Plant litter diversity increases microbial abundance, fungal diversity, and carbon and nitrogen cycling in a Mediterranean shrubland

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Abstract

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

Keywords

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

1. Introduction

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

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

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

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

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

2. Materials and Methods

2.1. Study site

The study site was located in the Massif de l'Etoile near Marseille, France (43° 22′ N, 5°25′ E) at 275 m above sea level (see Montès et al. (2008) for a detailed description of the study site). The mean annual precipitation is 552 mm and the mean annual temperature is 14.6 °C (mean values over the period 2002-2012 averaged across the two meteorological stations in Marignane (43°26'N, 5°12'E) and Marseille (43°15'N, 5°22'E) closest to our study site). The soil is classified as shallow rendzina developed over limestone bedrock with with 66 % of 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 m^2 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 ± 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 × 2 precipitation treatments × 3 plots (4 plots
175 for 4-species mixture) × 4 replicates per plot) were installed *in situ* across all plots in the central

176 4 m^2 part of the 16 m^2 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 concentraction 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

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

2.4. Bacterial and fungal densities

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

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

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

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

2.5. Bacterial and fungal community diversity

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

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

2.6. Statistical analyses

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

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

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

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

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

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

3. Results

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

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

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

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

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

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

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

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

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

Bacterial and fungal community dissimilarities were also affected by litter mixture composition and richness (PERMANOVA, Pseudo-F = 4.83 to 11.07, $P < 0.0001$). Reduced precipitation led to a shift in bacterial and/or fungal community in 11 of the 15 litter treatments between control and reduced precipitation treatments (Table S3; 7 out of 15 litter treatments for 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 ± 2.2% in *Ulex* litter to 40.2 ± 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 ± 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 ±
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 ± 1.9% compared to 36.9 ± 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 ± 0.6% on average with less rainfall compared to 12.1 ± 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).

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

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

4. Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

613 **Acknowledgements**

614 We thank our colleagues from CEFE UMR5175 (Montpellier) and from IMBE UMR
615 7263 (Marseille) for their contribution to the set-up of the experiment and the field work. We
616 specially thank Sylvie Dupouyet for her tireless assistance during the field work, Caroline
617 Lecareux and Germain Boungou for their assistance to the chemical analyses, Jean-Philippe
618 Mévy for the acquisition of meteorological and soil humidity data, Elise Courvosier-Dezord
619 from the “Plateforme Analyse et Valorisation de la Diversité” for her assistance in qPCR

analysis and the staff of the “Plateforme Génomique du Génopole de Toulouse Midi-Pyrénées” for technical assistance with the sequencing facilities. Funding was provided by the Agence Nationale de la Recherche (ANR) through the project CLIMED (ANR-09-CEP-007).

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803

TABLES

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

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

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

		Bacterial abundance		Fungal abundance		Bacterial diversity		Fungal diversity		Carbon release		Nitrogen release	
	$d.f._{num}$	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
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	

