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

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ARTICLE INFO

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.

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1. Introduction

Decomposition of plant material is a key ecosystem function

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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 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 cmol kg^{-1} (means from 92 different soil samples taken in the study plots; see Shihan et al. (2017) for further details).

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

2.2. Experimental setup

The study site is characterized by a natural small-scale mosaic of assemblages of distinct compositions of the four dominating woody shrub species (i.e. *Quercus, Cistus, Rosmarinus* and *Ulex*) that allowed establishing a series of plots varying in species richness of these four species. Ninety-two 4 × 4 m plots were selected based on plant community composition in order to include all 15 possible combinations of the four dominant shrub species (i.e. 4 single-species litter, 6 two-species mixtures, 4 three-species mixtures, and one four-species mixture). The distance between plots varied from 1 to 30 m (Fig. S1). Each of the 15 different plant combinations was replicated six times, except for the four-species mixture that was replicated eight times. All plots were equipped with a 4 m × 4 m solid aluminum frame, held 2 m above the ground by aluminum posts at the outer border of the 16 m^2 plot area and fixed to the ground with reinforcing bars. Stainless steel gutters were mounted on top of the aluminum frame. Contrary to the rain exclusion plots, the gutters in the control plots were mounted upside down in order to let the precipitation fall on the vegetation. A supplementary PVC gutter and a pipe mounted at the border of the frame allowed to evacuate the rainwater away from the plots. Over the 6 replicates for each plant combinations (8 for the four-species mixture), 3 replicates were randomly assigned to control plots and the other 3 to rain exclusion plots (4 of each for the four-species mixture). The experimental rain exclusion was set up in October 2011. The exact amount of precipitation excluded was estimated using (i) rain gauges installed at ground level underneath the gutters in both control and rain-excluded plots and (ii) TDR100 probes (Campbell Scientific Inc., Logan, Utah) installed in seven control and eight rain-excluded plots at 10 cm soil depth and by (iii) determining the gravimetric humidity in the soil sampled in control and excluded plots. Compared to control plots, the rain exclusion plots received on average 12± 2% less rainfall. This exclusion resulted in an average lower soil humidity of −6.5% (that could reach between −13 and −24% during rain events) at 10 cm soil depth between control and rain exclusion plots (determined by

permanently installed TDR probes; Fig. S2).

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

In order to mimic natural conditions during litter decomposition, leaf litter mixtures were placed in specifically designed field mesocosms, consisting of "open-bottom" PVC cylinders (5 cm tall) covered with 1 mm mesh net on top and with windows on the side covered with 10 mm mesh net, similar to those used in Barantal et al. (2011). We constructed these field mesocosms in order to allow direct contact of the litter with the soil surface and full access of the soil fauna without flattening the litter material as is typically the case with traditional litterbags. Mesocosms were filled with a total of six grams of air-dried leaf litter reflecting the species composition of the respective plot (i.e. 4 single-species litters and 11 different litter mixtures), with equal proportions of the different litter species in the mixtures. In December 2011, a total of 368 cylinders (15 litter treatments × 2 precipitation treatments × 3 plots (4 plots for 4-species mixture) × 4 replicates per plot) were installed *in situ* across all plots in the central 4 m^2 part of the 16 m^2 plot area to avoid edge effects. Mesocosms were placed in nearly flat area and fixed onto the soil surface with nails, avoiding rock outcrops and very shallow soil.

