-species mixture) \times 4 replicates per plot) were installed *in situ* across all plots in the central

176 4 m² part of the 16 m² plot area to avoid edge effects. Mesocosms were placed in nearly flat
177 area and fixed onto the soil surface with nails, avoiding rock outcrops and very shallow soil.

178 After 12 months of field exposure, the remaining litter material was collected from each
179 mesocosm, put in plastic bags, and immediately transferred to the laboratory. Leaf litter was
180 separated into species, thoroughly brushed to remove adhering soil particles, freeze-dried
181 (Lyovac GT2®) and weighed to obtain litter dry mass data of each species in each mesocosm.
182 After weighing the component litter species, all litter from an individual field mesocosm was
183 again put together and then ground using a ball mill to a fine powder before chemical and
184 microbial analyses.

185

186 ***2.3. Litter measurements***

187 Initial litter quality was determined from four subsamples of each species-specific litter
188 batch (Table 1). In addition, we analyzed carbon (C) and nitrogen (N) concentrations in
189 aliquotes from all litter samples retrieved from the field mesocosms at the end of the
190 experiment. Carbon and N concentrations of initial and remaining litter materials were
191 determined by thermal combustion on a Flash EA 1112 series C/N elemental analyzer (Thermo
192 Scientific, Waltham, MA, USA). Based on the litter dry mass data and the initial and final C
193 and N concentrations, we calculated the percentage of absolute C and N release for each
194 mesocosm as the ratio of remaining to initial C and N contents. Phosphorus (P) concentration
195 was measured colorimetrically using the method of Santonja et al. (2015a). Lignin
196 concentration was determined according to the van Soest extraction protocol (van Soest and
197 Wine 1967) using a fiber analyzer (Fibersac 24; Ankom, Macedon, NJ, USA). Phenolic
198 concentration was measured colorimetrically using the method of Santonja et al. (2015a) with
199 gallic acid as a standard. To determine the water holding capacity (WHC), intact leaf litter
200 samples were soaked in distilled water for 24 h, drained and weighed. The dry weight was

201 determined after drying samples at 60 °C for 48 h. WHC was calculated according to the
202 formula: moist weight / dry weight × 100% (Santonja et al., 2015a).

203

204 ***2.4. Bacterial and fungal densities***

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

213 The fungal and bacterial abundances were estimated by quantitative real-time PCR
214 (qPCR) using the LightCycler 480 system (Roche Applied Science). A fragment of the bacterial
215 16S rDNA gene was amplified using the primers Eub338 (5'-
216 ACTCCTACGGGAGGCAGCAG-3') and Eub518 (5'-ATTACCGCGGCTGCTGG-3')
217 (Fierer et al., 2005). The fungal 18S-5,8S intergenic spacer (ITS1) was amplified using the
218 primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and 5,8S (5'-
219 CGCTGCGTTCTTCATCG-3') (Fierer et al., 2005). Standard solution of bacterial 16S rDNA
220 gene was generated from a pGEM-T cloned 16S rDNA gene of a soil representative strain
221 (Henry et al., 2006). A solution of the fungal ITS marker was generated using genomic DNA
222 of a representative strain of *Aspergillus niger* (personal collection), and serial decimal dilutions
223 were used as standards. The DNA concentration of the standard solutions was determined using
224 NanoDrop 2000c UV-Vis spectrophotometer (Thermo Fisher Scientific Inc.). Annealing
225 temperatures were optimized for each primer pair following gradient PCR tests in a Mycycler

226 Thermal Cycler (Biorad). PCR mixtures (20 μ l) contained 200 μ M dNTPs, 5 % (v:v) DMSO,
227 1 μ M of each primer and 0.25 units GoTaq DNA-polymerase with the corresponding 5X PCR
228 buffer (Promega) and used the same program as for qPCR but with denaturing, annealing and
229 extension steps of 30 s. Amplification products were verified by using 2% Nusieve agarose gel
230 electrophoresis.

231 For real time quantitative PCR, the 20 μ l -mixtures contained 10 μ l of SYBR green I
232 master mix (Roche Applied Science), 1 μ M each primer (final concentration), 2 μ l of DNA
233 template and ultrapure water for the balance. Reaction conditions included an initial
234 denaturation step of 10 minutes at 95°C, followed by 50 cycles of amplification consisting of
235 15 seconds denaturation at 95°C, 15 seconds of primer annealing, and 10 seconds of extension
236 at 72°C; the final step was set up to determine the melting temperature of the amplified product
237 through slow increase of temperature (0.2°C s⁻¹) between 60°C and 95°C. Microbial density
238 values, hereafter named “microbial abundance”, were expressed as 16S rDNA and 18S rDNA
239 gene copy numbers per g of dry litter for bacteria and fungi, respectively.

240

241 ***2.5. Bacterial and fungal community diversity***

242 We used Automated Ribosomal Intergenic Spacer Analysis (ARISA) to characterize
243 bacterial communities (Ranjard et al., 2001), and terminal Restriction Fragment Length
244 Polymorphism (tRFLP) to characterize fungal communities (Liu et al., 1997). The bacterial
245 16S-23S rDNA Intergenic Spacer (ITS) was amplified using the primers [6fam]-s-d-Bact-1522-
246 b-S-20 (5'- TGCGGCTGGATCCCCTCCTT-3') and L-D-Bact-132-a-A-18 (5'-
247 CCGGGTTTCCCCATT CGG-3'). The fungal 18S-5,8S ITS1 region was amplified using the
248 primers [HEX]-ITS1-F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-
249 TCCTCCGCTTATTGATATGC-3'). Both PCR reactions were performed in 20 μ l mixtures
250 containing 200 μ M dNTPs, 5 % (v:v) DMSO, 0.5 μ M of each primer and 0.25 units GoTaq

251 DNA-polymerase with the corresponding 5X PCR buffer (Promega). After initial denaturation
252 at 94°C for 5 minutes, we ran 35 cycles of denaturation at 94°C for 1 minute, followed by
253 annealing at 55°C for 1 minute, elongation at 72°C for 1 minute and a final elongation at 72°C
254 for 10 minutes. PCR products of the fungal ITS were digested by adding 5 U of *HinfI* restriction
255 enzyme and 2 µl of the corresponding restriction buffer (Fermentas). Following verification of
256 the ARISA and tRFLP products by using 2% NuSieve-agarose gel electrophoresis, these
257 products were diluted with sterile distilled water (1/20), and analyzed with capillary sequencer
258 ABI 3730 (Applied Biosystem). One microliter of the diluted sample was mixed with 0.8 µl of
259 GeneScan-2500 ROX-labelled size standard and 8 µl of deionized formamide; denaturation was
260 completed at 95°C for 5 min before capillary electrophoresis in the POP-7 polymer, during 3 h
261 with 7.5 kV run-voltage. The output series of peak-sizes corresponding to bacterial or fungal
262 operating taxonomic units (OTUs) were analyzed by using the GeneMapper® v4.1 program
263 (Applied Biosystem). Size standard peaks were defined individually, parameters of the internal
264 AFLP-method were set up in order to detect peak-sizes in the 300-1200 bp range, with bin-
265 windows set up to one bp and lower detection limits of peak-heights fixed individually at values
266 ranging from 1 to 100 Raw Fluorescent Units (RFUs).

