



Temporal Shifts in Plant Diversity Effects on Carbon and Nitrogen Dynamics During Litter Decomposition in a Mediterranean Shrubland Exposed to Reduced Precipitation

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TITLE: Temporal shifts in plant diversity effects on carbon and nitrogen dynamics during litter decomposition in a Mediterranean shrubland exposed to reduced precipitation

RUNNING HEAD: Litter diversity effects on C and N dynamics

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ABSTRACT

Climate and plant diversity are major determinants of carbon (C) and nitrogen (N) dynamics in decomposing plant litter. However, the direction and extent to which these dynamics are affected by combined changes in climate and biodiversity is not well understood. We used a field experiment in a Mediterranean shrubland ranging from one to four shrub species with partial rain exclusion (-12%) to test how lower precipitation interacts with shrub species diversity to influence C and N release during decomposition. We also distinguished between first-year (0-12 months) and second-year decomposition (12-24 months) to test the hypothesis of stronger diversity effects at the beginning of the decomposition process. Litter C and N release increased with litter species richness during the first year, but not during the second year of decomposition. However, these richness effects were weak and less consistent than litter composition effects, which persisted over time and became even stronger for C release after two years of decomposition. Partial rain exclusion reduced N release by 17% only during the first year and had no effect on C release in either year. Community weighted mean (CWM) traits and functional dissimilarity (FD) of litter traits contributed both to explain litter species composition effects. These litter trait effects were not altered by partial rain exclusion, but were more important after two years than after one year of decomposition. Our findings suggest increasing trait legacy effects with ongoing decomposition. More generally, our data showed that changes in the diversity of dominant shrub species had stronger effects on C and N release during litter decomposition than a moderate reduction in precipitation.

KEY WORDS

Biodiversity-ecosystem functioning; community weighted means; decomposition stage; functional trait dissimilarity; garrigue; litter functional traits; rainfall exclusion

47 **HIGHLIGHTS**

- 48 - Changing plant diversity affected litter decomposition more than reduced rainfall
- 49 - Less rainfall did not change C but reduced N release after one year of decomposition
- 50 - Legacy effects of litter traits on C and N release increased during decomposition
- 51

INTRODUCTION

Ongoing climate change and biodiversity loss are projected to considerably alter the structure and function of Earth's ecosystems (Bellard and others 2012). The degree of these alterations and their consequences for human societies are difficult to predict and may strongly differ depending on the specific geographical area (Pearce-Higgins and others 2015). With its position at the transition from the European temperate zone to the dry subtropical zone in Africa and the Middle East, the Mediterranean basin may be particularly sensitive to climate change, especially to shifts in the precipitation regime (Giorgi 2006; Mariotti and others 2008). Indeed, regional climate models predict a decrease in the amount of precipitation, and an increase in both temperature and the periods without precipitation in the Mediterranean region in the near future (Giorgi and Lionello 2008; Dubrovsky and others 2014; Polade and others 2014). The geographical position between two major climate zones, among other factors such as geological complexity and evolutionary history, also contributes to the exceptionally rich Mediterranean biological diversity (Cowling and others 1996; Thompson 2005), making the Mediterranean basin one of the ten biodiversity hotspots worldwide (Médail and Quézel 1999; Myers and others 2000).

Climate and the diversity of plant communities (i.e. the richness and the composition of species) are two major drivers of ecosystem processes such as productivity and decomposition. Yet, the combined effects and relative importance of changes in climatic conditions and biodiversity are poorly understood and have only rarely been addressed experimentally (Vogel and others 2013). Decomposition of dead organic matter governs the rate at which the carbon and nutrients immobilized in dead tissues are made available for microbial and plant growth (Swift and others 1979). Because microbial activity depends strongly on temperature and moisture conditions, climatic variables are long known as key drivers of decomposition (Swift and others 1979; Berg and others 1993; Coûteaux and others 1995). Recent studies showed that

variation in microclimate at local scales is a more important factor explaining differences in decomposition rates than the traditionally considered continental-scale variation in macroclimate (Bradford and others 2016; Joly and others 2017). Moreover, site-specific differences in litter quality among co-occurring plant species account overall for more variation in litter decomposition than differences in environmental factors across broad latitudinal gradients (Cornwell and others 2008). Therefore, relatively small changes in microclimatic conditions and shifts in plant species composition may strongly affect C and nutrient dynamics during decomposition at small spatial scales.