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

2.3. Litter measurements

Initial litter quality was determined from four subsamples of each species-specific litter batch (Table 1). In addition, we analyzed carbon (C) and nitrogen (N) concentrations in aliquotes from all litter samples retrieved from the field mesocosms at the end of the experiment. Carbon and N concentrations of initial and remaining litter materials were determined by thermal combustion on a Flash EA 1112 series C/N elemental analyzer (Thermo Scientific, Waltham, MA, USA). Based on the litter dry mass data and the initial and final C and N concentrations, we calculated the percentage of absolute C and N release for each mesocosm as the ratio of remaining to initial C and N contents. Phosphorus (P) concentration was measured

colorimetrically using the method of Santonja et al. (2015a). Lignin concentration was determined according to the van Soest extraction protocol (Van Soest and Wine, 1967) using a fiber analyzer (Fibersac 24; Ankom, Macedon, NJ, USA). Phenolic concentration was measured colorimetrically using the method of Santonja et al. (2015a) with gallic acid as a standard. To determine the water holding capacity (WHC), intact leaf litter 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 centrifuged 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′- ACTCCTACGGGAGG-CAGCAG-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.

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

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 min at 95 °C, followed by 50 cycles of amplification consisting of 15 s denaturation at 95 °C, 15 s of primer annealing, and 10 s 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′-CTTGGTCATTTA-GAGGAAGTAA-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 min, we ran 35 cycles of denaturation at 94 °C for 1 min, followed by annealing at 55 °C for 1 min, elongation at 72 °C for 1 min and a final elongation at 72 °C for 10 min. 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' = -∑ p_i × 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 × 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

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.

	d.f._num	Bacterial abundance		Fungal abundance		Bacterial diversity		Fungal diversity		Carbon release		Nitrogen release	
		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	

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 (Fig. 2a and b, Fig. 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 (Fig. 2a and b, Fig. 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) (Fig. 2a and b). 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 (Fig. 2a and b).

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 (Fig. 2c and d), 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

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	1.6 ± 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
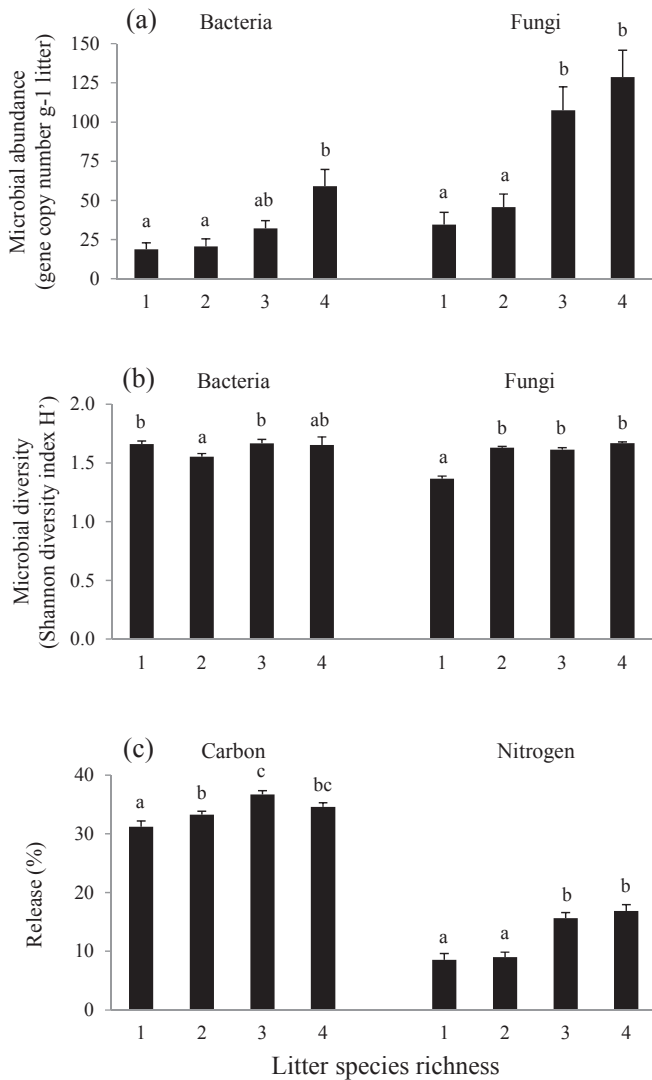
Fig. 1. Microbial abundance (a), microbial diversity (b), and carbon and nitrogen release (c) according to the four levels of litter species richness. Different letters denote significant differences among species richness levels.