267

268 **2.6. Statistical analyses**

269 Statistical analyses were performed with the PRIMER-E software (version 6.1, Primer-
270 E Ltd, Plymouth, United Kingdom) for multivariate analyses, and with the R software (version
271 3.3.1, The R Foundation for Statistical Computing, Vienna, Austria) for univariate analyses.

272 Bacterial and fungal OTU - abundance matrices based on ARISA and tRFLP
273 fingerprints were analyzed with PRIMER-E. The DIVERSE routine was run to obtain the
274 Shannon's index of diversity ($H' = -\sum p_i \times \log_{10} p_i$, where p_i is the proportion of the total
275 abundance arising from the i^{th} species), hereafter named "microbial diversity". The abundance

276 value of each OTU (i.e. the peak-height value) was standardized by total abundance of the
277 sample and then log-transformed ($\text{Log } X+1$) before generating Bray-Curtis similarity matrices
278 (multivariate measures based on pairwise resemblances among sample units) with minimum
279 transformed-value as a dummy variable. High Bray-Curtis similarity values between
280 fingerprints indicate similar microbial community composition, whereas, low values indicate
281 no or few common OTU (Clarke and Warwick, 2001). We assessed whether microbial
282 community structure differed according to plant litter diversity and precipitation conditions by
283 using multivariate analysis of variance (PERMANOVA) that was set up with 9999
284 permutations.

285 We used linear mixed-effects models to test for the effects of precipitation change
286 (control vs rain exclusion) and litter diversity (separated in i) litter species composition, ii) litter
287 species richness and iii) litter species identity) on microbial (bacteria and fungi) abundance and
288 diversity and on the release of C and N. For each of the 6 response variables, we used three
289 distinct statistical models. The first model tested the impact of litter species composition (i.e.
290 15 litter treatments), precipitation treatments, and their interactions. The second model tested
291 the impact of litter species richness (i.e. 1, 2, 3 or 4 species), precipitation treatments, and their
292 interactions. The third model tested the impact of litter species identity (i.e. the
293 presence/absence of each species), precipitation treatments, and their interactions. The four
294 replicate mesocosms per plot were nested within plots, and plots were included as random
295 factors in all models. To take into account the effects of soil heterogeneity between plots, we
296 used the scores of the first axis of the PCA analysis of plot-specific soil characteristics (i.e. soil
297 texture, pH, cation exchange capacity (CEC), and the concentrations of carbon, nitrogen,
298 calcium, magnesium, sodium, potassium, iron, manganese and aluminum; Table S1, Fig. S3,
299 Shihan et al. 2017) that were fitted as a supplementary variable (named “Soil”) in the models.

300 Based on the average microbial abundance and diversity and of C and N release
301 measured in single species plots we calculated the expected values (E) for litter mixtures in
302 mixed species plots within the corresponding precipitation treatments. From these expected
303 values (E) and the observed values (O) we calculated the relative mixture effects (RME) of
304 litter mixing on response variables as follows: $RME = (O - E) / E \times 100\%$ (Wardle et al., 1997).
305 We used one-sample Student's t-tests to test whether RME significantly differs from zero for
306 each litter mixture in either precipitation conditions.

307 Finally, we performed Pearson correlations to test the relationships between microbial
308 abundance/diversity and RME of C and N release. We also tested the relationships between the
309 microbial community dissimilarity matrices (calculated as $100 - \text{Bray-Curtis similarity}$) and
310 RME of C and N release (transformed in Euclidian-distance based matrices) by using the
311 RELATE routine of PRIMER-E (i.e. a non-linear alternative test to the Mantel test based on
312 the null hypothesis of non-relationship between two distance matrices) with Spearman rank
313 correlations (ρ) set up with 9999 permutations.

314

315 **3. Results**

316

317 ***3.1. Response of microbial abundance to litter diversity and precipitation change***

318 Microbial abundances differed among litter treatments (both composition and richness;
319 Table 2; Fig. 1a), but not in response to a change in precipitation (Table 2). The lowest and the
320 highest values of bacterial and fungal abundances ranged from 10^5 to 10^8 16S or 18S rDNA
321 gene copy number g^{-1} litter, respectively (Table 3). Irrespective of the precipitation treatment,
322 bacterial and fungal abundances were positively correlated across the 15 different litter
323 combinations ($R^2 = 0.64$, $P < 0.0001$). Microbial abundances increased with increasing litter

324 species richness, showing particularly marked differences when increasing the species number
325 from 2 to 3 (Fig. 1a).

326 Likewise, the relative mixture effects (RME) that compare microbial abundances
327 observed in litter mixtures to those expected based on single-species litter were amplified with
328 increasing litter species richness (Figs. 2a, 2b and S4a). RME increased from - 29% and + 19%
329 on average in the two-species litter mixtures, to + 74% and + 149% on average in the three-
330 species litter mixtures, and to + 209% and + 282% on average in the four-species litter mixtures
331 for bacterial and fungal abundances, respectively (Figs. 2a, 2b and S4a). RME on microbial
332 abundance were null or antagonistic in the two-species mixtures (for 6 out of 12 combinations,
333 *i.e.* 6 two-species mixtures \times 2 precipitation treatments) and for 3 out of 12 combinations for
334 bacteria and fungi, respectively) (Figs. 2a and 2b). In contrast, three- and four-species litter
335 mixtures showed some synergistic effects on microbial abundance, for 2 out of 10 combinations
336 for bacteria and for 4 out of 10 combinations for fungi (Figs. 2a and 2b).

337 As indicated by the significant litter composition \times precipitation treatment interaction,
338 the relationship between litter composition and bacterial abundance differed according to the
339 precipitation treatment (Table 2). This interaction was mainly driven by the two-species litter
340 mixtures containing *Quercus*, with *Quercus/Rosmarinus* and *Quercus/Ulex* mixtures, both
341 showing lower bacterial abundance with reduced precipitation compared to the control, and
342 conversely, the *Cistus/Quercus* mixture showing higher bacterial abundance with reduced
343 precipitation than in the control (Table 3). Analyses on the relative importance of each litter
344 species in the mixtures pointed out the importance of *Quercus*, which was related to higher
345 microbial abundances when present compared to when its litter was absent (Table S2). The
346 presence of *Rosmarinus* enhanced microbial abundance, while the presence of *Ulex* decreased
347 bacterial abundance.