Changing plant species composition can modify litter quality control over decomposition in two ways. First, according to the “mass-ratio hypothesis” (Grime 1998; Garnier and others 2004) litter trait control over decomposition shifts along with the change in the average litter quality, expressed as community-weighted mean (CWM) traits (Quested and others 2007; Mokany and others 2008; Laughlin 2011). Second, according to the “niche complementarity hypothesis” (Petchey and Gaston 2006; Diaz and others 2007) a change in the functional diversity (FD) of trait-values affects the degree at which complementary resource use occurs in the decomposer community (Wardle and others 1997; Vos and others 2013; Barantal and others 2014; Handa and others 2014). These two mechanisms can operate simultaneously in affecting litter decomposition following a shift in plant (litter) composition (Garcia-Palacios and others 2017). Moreover, the relative importance of CWM-trait and FD-trait control may strongly differ for C and N release patterns from decomposing litter mixtures (Garcia-Palacios and others 2017), suggesting that C and N dynamics are distinctly affected by changes in plant community composition. However, C and N dynamics are rarely addressed together in studies evaluating biodiversity effects on decomposition, especially in Mediterranean ecosystems (but see Handa and others 2014; Garcia-Palacios and others 2017),

and it remains unknown how C and N dynamics respond to a concomitant change in plant diversity and precipitation.

Because it is generally assumed that initial differences in litter quality among different plant species converge during litter decomposition (e.g. [Moore and others 2005](#); [Preston and others 2009](#)), trait mediated litter diversity control on decomposition should be maximal during the initial stage of decomposition, but is expected to decrease in importance during later decomposition stages. However, this hypothesis has not been specifically tested so far. A recent experimental test of how the relative importance of microclimate, litter quality and decomposer community control over decomposition changes over time showed that abiotic control factors increase in their relative importance in later compared to earlier decomposition stages ([Garcia-Palacios and others 2016](#)). Based on these results, we expect that the relative importance of the effects of trait-mediated litter diversity and decreased precipitation would shift with ongoing decomposition towards less litter diversity but more microclimate control. The disentangling of the relative importance of a combined change in precipitation and plant community diversity on C and N dynamics during different stages of decomposition would improve our understanding of the consequences of ongoing climate and biodiversity change on biogeochemical cycling.

In a large field experiment, we established 92 plots of all possible combinations of four dominant woody species, half of them with partial rain exclusion, to test how simultaneous changes in plant diversity and precipitation affect decomposition and other ecosystem processes in a Mediterranean shrubland in Southern France. Here we report the data from a 2-year decomposition experiment that was set up to evaluate how C and N dynamics are affected by changing plant diversity and precipitation during different decomposition stages. Two previous studies using the same decomposition experiment investigated the effects of plant diversity and precipitation changes on the abundance and diversity of microbial communities in the litter

after one year of decomposition ([Santonja and others 2017a](#)), and on functional diversity of soil microorganisms after one and two years of decomposition ([Shihan and others 2017](#)). Here we addressed two entirely different hypotheses using new data. We hypothesized that H1: decreasing plant species richness will have a negative effect on C and N release after one year rather than after two years of decomposition, and H2: experimental reduction of precipitation will have a negative effect on C and N release after two years rather than after one year of decomposition.

MATERIALS AND METHODS

Study site

The study was carried out in the Massif de l'Etoile located at the northern end of the Marseille city in southern France (43° 22' N, 5°25' E). The study site is at 275 m a.s.l. with a mean annual precipitation of 552 mm and a mean annual temperature of 14.6 °C (see [Santonja and others \(2017a\)](#) for further details). The soil is a shallow and stony rendzina (66 % of stones in the top 50 cm) on limestone, with a mean depth of <20 cm, mean pH of 7.9, mean C:N ratio of 18:1 and mean CEC of 36.8 cmol.kg⁻¹ (see [Shihan and others \(2017\)](#) for further details). The vegetation is a woody shrub-dominated “garrigue”, with shrub heights ranging between 0.2 and 1.4 m and a total cover from 25% to 95% ([Montès and others 2008](#)). Four woody shrub species dominate the community and account for 73% of total vegetation cover: *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%) (see [Rodriguez-Ramirez and others \(2017\)](#) for further details).