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).

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

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

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

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

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

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

Bacterial abundance was positively, but weakly, correlated to RME on C and on N release under both precipitation conditions (Table 4). Weak positive correlations were also found between fungal abundance and RME on C release — but only with reduced precipitation, and between fungal abundance and RME on N release under both precipitation conditions (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
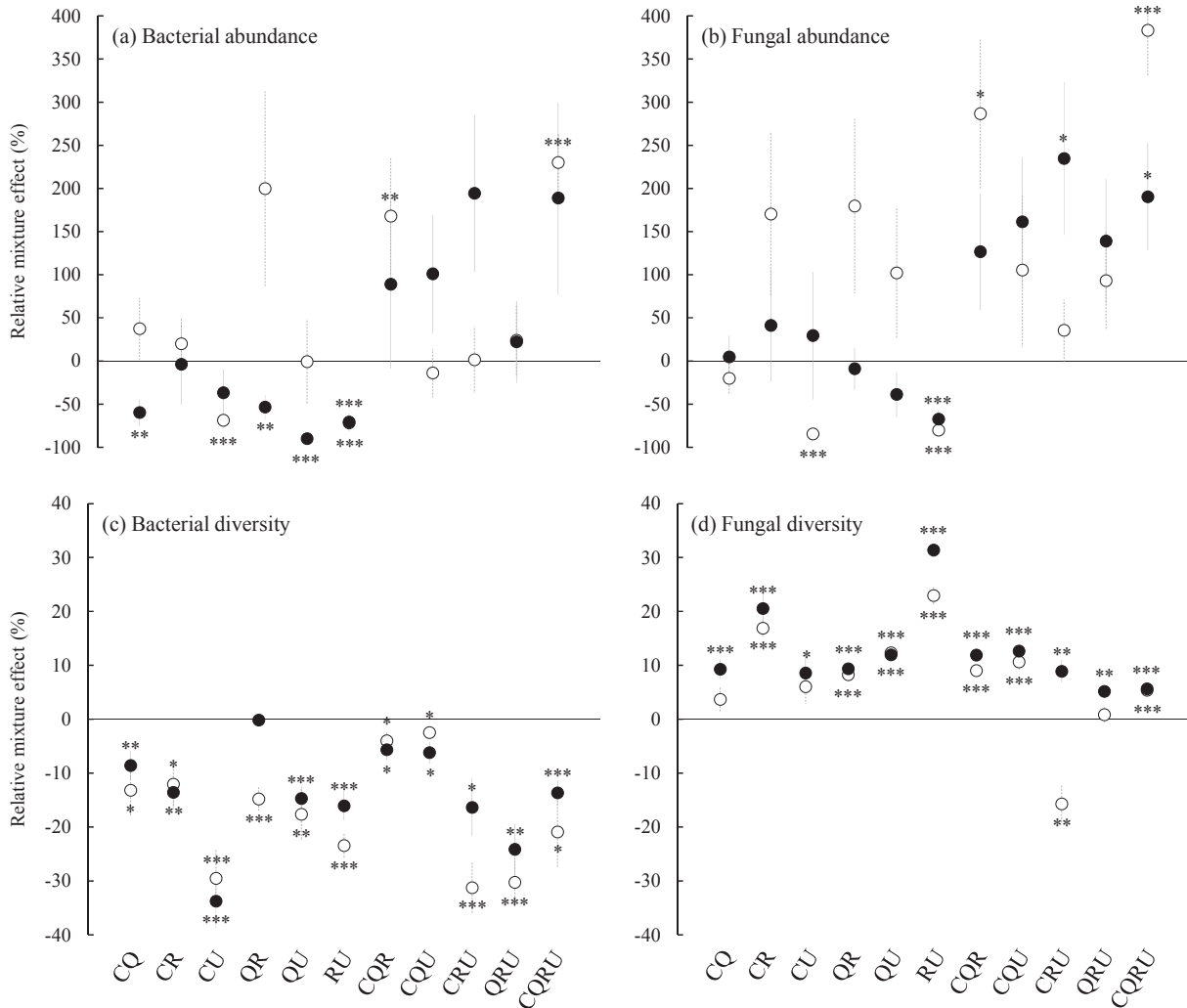