348

349 **3.2. Response of microbial diversity to litter diversity and precipitation change**

350 The Shannon diversity index H' ranged from 1.1 to 1.9 for bacteria and from 1.2 to 1.8
351 for fungi across precipitation and litter treatments (Table 3). It was significantly affected by
352 both litter species richness and composition for both microbial groups (Table 2; Fig. 1b), as
353 well as by precipitation change for bacteria (Table 2).

354 We observed higher bacterial diversity in the single-species litters and the three-species
355 litter mixtures compared to the two-species litter mixtures, while fungal diversity increased
356 mostly between single-species litter and two-species litter mixtures (Tables 2 and 3; Fig. 1b).
357 Accordingly, the RME on diversity differed markedly between bacteria and fungi (Figs. 2c and
358 2d), with mostly antagonistic effects of litter mixing on bacterial diversity (for 21 of the 22
359 combinations), and generally synergistic effects on fungal diversity (for 18 of the 22
360 combinations). RME on fungal diversity were higher in the two-species litter mixtures (+13.5%
361 on average) than in the three- and the four-species litter mixtures (+5.5 and +5.6% on average,
362 respectively) (Fig. S4b).

363 A decrease in precipitation led to an overall higher bacterial diversity compared to
364 control condition, but had no main effect on fungal diversity (Tables 2 and 3). However, fungal
365 diversity was interactively affected by a change in precipitation and litter composition (Table
366 2). This interaction was largely driven by the overall higher fungal diversity in the three-species
367 litter mixtures under rainfall exclusion compared to control condition (Table 3).

368 Bacterial and fungal community dissimilarities were also affected by litter mixture
369 composition and richness (PERMANOVA, Pseudo- $F = 4.83$ to 11.07 , $P < 0.0001$). Reduced
370 precipitation led to a shift in bacterial and/or fungal community in 11 of the 15 litter treatments
371 between control and reduced precipitation treatments (Table S3; 7 out of 15 litter treatments for
372 bacterial communities and 8 out of 15 litter treatments for fungal communities).

373 Analyzing the relative importance of the presence of each litter species on microbial
374 diversity showed essentially the same effects as those reported for microbial abundances.
375 *Quercus* leaf litter had the strongest (positive) effect on bacterial and fungal diversity (Table
376 S2). The presence of *Rosmarinus* did not affect bacterial diversity, but had a positive effect on
377 fungal diversity (Table S2). *Ulex* leaf litter decreased bacterial diversity, but – and this was the
378 only difference with species presence effects on microbial abundance – it increased fungal
379 diversity (Table S2). The presence of *Cistus* leaf litter had no influence on microbial diversity
380 (Table S2).

381

382 **3.3. Response of C and N release to litter diversity and precipitation change**

383 After 1 year of decomposition, we found large differences in C release from mesocosms
384 ranging from $19.5 \pm 2.2\%$ in *Ulex* litter to $40.2 \pm 2.1\%$ in the *Cistus/Quercus/Rosmarinus* litter
385 mixture (both under control condition, Table 3), and in N release that ranged from an apparent
386 net N uptake of $5.4 \pm 2.5\%$ in the *Cistus/Ulex* litter mixture (higher final than initial litter N
387 content was also observed in *Rosmarinus/Ulex* litter mixture) and apparent N release of $21 \pm$
388 1.9% in the *Cistus/Quercus/Rosmarinus* litter mixture (both under rain exclusion, Table 3).
389 Both litter species richness and composition had significant effects on C and N release (Table
390 2; Fig. 1c). In general, increasing litter species richness increased C release (single-species <
391 multi-species litter mixtures) as well as N release (single- and two-species litter mixtures <
392 three- and four-species litter mixtures) (Fig. 1c). Accordingly, RME on C release were mostly
393 positive (for 15 of the 22 combinations) and rather homogenous among the different litter
394 mixtures (Fig. 3a). On the other hand, RME on N release varied somewhat more among litter
395 mixtures with overall less positive effects (for 10 out of 22 combinations) compared to those
396 observed for C release (Fig. 3b).

397 The effect of reduced precipitation on net C release depended on litter composition,
398 mainly due to the negative effects on *Rosmarinus* litter ($25.3 \pm 1.9\%$ compared to $36.9 \pm 2.2\%$
399 in the control, Table 3), and positive effects in some litter treatments including *Ulex* litter (*Ulex*
400 singly, *Quercus/Ulex*, and *Cistus/Rosmarinus/Ulex*, although not significant when tested for the
401 specific litter treatments individually). These distinct responses to reduced precipitation
402 resulted in overall larger RME on C release (+3.2% in control to +7.5% under precipitation
403 change, on average), which were more frequently synergistic (for 9 of the 11 combinations)
404 compared to control condition (for 6 of the 11 combinations, Fig. 3a). The net N release
405 decreased to $10.0 \pm 0.6\%$ on average with less rainfall compared to $12.1 \pm 0.6\%$ measured in
406 control plots (Tables 2 and 3), regardless of litter treatment (i.e. no interactions with litter
407 species richness or composition).

408 The presence of particular litter species explained some of the observed litter
409 composition effects on C and N release (Table S2). The presence of *Quercus*, *Cistus*, and
410 *Rosmarinus* (in the order of decreasing importance) had a positive effect on C release. In
411 contrast, the presence of *Ulex* had a negative effect on C release. N release increased only in
412 the presence of *Quercus* leaf litter, but this effect was quite strong (Table S2). Similar to its
413 negative effect on C release, the presence of *Ulex* also decreased N release.

414

415 ***3.4. Relationships between microbial community characteristics and C and N release***

416 Bacterial abundance was positively, but weakly, correlated to RME on C and on N
417 release under both precipitation conditions (Table 4). Weak positive correlations were also
418 found between fungal abundance and RME on C release – but only with reduced precipitation,
419 and between fungal abundance and RME on N release under both precipitation conditions
420 (Table 4).

421 RME on C and N release increased with increasing bacterial diversity under both
422 precipitation conditions (Table 4). Weak positive relationships were also observed between
423 RME on C and N release and fungal diversity under control condition, but not with reduced
424 precipitation (Table 4).

425 Bacterial community dissimilarity was positively, but weakly, correlated to RME on C
426 and N release under reduced precipitation (Table 4), suggesting that more heterogeneous
427 bacterial communities were correlated to higher C and N release under drier conditions. Fungal
428 community dissimilarity was also weakly positively correlated with RME on N release under
429 both precipitation treatments (Table 4).