Experimental setup

The experimental setup has been described previously ([Santonja and others 2017a](#)), but briefly we selected plots of different combinations of the four dominant woody shrub species. Those included all 15 possible combinations of single-, two-, three- and four-species mixtures with a replication of six plots per species combination (except for the four-species mixture that was replicated eight times), yielding a total of 92 plots. Half of the plots of each species combination were randomly assigned to a control treatment, and the other half to a partial rain exclusion treatment. The 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 m² plot area and fixed to the ground with reinforcing bars in October 2011. We mounted stainless steel gutters on top of the aluminum frame. Contrary to the partial rain exclusion plots, the gutters in the control plots were mounted upside down, which means they did not intercept rainfall but affected other microclimatic variables in the same way (e.g. light interception, wind patterns). The rainwater was channeled away from the plots via a supplementary PVC gutter and a pipe fixed at the border of the frame. The density of the gutters was chosen to target an average exclusion of 25 to 30% of the total annual rainfall. This corresponds to the mean of predicted changes during the dry season in the Mediterranean area at the end of the 21st century (i.e. 20-30% decrease, [Giorgi 2006](#); [Giorgi and Lionello 2008](#); [Mariotti and others 2008](#); [Dubrovsky and others 2014](#)). However, it would be more than the average change predicted for an entire year. For example, by using CMIP3 multi-model simulations, [Mariotti and others \(2008\)](#) reported that climate projections for the Mediterranean basin predict a 15% decrease per year, with a higher decrease during the dry season (-23%) compared to the wet season (-10%) when most of the total rainfall occurs.

We quantified the exact amount of excluded rainfall in three ways: (i) with permanently installed TDR100 probes (Campbell Scientific Inc., Logan, Utah) at 10 cm soil depth in seven control and eight rain-excluded plots, respectively, (ii) using rain gauges at ground level

underneath the gutters in both control and rain-excluded plots, and (iii) by determining the gravimetric soil water content in soil samples from control and exclusion plots. These measurements indicated that we only occasionally reached the target value and that our exclusion system reduced the average annual precipitation by only $12 \pm 2\%$ compared to the control plots. This seems to be mostly the consequence of wind turbulence during rainfall rendering the gutters covering the relatively small plot area of 16 m^2 at a height of about 2 m above the ground not as effective as we anticipated. An average of 12% less rainfall, however, is close to the 15% mean annual decrease reported by the projections of 14 CMIP3 global climate models for the Mediterranean Basin (Mariotti and others 2008) and to the 10% mean annual decrease reported by the projections of 28 CMIP5 global climate models for southern France (Polade and others 2014). Moreover, we stress that a given percentage of excluded rainfall does not readily translate into a similarly reduced soil water content, which depends also on the total amount of precipitation during a single rainfall event and how these events are distributed. As a consequence, during certain rain events we measured between -13% and -24% lower soil volumetric water content at 10 cm soil depth in plots with partial rain exclusion compared to control plots (Supplementary Fig. S1).

Freshly fallen leaf litter of the four shrub species were collected over the whole period of maximum litterfall (June to July, 2011) using suspended litter traps. Every two days, leaf litter was retrieved from litter traps, air-dried at room temperature and stored until the beginning of the experiment. For the exposure of litter in the experimental plots we constructed “open-bottom” PVC cylinders (5 cm tall) covered with 1 mm mesh net on top and with windows on the side covered with a 10 mm mesh. These cylinders allow direct contact of the litter with the soil surface and full access of the soil fauna without flattening the litter material as was suggested by Barantal and others (2011). Cylinders were filled with a total of six grams of air-dried leaf litter reflecting the species composition of the respective plot, with equal proportions

of the different litter species in the 11 multi-species litter mixtures. We installed seven cylinders in the central 4 m² part of each of the 92 plots, yielding a total of 644 cylinders. To place the cylinders we avoided rock outcrops and very shallow soil.