Fig. 2. Relative Mixture Effects (RME) on microbial abundance (a and b) and diversity (c and d) from multi-species litter mixtures according to control (open symbol) and precipitation change (close symbol) conditions. RME significantly different from 0 are indicated with the respective symbols * for $P < 0.05$, ** for $P < 0.01$, and *** for $P < 0.001$. C = *Cistus*, Q = *Quercus*, R = *Rosmarinus*, U = *Ulex*. Combinations of capital letters correspond to combinations of plant species in litter mixtures.
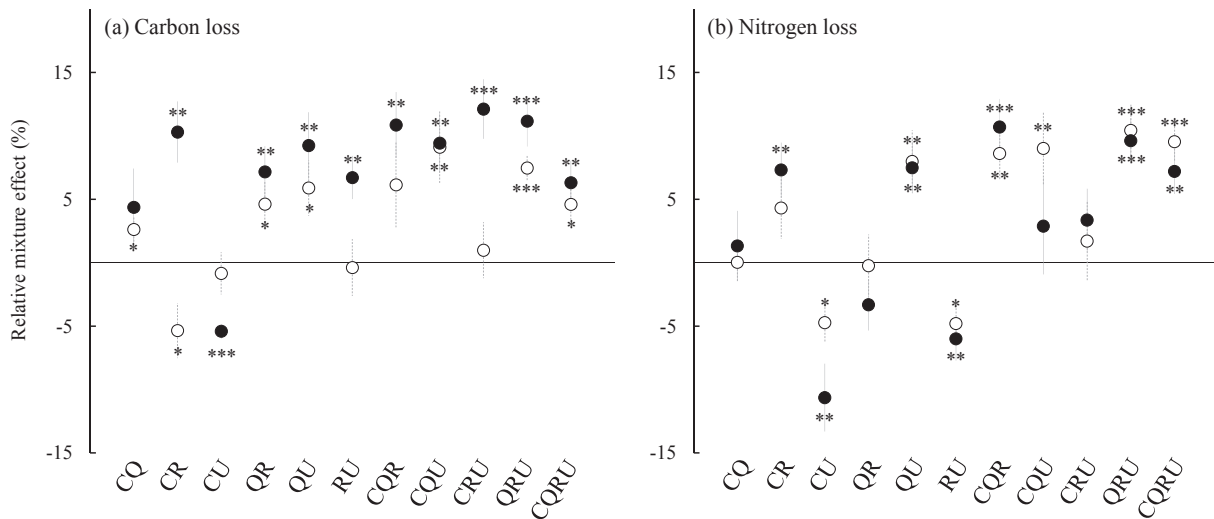
Fig. 3. Relative Mixture Effects (RME) on carbon (a) and nitrogen (b) release from multi-species litter mixtures according to control (open symbol) and precipitation change (close symbol) conditions. RME significantly different from 0 are indicated with the respective symbols * for $P < 0.05$, ** for $P < 0.01$, and *** for $P < 0.001$. C = *Cistus*, Q = *Quercus*, R = *Rosmarinus*, U = *Ulex*. Combinations of capital letters correspond to combinations of plant species in litter mixtures.