430

431 **4. Discussion**

432 With the present study, we addressed the question of how plant litter diversity and
433 reduced precipitation affect microbial abundance and diversity, as well as C and N release from
434 decomposing litter in a Mediterranean shrubland. After one year of decomposition in the field,
435 we found that the abundance and diversity of microbial communities within multi-species litter
436 mixtures differed from that predicted from the component species decomposing singly.
437 Likewise, and in line with the majority of litter diversity experiments in various ecosystems
438 (Gartner and Cardon, 2004; Hättenschwiler et al., 2005), leaf litter mixing generally resulted in
439 higher, although moderate, C and N release rates than expected from data of single-species
440 litter.

441

442 ***4.1. Litter diversity effects on microbial communities and C and N release***

443 In line with our first hypothesis, the majority of the litter mixtures had microbial
444 abundances distinct from those predicted from the respective single-species litter. Bacterial and
445 fungal abundances tended to increase with an increasing number of litter species in mixtures.

446 This may indicate that a higher diversity of resources provided by the mixing of litter from
447 different species increases overall microbial abundance through resource complementarity,
448 similar to what was reported in previous studies in both aquatic (Kominoski et al., 2007;
449 Kominoski et al., 2009) and terrestrial (Chapman and Newman, 2010; Chapman et al., 2013)
450 ecosystems. However, this mechanism would not explain why the two-species litter mixtures
451 often showed lower abundances (mostly for bacteria) than expected from single-species litter.
452 Perhaps competitive interactions between the two bacterial communities developing on
453 monospecific leaf litter predominated in two-species mixtures compared to mixtures with more
454 than two species. Jousset et al. (2011) proposed such competitive interactions for resources and
455 habitats between microorganisms from distinct communities, but with low genotypic
456 dissimilarity, while positive effects of complementary resource use prevail when populations
457 are more dissimilar. In the present study, the addition of litter from a third plant species to the
458 two-species mixtures may have increased sufficiently resource and habitat diversity and/or
459 community dissimilarity to decrease competition between species-specific microbial
460 communities. For example, the addition of *Quercus* litter to the two-species mixtures
461 *Cistus/Ulex* or *Rosmarinus/Ulex* had a notably strong effect on microbial abundance, with a
462 switch from lower to rather higher abundances than predicted from the respective single species.
463 However, a finer temporal resolution of microbial dynamics and interactions is required to
464 corroborate potential mechanisms underlying these responses.

465 The responses in microbial diversity to litter mixing differed from those observed for
466 abundance. Fungal diversity was generally higher in litter mixtures compared to that predicted
467 from single-species litter, while bacterial diversity was lower. This finding clearly shows that
468 litter mixing can have contrasting effects on the diversity of decomposer communities under
469 the exactly same environmental conditions. So far, fungal and bacterial diversity have rarely
470 been assessed in conjunction in the same study, and it is thus difficult to evaluate how general

471 such opposite litter mixing effects on the diversity of fungal and bacterial communities might
472 be. As an example, Lunghini et al. (2013) reported similar results with higher fungal diversity
473 in leaf litter mixtures than expected from the respective single species in a Mediterranean
474 shrubland in southern Italy. Kominoski et al. (2009) reported both synergistic and antagonistic
475 effects of litter mixing on the number of bacterial and fungal ribotypes associated with tree litter
476 species decomposing in a temperate stream. However, these aquatic microbial communities are
477 quite different from those we studied in terrestrial ecosystem.

478 The majority of litter mixtures also exhibited C and/or N release patterns that differed
479 from those expected from litter species decomposing singly. On average, C and N release across
480 all litter mixtures was 3.3% and 4.1% greater, respectively, than expected from single species
481 after one year of decomposition. These modest increases in C and N release in our
482 Mediterranean shrubland are in accordance with recent findings of moderate effects of litter
483 mixing on decomposition in forest ecosystems from five different biomes (Handa et al., 2014).
484 In agreement with previous studies, we observed predominantly non-additive interactions
485 during decomposition, with a majority of synergistic effects that increased with increasing litter
486 species richness (Gartner and Cardon 2004; Lecerf et al., 2007; Handa et al., 2014; Santonja et
487 al., 2015b). Our data also highlighted that the litter diversity effects on decomposition are
488 mediated by species identity rather than species richness (Kominoski et al., 2007; Vivanco and
489 Austin, 2008; Schindler and Gessner, 2009; Santonja et al. 2015b), with the presence of
490 *Quercus* increasing C and N release from litter mixtures while the presence of *Ulex* had the
491 opposite effect. Litter from *Quercus*, which is better represented in the study site than *Ulex* (i.e.
492 36 % vs. 10% of average cover, respectively), might be preferred by the soil decomposers.
493 Differences in litter traits between these two species could also explain their contrasting effects.
494 Overall, *Quercus* has a rather better litter quality compared to *Ulex*, with lower lignin/N and
495 lignin/P ratios. Previous studies suggested that the presence of high-quality litter in the mixtures

496 enhances the decomposition of the other litters (Wardle et al., 1997; Hättenschwiler et al., 2005;
497 Santonja et al. 2015b). Moreover, *Quercus* has a higher WHC than *Ulex*, a physical trait directly
498 affecting litter humidity that is highly important for decomposer activity (Hättenschwiler et al.
499 2005; Makkonen et al. 2013; Santonja et al., 2015b). For example, Makkonen et al. (2013)
500 showed that the relative mixture effects on mass loss increased when litter and soil moisture
501 conditions became more favorable for plant litter decomposition.

502

503 ***4.2. Relationships between microbial community characteristics and C and N release***

504 When testing the relationship between microbial community characteristics and
505 decomposition parameters, we showed that both microbial abundance and diversity were
506 positively, but weakly, correlated to the relative mixture effect (RME) on C and N release. Such
507 correlations are in line with theoretical predictions of more efficient resource exploitation with
508 more abundant and diverse microbial communities (Loreau 2001), and the general expectation
509 that more diverse soil decomposer communities would increase the rate of litter decomposition
510 through mechanisms such as facilitation or complementary resource use (Gessner et al., 2010).
511 The few studies that specifically assessed relationships between the microbial decomposer
512 community and decomposition efficiency reported enhanced decomposition rates with higher
513 microbial diversity (Robinson et al., 1993; Setälä and McLean, 2004; Tiunov and Scheu, 2005;
514 Chapman et al., 2013). For example, Setälä and McLean (2004) observed that the efficiency of
515 fungal communities to degrade raw coniferous forest humus increased with the number of
516 fungal taxa, but only at the low end of the species richness gradient. Tiunov and Scheu (2005)
517 using five fungal species, showed that the rate of cellulose decomposition was positively
518 associated with species richness. Our study is the first to compare such relationships between
519 both fungal and bacterial abundance and diversity, and C and N release in the same field
520 experiment. Interestingly, bacterial and fungal communities displayed contrasting relationships

521 to litter mixture effects on C and N release, with stronger relationships between fungal diversity
522 and RME compared to bacterial diversity.