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

	Bacterial abundance (10^6)		Fungal abundance (10^6)		Bacterial diversity		Fungal diversity		Carbon release (%)		Nitrogen release (%)	
	Control	PC	Control	PC	Control	PC	Control	PC	Control	PC	Control	PC
C	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.2	0.1 ± 0.01	1.5 ± 0.11	1.6 ± 0.05	1.2 ± 0.05	1.2 ± 0.08	39.5 ± 1.6	36.9 ± 1.5	8.2 ± 1.8	8.7 ± 2.2
Q	69.9 ± 17.6	65.8 ± 11.5	120.4 ± 30.5	123.3 ± 24.0	1.9 ± 0.02	1.9 ± 0.02	1.6 ± 0.02	1.6 ± 0.01	32.6 ± 2.0	35.8 ± 2.2	15.7 ± 3.5	18.5 ± 1.8
R	5.6 ± 1.5	7.7 ± 1.4	6.0 ± 1.2	10.7 ± 2.2	1.7 ± 0.06	1.7 ± 0.02	1.3 ± 0.03	1.4 ± 0.03	36.9 ± 2.2	25.3 ± 1.9	8.5 ± 2.3	1.8 ± 2.0
U	2.8 ± 0.9	3.2 ± 1.0	8.4 ± 2.6	3.2 ± 0.9	1.5 ± 0.09	1.6 ± 0.08	1.4 ± 0.04	1.3 ± 0.10	19.5 ± 2.2	22.8 ± 2.0	5.2 ± 2.3	1.4 ± 3.9
CQ	14.8 ± 5.7	46.7 ± 12.2	50.5 ± 11.1	69.0 ± 16.5	1.7 ± 0.09	1.8 ± 0.05	1.6 ± 0.03	1.6 ± 0.03	37.6 ± 0.6	39.1 ± 2.0	13.2 ± 1.3	16.3 ± 2.3
CR	3.7 ± 1.0	4.5 ± 2.2	9.0 ± 3.2	8.2 ± 3.8	1.6 ± 0.07	1.5 ± 0.06	1.5 ± 0.02	1.5 ± 0.03	34.9 ± 1.3	37.9 ± 1.7	12.2 ± 2.2	11.7 ± 2.0
CU	0.5 ± 0.1	1.2 ± 0.5	0.8 ± 0.2	2.3 ± 1.3	1.2 ± 0.09	1.1 ± 0.09	1.5 ± 0.05	1.5 ± 0.05	28.3 ± 1.2	25.0 ± 0.4	1.8 ± 1.4	-5.4 ± 2.5
QR	114.1 ± 43.0	16.6 ± 5.0	178.2 ± 64.4	60.2 ± 16.2	1.7 ± 0.08	1.7 ± 0.04	1.7 ± 0.02	1.7 ± 0.02	37.8 ± 1.1	35.5 ± 1.4	12.9 ± 2.1	9.6 ± 1.8
QU	33.4 ± 16.3	3.1 ± 0.5	120.9 ± 46.0	36.7 ± 15.7	1.6 ± 0.10	1.7 ± 0.05	1.7 ± 0.01	1.7 ± 0.02	30.2 ± 1.6	35.3 ± 1.9	17.8 ± 2.2	17.6 ± 1.8
RU	1.2 ± 0.3	1.7 ± 0.6	1.4 ± 0.4	2.2 ± 0.7	1.5 ± 0.04	1.6 ± 0.05	1.7 ± 0.02	1.7 ± 0.02	27.9 ± 1.6	28.7 ± 1.3	2.0 ± 1.7	-3.5 ± 1.7
CQR	70.3 ± 17.9	46.7 ± 24.1	169.8 ± 37.8	227.3 ± 70.8	1.9 ± 0.03	1.9 ± 0.04	1.7 ± 0.01	1.7 ± 0.01	40.2 ± 2.1	39.8 ± 1.8	19.5 ± 1.8	21.0 ± 1.9
CQU	20.5 ± 6.7	44.9 ± 15.3	87.1 ± 37.6	141.3 ± 43.2	1.9 ± 0.02	1.8 ± 0.04	1.7 ± 0.02	1.8 ± 0.02	36.5 ± 2.0	37.7 ± 1.6	18.2 ± 2.6	13.0 ± 3.4
CRU	3.0 ± 1.2	12.2 ± 3.8	7.1 ± 1.9	16.3 ± 4.3	1.3 ± 0.09	1.5 ± 0.10	1.2 ± 0.05	1.5 ± 0.03	32.4 ± 1.5	36.4 ± 1.7	8.4 ± 2.9	7.1 ± 2.4
QRU	30.9 ± 10.6	29.3 ± 11.5	83.3 ± 24.8	104.0 ± 31.4	1.4 ± 0.09	1.5 ± 0.10	1.6 ± 0.02	1.6 ± 0.02	34.9 ± 0.7	35.6 ± 1.4	19.5 ± 1.9	17.7 ± 1.6
CQRU	64.3 ± 6.5	53.9 ± 20.7	162.5 ± 18.1	98.3 ± 21.1	1.6 ± 0.13	1.7 ± 0.05	1.7 ± 0.02	1.7 ± 0.02	35.0 ± 0.8	34.1 ± 1.2	18.4 ± 1.3	15.4 ± 1.6