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

	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*

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

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

4.3. Effects of a change in precipitation

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

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

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

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

Acknowledgements

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2017.04.006.

References

Aerts, R., 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. Oikos 79, 439–449.

Angel, R., Soares, M.I.M., Ungar, E.D., Gillor, O., 2010. Biogeography of soil archaea and bacteria along a steep precipitation gradient. ISME Journal 4, 553–563.

Barantal, S., Roy, J., Fromin, N., Schimann, H., Hättenschwiler, S., 2011. Long-term presence of tree species but not chemical diversity affect litter mixture effects on decomposition in a neotropical rainforest. Oecologia 167, 241–252.

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

Bardgett, R.D., 2005. The Biology of Soil: a Community and Ecosystem Approach. Oxford University Press, Oxford.

Berard, A., Bouchet, T., Sevenier, G., Pablo, A.L., Gros, R., 2011. Resilience of soil microbial communities impacted by severe drought and high temperature in the context of Mediterranean heatwaves. European Journal of Soil Biology 47, 333–342.

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

Cadish, G., Giller, K.E., 1997. Driven by Nature: Plant Litter Quality and Decomposition. CAB international, Wallingford.

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

Chapman, S.K., Newman, G.S., Hart, S.C., Schweitzer, J.A., Koch, G.W., 2013. Leaf litter mixtures alter microbial community development: mechanisms for non-additive effects in litter decomposition. PLoS One 8, e62671.

Clarke, K.R., Warwick, R.M., 2001. A further biodiversity index applicable to species lists: variation in taxonomic distinctness. Marine Ecology Progress Series 216, 265–278.

Cregger, M.A., Schadt, C.W., McDowell, N.G., Pockman, W.T., Classen, A.T., 2012. Response of the soil microbial community to changes in precipitation in a semiarid ecosystem. Applied and Environmental Microbiology 78, 8587–8594.

Coulis, M., Fromin, N., David, J.-F., Gavinet, J., Clet, A., Devidal, S., Roy, J., Hättenschwiler, S., 2015. Functional dissimilarity across trophic levels as a driver of soil processes in a Mediterranean decomposer system exposed to two moisture levels. Oikos 124, 1304–1316.

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

Dubrovsky, M., Hayes, M., Duce, P., Trnka, M., Svoboda, M., Zara, P., 2014. Multi-GCM projections of future drought and climate variability indicators for the Mediterranean region. Regional Environmental Change 14, 1907–1919.

Fierer, N., Craine, J.M., McLaughgan, K., Schimel, J.P., 2005. Litter quality and the temperature sensitivity of decomposition. Ecology 86, 320–326.

Garcia-Palacios, P., Prieto, I., Ourcival, J.-M., Hättenschwiler, S., 2016. Disentangling the litter quality and soil microbial contribution to leaf and fine root litter decomposition responses to reduced rainfall. Ecosystems 19, 490–503.

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

Gessner, M.O., Swan, C.M., Dang, C.K., Mckie, B.G., Bardgett, R.D., Wall, D.H., Hättenschwiler, S., 2010. Diversity meets decomposition. Trends in Ecology and Evolution 25, 372–380.

Giorgi, F., Lionello, P., 2008. Climate change projections for the Mediterranean region. Global and Planetary Change 63, 90–104.

Handa, I.T., Aerts, R., Berendse, F., Berg, M.P., Bruder, A., Butenschoen, O., Chauvet, E., Gessner, M.O., Jabiol, J., Makkonen, M., McKie, B.G., Malmqvist, B., Peeters, E.T.H.M., Scheu, S., Schmid, B., Ruijven, J.V., Vos, V.C.A., Hättenschwiler, S., 2014. Consequences of biodiversity loss for litter decomposition across biomes. Nature 509, 218–221.

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

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

Henry, S., Bru, D., Stress, B., Hallet, S., Philippot, L., 2006. Quantitative detection of the nosZ gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, narG, nirK, and nosZ genes in soils. Applied and Environmental Microbiology 72, 5181–5189.

Hobbie, S.E., 1996. Temperature and plant species control over litter decomposition in Alaskan tundra. Ecological Monographs 66, 503–522.