523 The relationships between decomposition and microbial abundance and diversity were
524 also dependent on the identity of the plant species included in the litter mixture. When *Quercus*
525 that exhibited the highest microbial abundance and diversity on single-species litter was present
526 in the mixture, the synergistic effects on C and N release were particularly frequent. In contrast,
527 *Cistus* that showed the lowest microbial abundance and diversity in single-species litter showed
528 neither synergistic nor antagonistic effects on C and N release. This could suggest that high
529 microbial abundance and diversity supported by a particular leaf litter species can lead to a
530 stimulation of decomposition of associated leaf litter species present in the same mixture.
531 However, for the support of such a mechanism, the bacterial and fungal species and their
532 functional diversity would need to be determined in more detail, for example by the use of
533 sequencing.

534

535 ***4.3. Effects of a change in precipitation***

536 Because humidity is a key environmental factor for microbial activity, reduced
537 precipitation is expected to have negative effects on decomposition. Previous studies generally
538 reported reduced decomposition rates under drier conditions (Saura-Mas et al., 2012; Vogel et
539 al., 2013; Walter et al., 2013; Coulis et al., 2015; Santonja et al., 2015a; Santonja et al., 2017).
540 However, the extent of such effects depends on the actual precipitation regime, the relative
541 amount of precipitation change, and the type of ecosystem. In agreement with previous studies,
542 we observed an overall 17% reduction of N release under drier compared to control conditions,
543 while there was no overall effect of reduced precipitation on C release, which nevertheless was
544 distinctly affected depending on litter composition. More negative effects on N than on C
545 release could lead to a decoupling between C and N dynamics under drier climate. We could

546 hypothesize that less precipitation enhances microbial N immobilization (Lodge et al., 1994),
547 and consequently affects to a larger extent the N- compared to the C-release. Because
548 Mediterranean soils are characterized by a rather low fertility (Sardans and Peñuelas, 2013), a
549 reduced N release during litter decomposition under drier conditions may imply higher N
550 limitation of plant growth, which could reinforce the negative effects of lower soil water
551 availability.

552 There were only small effects of reduced precipitation on microbial abundance, with an
553 increase in both fungal and bacterial diversities with less precipitation in only three-species
554 litter mixtures. This may be not too surprising given the relatively small amount of precipitation
555 excluded in our study. Most other studies that evaluated microbial diversity in response to a
556 change in precipitation also reported only minor effects on soil bacterial and fungal diversities
557 in Mediterranean ecosystems even when the amount of rainfall excluded was larger than in our
558 study (Wilkinson et al., 2002; Berard et al., 2011; Yuste et al., 2011; Sherman et al., 2012;
559 Yuste et al., 2014; Sherman et al., 2014), suggesting that the soil microbial communities are
560 already well adapted to rather dry conditions that regularly occur during the hot and dry
561 summer. However, Cregger et al. (2012), working in a semiarid ecosystem in central New
562 Mexico, found that a 50% reduced rainfall altered soil bacterial and fungal community
563 composition and reduced fungal abundance. Likewise, a 29% reduction of rainfall over 11 years
564 in a Mediterranean *Quercus ilex* forest resulted in a 27% lower soil microbial biomass and an
565 altered microbial physiological profile (Garcia-Palacios et al., 2016). Such changes in microbial
566 community structure, composition (i.e changes in the identity, relative abundance and/or
567 biomass), and functions are likely explained by physiological capacities to tolerate water stress
568 that vary among species and affect their dominance within the community (Schimel et al.,
569 2007). The discrepancy of results on the consequences of precipitation change for microbial
570 communities reported in the literature is also likely related to different experimental setups,

571 distinct amounts of rainfall that are excluded (ranging from 12% (our experiment) to 50%
572 (Cregger et al., 2012)), and the different ecosystems that respond distinctly to precipitation
573 change. As suggested by Yuste et al. (2014), the strong seasonal differences in temperature and
574 soil water availability characteristic for the Mediterranean region could represent an adaptative
575 selection pressure for microbial communities with an overall higher drought tolerance
576 compared to microbial communities in ecosystems of no or less severe drought occurrence.

577 Our data did not fully confirm our last hypothesis predicting attenuated litter mixture
578 effects under drier conditions. Some relationships between microbial communities and
579 decomposition disappeared under precipitation change, but others were maintained and new
580 ones appeared. In contrast to our hypothesis, synergistic effects on C release were more frequent
581 and overall greater under reduced precipitation. In fact, 82% of all litter mixtures showed
582 synergistic effects under drier conditions, compared to 55% under control conditions. Such an
583 increase in synergistic interactions with increasing drought is difficult to interpret, but may be
584 related to a drought-induced change of the microbial community that is more complementary
585 in its resource use. In fact, we could hypothesize a decrease in competitive interactions for
586 resources and habitats under drier conditions by a more drought-tolerant microbial assemblage.
587 However, in contrast to our findings, Santonja et al. (2015a) reported a strong decrease of
588 synergistic litter mixture interactions in a Mediterranean forest subjected to rainfall exclusion
589 in an earlier study, suggesting the opposite mechanism. Taken together, these findings indicate
590 that the change in environmental conditions predicted for the future may lead to a marked shift
591 in both intensity and direction of litter mixture interactions, which, however, at present seems
592 difficult to predict.

593

594 **5. Conclusion**

595 Our results from a field experiment clearly demonstrated that changes in the diversity
596 and composition of the plant community and a moderate alteration in the amount of
597 precipitation in a Mediterranean shrubland altered litter microbial abundance and diversity, and
598 C and N release during decomposition. Multi-species litter mixtures showed higher microbial
599 abundance, lower bacterial diversity and higher fungal diversity compared to single-species
600 litter. C and N release increased with increasing litter species richness. Microbial abundance
601 and diversity were positively, but weakly, correlated with the relative litter mixture effects on
602 C and N release. This could suggest a potential mechanistic link between resource diversity,
603 microbial abundance and diversity, and rates of C and N mineralization. Drier conditions
604 increased microbial diversity, reduced net N release and led to higher and more frequent
605 synergistic effects on C release. These effects induced by less overall rainfall, however, will
606 depend on plant species composition that may also be directly affected by climate change. We
607 also underscored the importance of *Quercus coccifera* in structuring microbial communities
608 and driving synergistic effects on C and N release, suggesting a key role of this species in the
609 functioning of the type of Mediterranean shrubland we studied here. Any specific effects of
610 future climate change on the distribution and/or abundance of *Quercus coccifera* could thus
611 have disproportionate indirect effects on ecosystem functioning.