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

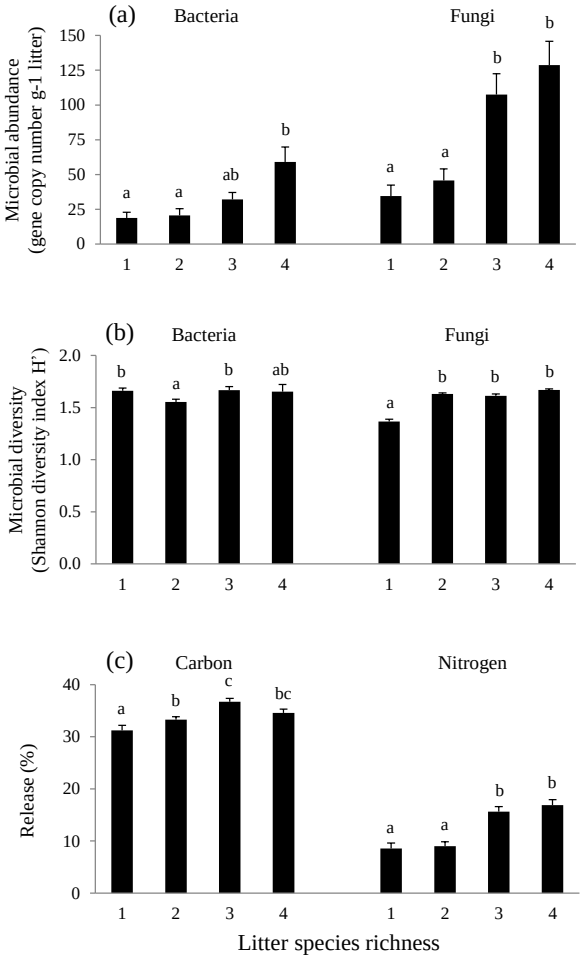
848 **Fig. 2.** Relative Mixture Effects (RME) on microbial abundance (a and b) and diversity (c and
849 d) from multi-species litter mixtures according to control (open symbol) and precipitation
850 change (close symbol) conditions. RME significantly different from 0 are indicated with the
851 respective 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.