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

Jousset, A., Schmid, B., Scheu, S., Eisenhauer, N., 2011. Genotypic richness and dissimilarity opposingly affect ecosystem functioning. Ecology Letters 14, 537–545.

Kaisermann, A., Roguet, A., Nunan, N., Maron, P.A., Ostle, N., Lata, J.C., 2013. Agricultural management affects the response of soil bacterial community structure and respiration to water-stress. Soil Biology and Biochemistry 66, 69–77.

Kominoski, J.S., Pringle, C.M., Ball, B.A., Bradford, M.A., Coleman, D.C., Hall, D.B., Hunter, M.D., 2007. Nonadditive effects of leaf litter species diversity on breakdown dynamics in a detritus-based stream. Ecology 88, 1167–1176.

Kominoski, J.S., Hoellein, T.J., Kelly, J.J., Pringle, C.M., 2009. Does mixing litter of different qualities alter stream microbial diversity and functioning on individual litter species? Oikos 118, 457–463.

Knapp, A.K., Beier, C., Briske, D.D., Classen, A.T., Luo, Y., Reichstein, M., Smith, M.D., Smith, S.D., Bell, J.E., Fay, P.A., Heisler, J.L., Leavitt, S.W., Sherry, R., Smith, B., Weng, E., 2008. Consequences of more extreme precipitation regimes for terrestrial ecosystems. Bioscience 58, 811–821.

Lecerf, A., Risnoveanu, G., Popescu, C., Gessner, M.O., Chauvet, E., 2007. Decomposition of diverse litter mixtures in streams. Ecology 88, 219–227.

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

Lodge, D.J., McDowell, W.H., McSwiney, C.P., 1994. The importance of nutrient pulses in tropical forests. Trends in Ecology and Evolution 9, 384–387.

Loreau, M., 2001. Microbial diversity, producer–decomposer interactions and ecosystem processes: a theoretical model. Proceedings of the Royal Society B: Biological Sciences 268, 303–309.

Lunghini, D., Granito, V.M., Di Lonardo, D.P., Maggi, O., Persiani, A.M., 2013. Fungal

diversity of saprotrophic litter fungi in a Mediterranean maquis environment. Mycologia 105, 1499–1515.

Makkonen, M., Berg, M.P., van Logtestijn, R.S.P., van Hal, J.R., Aerts, R., 2013. Do physical plant litter traits explain non-additivity in litter mixtures? A test of the improved microenvironmental conditions theory. Oikos 122, 987–997.

Montès, N., Maestre, F.T., Ballini, C., Baldy, V., Gauquelin, T., Planquette, M., Greff, S., Dupouyet, S., Perret, J.B., 2008. On the relative importance of the effects of selection and complementarity as drivers of diversity productivity relationships in Mediterranean shrublands. Oikos 117, 1345–1350.

Pesaro, M., Nicollier, G., Zeyer, J., Widmer, F., 2004. Impact of soil drying-rewetting stress on microbial communities and activities and on degradation of two crop protection products. Applied and Environmental Microbiology 70, 2577–2587.

Polade, S.D., Pierce, D.W., Cayan, D.R., Gershunov, A., Dettinger, M.D., 2014. The key role of dry days in changing regional climate and precipitation regimes. Scientific Reports 4, 4364.

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

Robinson, C.H., Dighton, J., Frankland, J.C., Coward, P.A., 1993. Nutrient and carbon dioxide release by interacting species of straw-decomposing fungi. Plant and Soil 151, 139–142.

Santonja, M., Fernandez, C., Gauquelin, T., Baldy, V., 2015a. Climate change effects on litter decomposition: intensive drought leads to a strong decrease of litter mixture interactions. Plant and Soil 393, 69–82.

Santonja, M., Baldy, V., Fernandez, C., Balesdent, J., Gauquelin, T., 2015b. Potential shift in plant communities with climate change in a Mediterranean Oak forest: consequence on nutrients and secondary metabolites release during litter decomposition. Ecosystems 18, 1253–1268.