612

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623

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802 response to temperature. *Global Change Biology* 17, 1475-1486.

803

804 **TABLES**

805

806 **Table 1.** Initial litter characteristics of the four shrub species (means \pm SE, n = 4). All
 807 percentages are on a dry mass basis. C = Carbon, N = Nitrogen, P = Phosphorus, WHC = Water
 808 holding capacity.

809

	<i>Cistus albidus</i>	<i>Quercus coccifera</i>	<i>Rosmarinus officinalis</i>	<i>Ulex parviflorus</i>
C (%)	42.2 \pm 1.2	44.7 \pm 1.5	46.8 \pm 0.9	48.0 \pm 0.6
N (%)	0.4 \pm 0.02	0.9 \pm 0.03	0.5 \pm 0.03	0.8 \pm 0.03
P (%)	0.4 \pm 0.01	0.3 \pm 0.01	0.2 \pm 0.02	0.2 \pm 0.02
Phenolics (%)	3.9 \pm 0.2	9.2 \pm 0.8	4.8 \pm 0.3	1.5 \pm 0.1
Lignin (%)	18.0 \pm 1.5	13.0 \pm 0.4	13.3 \pm 1.0	20.9 \pm 0.3
WHC (%)	201.4 \pm 7.9	131.9 \pm 9.8	127.9 \pm 3.8	79.4 \pm 5.9
C:N ratio	105.6 \pm 4.9	52.3 \pm 3.2	102.8 \pm 6.7	60.4 \pm 3.0
N:P ratio	9.7 \pm 0.6	26.3 \pm 1.8	18.9 \pm 2.0	45.0 \pm 7.8
Lignin:N ratio	45.1 \pm 3.3	15.2 \pm 0.4	29.4 \pm 2.7	26.3 \pm 0.4
Lignin:P ratio	442.3 \pm 31.9	398.7 \pm 10.5	553.8 \pm 55.0	1184.6 \pm 110.2

810

811

812 **Table 2.** Output of linear mixed effects models testing for the effects of litter species
 813 composition and precipitation change on microbial abundance and diversity and on C and N
 814 release. Plot-specific soil characteristics (Soil) were included as co-variable (see ‘Statistical
 815 analyses’ section). d.f. = degrees of freedom. *F*-values and associated *P*-values (with the
 816 respective symbols * for $P < 0.05$, ** for $P < 0.01$, and *** for $P < 0.001$) are indicated.
 817

	d.f.num	Bacterial abundance		Fungal abundance		Bacterial diversity		Fungal diversity		Carbon release		Nitrogen release	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Soil	1	1.4		1.2		0.4		4.7	*	5.1	*	0.0	
Composition (C)	14	6.6	***	7.8	***	11.3	***	31.3	***	15.6	***	18.7	***
Precipitation (P)	1	1.6		0.3		4.0	*	1.4		0.0		5.6	*
C × P	14	2.2	*	1.1		0.6		2.2	*	2.6	**	1.1	

818

819

820 **Table 3.** Summary of microbial abundance and diversity and C and N release according to the
821 15 litter combinations and the two precipitation conditions (control and precipitation change
822 (PC)) (mean \pm SE, n = 12 (16 for the 4-species mixture)). Bacterial and fungal abundances are
823 expressed as 16S rDNA and 18S rDNA gene copy numbers g⁻¹ litter, respectively. Bacterial
824 and fungal diversity are expressed as the Shannon diversity indice H'. Carbon and nitrogen
825 release are expressed as percentage of initial values. C = *Cistus*, Q = *Quercus*, R = *Rosmarinus*,
826 U = *Ulex*. Combinations of capital letters correspond to combinations of plant species in litter
827 mixtures.