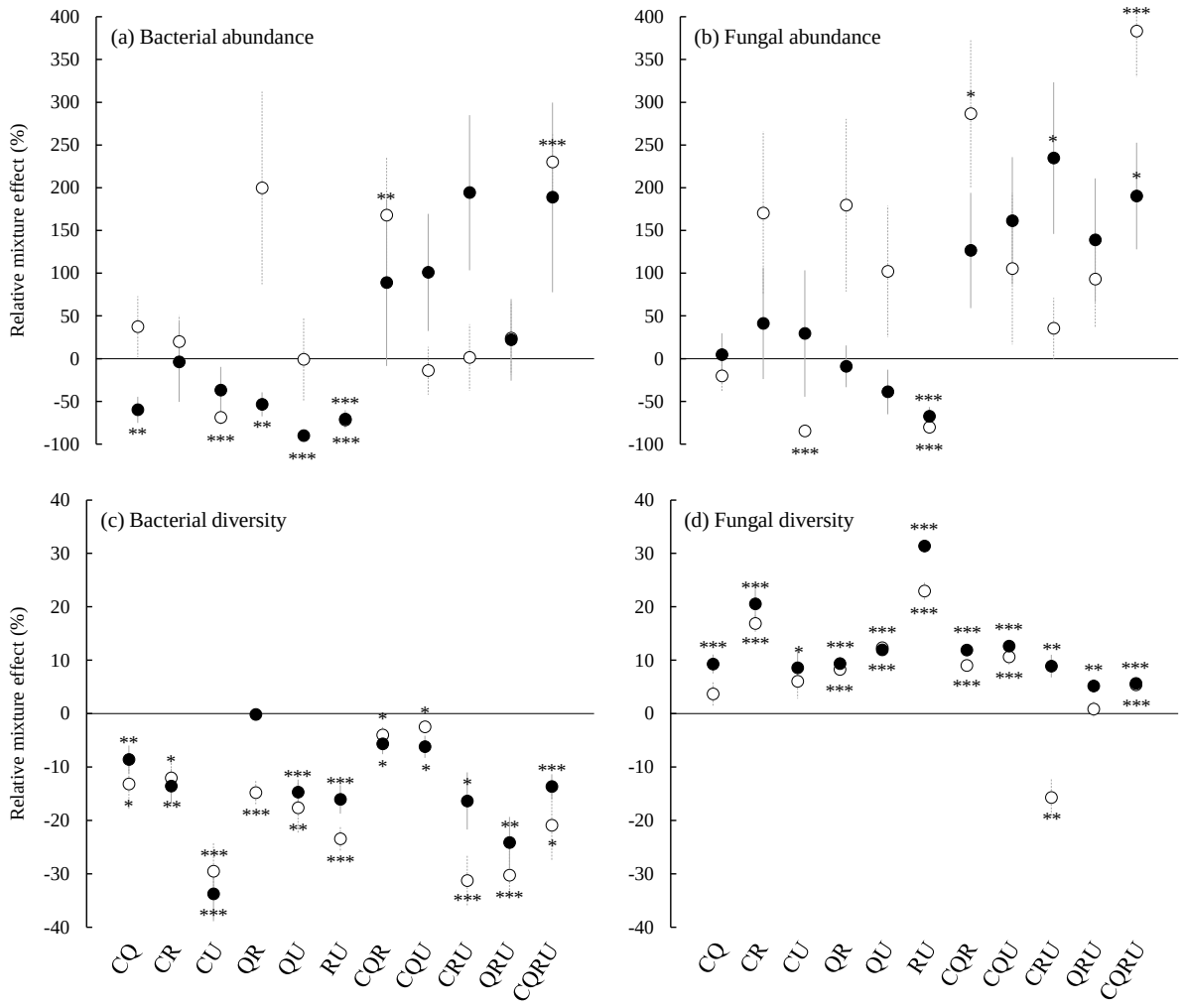
861

Fig. 1.


(a)
Bacteria
Fungi
Microbial abundance (gene copy number g-1 litter)
150
125
100
75
50
25
0
a
a
ab
b
a
a
b
b
1
2
3
4
(b)
Bacteria
Fungi
Microbial diversity (Shannon diversity index H')
2.0
1.5
1.0
0.5
0.0
b
a
b
ab
a
b
b
b
(c)
Carbon
Nitrogen
Release (%)
40
30
20
10
0
a
b
c
bc
a
a
b
b
Litter species richness

866 **Fig. 2.**

867



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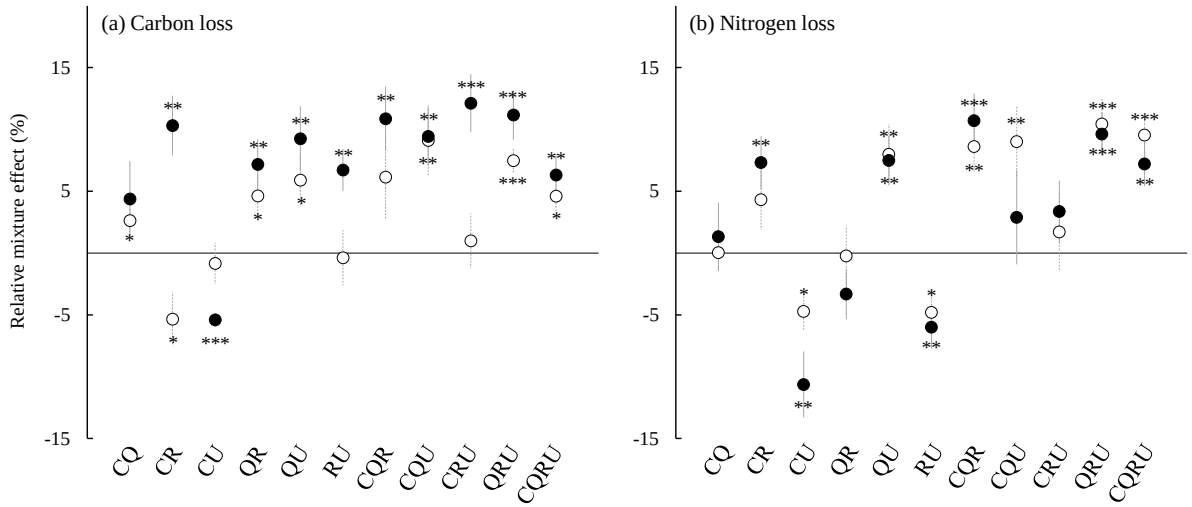
869

870 **Fig. 3.**

871



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