Santonja, M., Fernandez, C., Gers, C., Proffit, M., Gauquelin, T., Reiter, I., Cramer, W., Baldy, V., 2017. Plant litter mixture partly mitigates the negative effects of extended drought on soil biota and litter decomposition in a Mediterranean oak forest. Journal of Ecology. http://dx.doi.org/10.1111/1365-2745.12711.

Sardans, J., Peñuelas, J., 2013. Plant-soil interactions in Mediterranean forest and shrublands: impacts of climatic change. Plant and Soil 365, 1–33.

Saura-Mas, S., Estiarte, M., Peñuelas, J., Lloret, F., 2012. Effects of climate change on leaf litter decomposition across post-fire plant regenerative groups. Environmental and Experimental Botany 77, 274–282.

Schindler, M.H., Gessner, M.O., 2009. Functional leaf traits and biodiversity effects on litter decomposition in a stream. Ecology 90, 1641–1649.

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

Setälä, H., McLean, M.A., 2004. Decomposition rate of organic substrates in relation to the species diversity of soil saprophytic fungi. Oecologia 139, 98–107.

Sherman, C., Sternberg, M., Steinberger, Y., 2012. Effects of climate change on soil respiration and carbon processing in Mediterranean and semi-arid regions: an experimental approach. European Journal of Soil Biology 52, 48–58.

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

Sherman, C., Grishkan, I., Barness, G., Steinberger, Y., 2014. Fungal community-plant litter decomposition relationships along a climate gradient. Pedosphere 24, 437–449.

Tilman, D., Lehman, C.L., Thomson, K.T., 1997. Plant diversity and ecosystem productivity: theoretical considerations. Proceedings of the National Academy of Sciences of the United States of America 94, 1857–1861.

Tiunov, A.V., Scheu, S., 2005. Facilitative interactions rather than resource partitioning drive diversity functioning relationships in laboratory fungal communities. Ecology Letters 8, 618–625.

Van Soest, P.U., Wine, R.H., 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. Journal of the Association of Official Analytical Chemistry 50, 50–55.

Vivanco, L., Austin, A.T., 2008. Tree species identity alters forest litter decomposition through long-term plant and soil interactions in Patagonia, Argentina. Journal of Ecology 96, 727–736.

Vogel, A., Eisenhauer, N., Weigelt, A., Scherer-Lorenzen, M., 2013. Plant diversity does not buffer drought effects on early-stage litter mass loss rates and microbial properties. Global Change Biology 19, 2795–2803.

Walter, J., Hein, R., Beierkuhnlein, C., Hammerl, V., Jentsch, A., Schadler, M., Schuerings, J., Kreyling, J., 2013. Combined effects of multifactor climate change and land-use on decomposition in temperate grassland. Soil Biology and Biochemistry 60, 10–18.

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

Wilkinson, S.C., Anderson, J.M., Scardelis, S.P., Tisiafouli, M., Taylor, A., Wolters, V., 2002. PLFA profiles of microbial communities in decomposing conifer litters subject to moisture stress. Soil Biology and Biochemistry 34, 189–200.

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

Yuste, J.C., Fernandez-Gonzalez, A.J., Fernandez-Lopez, M., Ogaya, R., Peñuelas, J., Sardans, J., Lloret, F., 2014. Strong functional stability of soil microbial communities under semiarid Mediterranean conditions and subjected to long-term shifts in baseline precipitation. Soil Biology and Biochemistry 69, 223–233.

Yuste, J.C., Peñuelas, J., Estiarte, M., Garcia-Mas, J., Mattana, S., Ogaya, R., Pujol, M., Sardans, J., 2011. Drought-resistant fungi control soil organic matter decomposition and its response to temperature. Global Change Biology 17, 1475–1486.