828

	Bacterial abundance (10 ⁹)		Fungal abundance (10 ⁹)		Bacterial diversity		Fungal diversity		Carbon release (%)		Nitrogen release (%)	
	Control	PC	Control	PC	Control	PC	Control	PC	Control	PC	Control	PC
C	0.3 \pm 0.1	0.2 \pm 0.1	0.4 \pm 0.2	0.1 \pm 0.01	1.5 \pm 0.11	1.6 \pm 0.05	1.2 \pm 0.05	1.2 \pm 0.08	39.5 \pm 1.6	36.9 \pm 1.5	8.2 \pm 1.8	8.7 \pm 2.2
Q	69.9 \pm 17.6	65.8 \pm 11.5	120.4 \pm 30.5	123.3 \pm 24.0	1.9 \pm 0.02	1.9 \pm 0.02	1.6 \pm 0.02	1.6 \pm 0.01	32.6 \pm 2.0	35.8 \pm 2.2	15.7 \pm 3.5	18.5 \pm 1.8
R	5.6 \pm 1.5	7.7 \pm 1.4	6.0 \pm 1.2	10.7 \pm 2.2	1.7 \pm 0.06	1.7 \pm 0.02	1.3 \pm 0.03	1.4 \pm 0.03	36.9 \pm 2.2	25.3 \pm 1.9	8.5 \pm 2.3	1.8 \pm 2.0
U	2.8 \pm 0.9	3.2 \pm 1.0	8.4 \pm 2.6	3.2 \pm 0.9	1.5 \pm 0.09	1.6 \pm 0.08	1.4 \pm 0.04	1.3 \pm 0.10	19.5 \pm 2.2	22.8 \pm 2.0	5.2 \pm 2.3	1.4 \pm 3.9
CQ	14.8 \pm 5.7	46.7 \pm 12.2	50.5 \pm 11.1	69.0 \pm 16.5	1.7 \pm 0.09	1.8 \pm 0.05	1.6 \pm 0.03	1.6 \pm 0.03	37.6 \pm 0.6	39.1 \pm 2.0	13.2 \pm 1.3	16.3 \pm 2.3
CR	3.7 \pm 1.0	4.5 \pm 2.2	9.0 \pm 3.2	8.2 \pm 3.8	1.6 \pm 0.07	1.5 \pm 0.06	1.5 \pm 0.02	1.5 \pm 0.03	34.9 \pm 1.3	37.9 \pm 1.7	12.2 \pm 2.2	11.7 \pm 2.0
CU	0.5 \pm 0.1	1.2 \pm 0.5	0.8 \pm 0.2	2.3 \pm 1.3	1.2 \pm 0.09	1.1 \pm 0.09	1.5 \pm 0.05	1.5 \pm 0.05	28.3 \pm 1.2	25.0 \pm 0.4	1.8 \pm 1.4	-5.4 \pm 2.5
QR	114.1 \pm 43.0	16.6 \pm 5.0	178.2 \pm 64.4	60.2 \pm 16.2	1.7 \pm 0.08	1.7 \pm 0.04	1.7 \pm 0.02	1.7 \pm 0.02	37.8 \pm 1.1	35.5 \pm 1.4	12.9 \pm 2.1	9.6 \pm 1.8
QU	33.4 \pm 16.3	3.1 \pm 0.5	120.9 \pm 46.0	36.7 \pm 15.7	1.6 \pm 0.10	1.7 \pm 0.05	1.7 \pm 0.01	1.7 \pm 0.02	30.2 \pm 1.6	35.3 \pm 1.9	17.8 \pm 2.2	17.6 \pm 1.8
RU	1.2 \pm 0.3	1.7 \pm 0.6	1.4 \pm 0.4	2.2 \pm 0.7	1.5 \pm 0.04	1.6 \pm 0.05	1.7 \pm 0.02	1.7 \pm 0.02	27.9 \pm 1.6	28.7 \pm 1.3	2.0 \pm 1.7	-3.5 \pm 1.7
CQR	70.3 \pm 17.9	46.7 \pm 24.1	169.8 \pm 37.8	227.3 \pm 70.8	1.9 \pm 0.03	1.9 \pm 0.04	1.7 \pm 0.01	1.7 \pm 0.01	40.2 \pm 2.1	39.8 \pm 1.8	19.5 \pm 1.8	21.0 \pm 1.9
CQU	20.5 \pm 6.7	44.9 \pm 15.3	87.1 \pm 37.6	141.3 \pm 43.2	1.9 \pm 0.02	1.8 \pm 0.04	1.7 \pm 0.02	1.8 \pm 0.02	36.5 \pm 2.0	37.7 \pm 1.6	18.2 \pm 2.6	13.0 \pm 3.4
CRU	3.0 \pm 1.2	12.2 \pm 3.8	7.1 \pm 1.9	16.3 \pm 4.3	1.3 \pm 0.09	1.5 \pm 0.10	1.2 \pm 0.05	1.5 \pm 0.03	32.4 \pm 1.5	36.4 \pm 1.7	8.4 \pm 2.9	7.1 \pm 2.4
QRU	30.9 \pm 10.6	29.3 \pm 11.5	83.3 \pm 24.8	104.0 \pm 31.4	1.4 \pm 0.09	1.5 \pm 0.10	1.6 \pm 0.02	1.6 \pm 0.02	34.9 \pm 0.7	35.6 \pm 1.4	19.5 \pm 1.9	17.7 \pm 1.6
CQRU	64.3 \pm 6.5	53.9 \pm 20.7	162.5 \pm 18.1	98.3 \pm 21.1	1.6 \pm 0.13	1.7 \pm 0.05	1.7 \pm 0.02	1.7 \pm 0.02	35.0 \pm 0.8	34.1 \pm 1.2	18.4 \pm 1.3	15.4 \pm 1.6

829

830

831 **Table 4.** Relationships between microbial communities and relative mixture effect (RME) on
832 C and N release according to the two environmental conditions (control and precipitation
833 change (PC)). Pearson correlations were performed between microbial abundance and diversity
834 and RME on C and N release. Spearman rank correlations were performed between microbial
835 community structure and RME on C and N release. Correlation values can range from -1.0 to
836 1.0, with 1.0 indicating perfectly positive correlations and -1.0 indicating perfectly negative
837 correlations. Significant correlations are indicated with the respective symbols * for $P < 0.05$,
838 ** for $P < 0.01$, and *** for $P < 0.001$.

839

	Bacterial abundance		Fungal abundance		Bacterial diversity		Fungal diversity		Bacterial community structure		Fungal community structure	
	Control	PC	Control	PC	Control	PC	Control	PC	Control	PC	Control	PC
RME on C release	0.22*	0.25**	-	0.35***	0.22**	0.35***	0.19*	-	-	0.10**	-	-
RME on N release	0.30***	0.26**	0.35***	0.40***	0.22**	0.20*	0.18*	-	-	0.13**	0.09*	0.08*

840

841

842 **Figure legend**

843

844 **Fig. 1.** Microbial abundance (a), microbial diversity (b), and carbon and nitrogen release (c)
845 according to the four levels of litter species richness. Different letters denote significant
846 differences among species richness levels.

847

848 **Fig. 2.** Relative Mixture Effects (RME) on microbial abundance (a and b) and diversity (c and
849 d) from multi-species litter mixtures according to control (open symbol) and precipitation
850 change (close symbol) conditions. RME significantly different from 0 are indicated with the
851 respective symbols * for $P < 0.05$, ** for $P < 0.01$, and *** for $P < 0.001$. C = *Cistus*, Q =
852 *Quercus*, R = *Rosmarinus*, U = *Ulex*. Combinations of capital letters correspond to
853 combinations of plant species in litter mixtures.

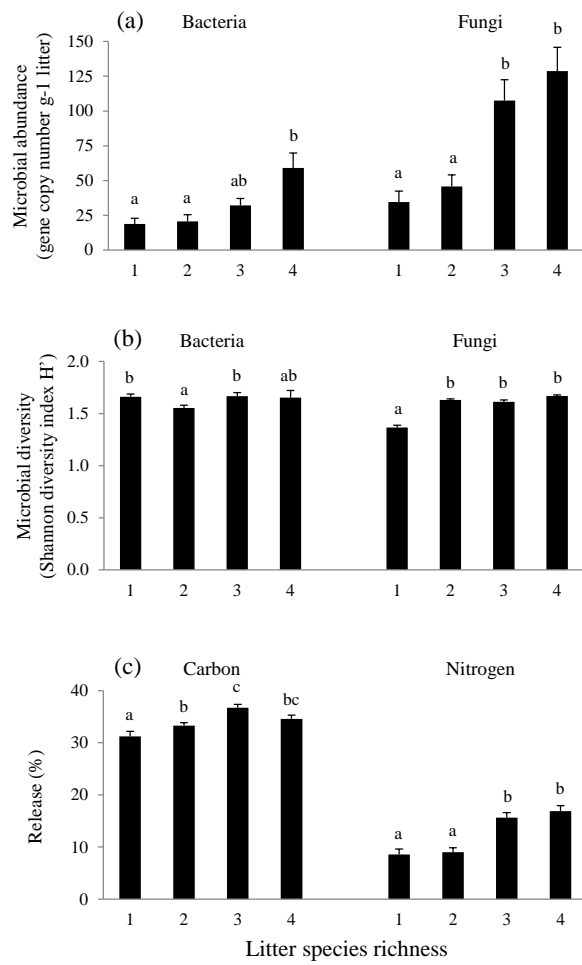
854

855 **Fig. 3.** Relative Mixture Effects (RME) on carbon (a) and nitrogen (b) release from multi-
856 species litter mixtures according to control (open symbol) and precipitation change (close
857 symbol) conditions. RME significantly different from 0 are indicated with the respective
858 symbols * for $P < 0.05$, ** for $P < 0.01$, and *** for $P < 0.001$. C = *Cistus*, Q = *Quercus*, R =
859 *Rosmarinus*, U = *Ulex*. Combinations of capital letters correspond to combinations of plant
860 species in litter mixtures.

861

862 **Fig. 1.**

863

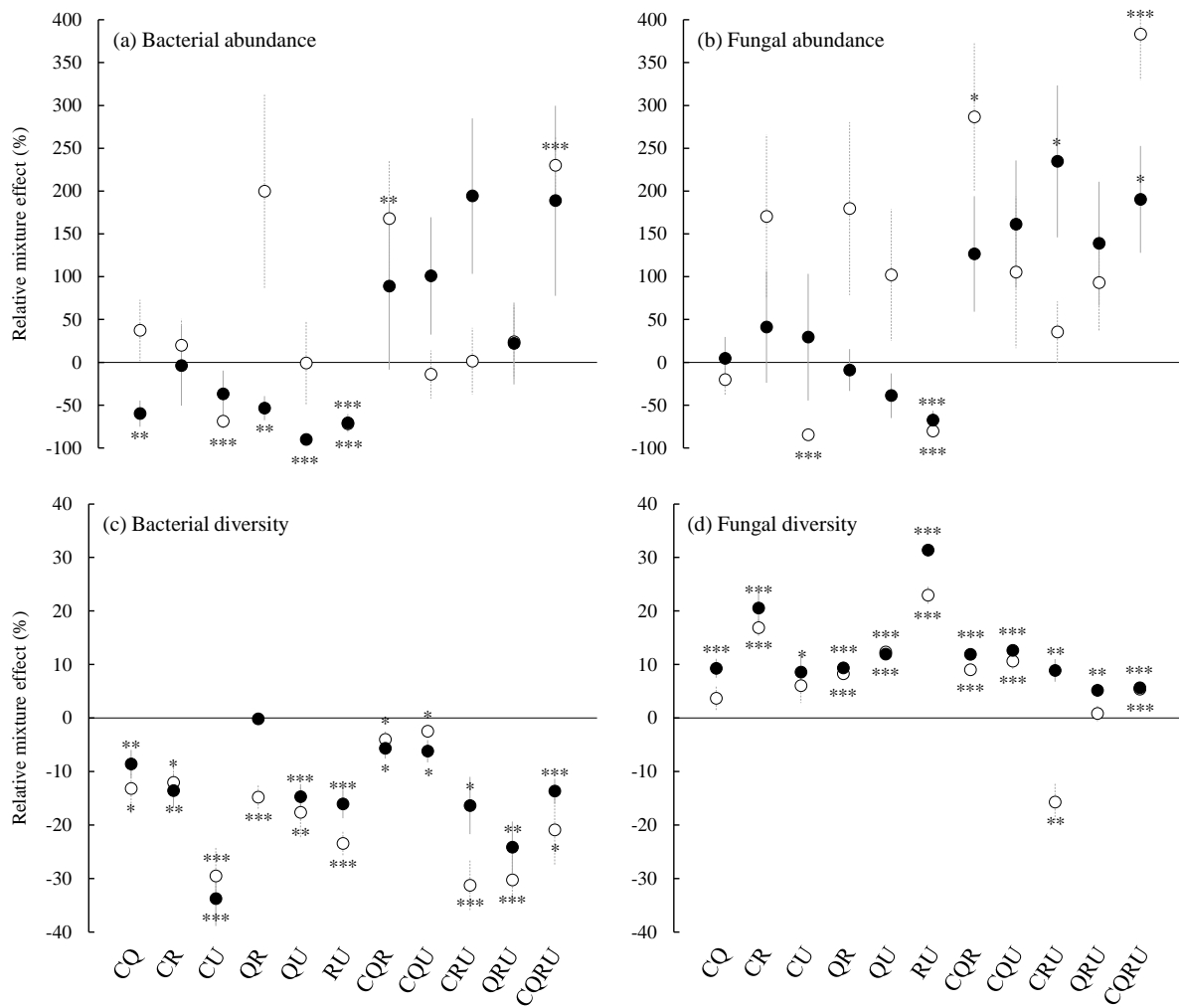


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865

866 **Fig. 2.**

867

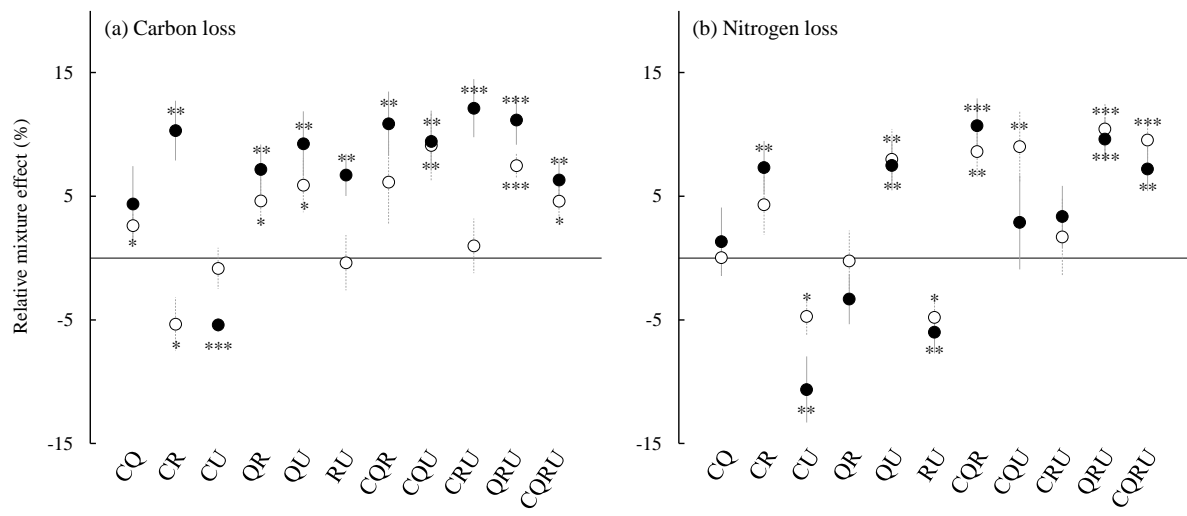


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870 **Fig. 3.**

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