Litter traits and decomposition

Initial litter quality was determined from four subsamples of each species-specific litter batch ([Supplementary Table S1](#)). Carbon and N concentrations were determined by thermal combustion using a Flash EA 1112 series C/N elemental analyzer (Thermo Scientific, USA). Phosphorus (P) concentrations were measured colorimetrically using the molybdenum blue method according to the protocol of [Santonja and others \(2015\)](#). 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). The concentration of phenolics was measured colorimetrically using the method of [Peñuelas and others \(1996\)](#) 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:

$$\text{moist weight} / \text{dry weight} \times 100\%.$$

The community-weighted mean traits (CWM) of litter mixtures were calculated as the average trait values of litter mixtures following [Garnier and others \(2004\)](#) as:

$$\text{Trait}_{\text{CWM}} = \sum_{i=1}^n p_i \times \text{trait}_i$$

where p_i is the relative abundance for species i and trait_i is the trait value for species i .

Functional dissimilarity (FD) was calculated according to Rao's quadratic entropy ([Botta Dukat 2005](#); [Epps and others 2007](#)) for each litter mixture as:

$$\text{Trait}_{\text{FD}} = \sum_{i=1}^n \sum_{j=1}^n p_i p_j * dij$$

where p_i and p_j are the relative abundance for shrub species i and j in the litter mixture, and d_{ij} the Euclidian distance between species i and j for the trait considered. Because the measured traits differ in their units, we used normalized values (using a z-scored standardization so as to get a mean of zero and a standard deviation of one) to calculate functional dissimilarity.

The remaining leaf litter was collected from the plastic cylinders after one and two years of field exposure. We considered the first year as an initial stage of decomposition that is dominated by leaching losses, which can account for up to 30% of initial mass depending on litter species (Berg and McClaugherty 2008). Mass loss in our study varied between 18.6% (*Ulex*) and 36.5% (*Cistus*) in the single species treatments after one year. The advanced decomposition during the second year, we defined here as a later stage of decomposition with a range of mass loss between 27.0% (*Ulex*) and 53.0% (*Quercus*) in the single species treatments after two years. We retrieved four replicates in December 2012 (368 mesocosms) and three replicates in December 2013 (276 mesocosms) of plot-specific leaf litter. The remaining leaf litter was 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 analyses. Carbon and N concentrations were measured from remaining litter material using the same procedure as described for initial concentrations. Initial and final concentrations of C and N after one year, and after two years of decomposition were multiplied with initial, and final litter mass after one and two years, respectively, for the calculation of the amount of C and N loss. The difference between initial amounts and those remaining after one year was used to calculate total C and N release during the first year of decomposition. The difference

between the amounts remaining after one year and after two years was used to calculate total C and N release during the second year of decomposition.

Statistical analyses

Statistical analyses were performed with the R software ([R Core Team 2016](#)) with significance levels indicated as * for $P < 0.05$, ** for $P < 0.01$ and *** for $P < 0.001$. We used a linear mixed-effects model approach (“nlme” package) to test the effect of diversity, partial rain exclusion, and decomposition (initial and later) stage and their interactions on C and N release. To take into account the fact that we had four and three replicate mesocosms per plot for first year and second year, respectively, the random part of the model indicated that the mesocosms were nested within plots with the following R syntax (“random = ~1|plot/mesocosm”). Due to the large number of potentially important predictors, we carried out three distinct statistical models to test for the litter diversity effect on C and N release. The first model tested the impact of litter species richness (i.e. the number of litter species decomposing together (1 to 4 species)), precipitation treatment, year of decomposition, and their interactions. As the “nlme” package does not permit inclusion of species richness and species composition simultaneously in the model because mixtures containing all four litter types also represent the same community composition, a second complementary model was used to test the impact of litter species composition (i.e. the specific combinations of litter species, corresponding to 15 litter treatments), precipitation treatment, year of decomposition, and their interactions. In a third model we tested the impact of litter species identity (i.e. the presence/ absence of a particular species), precipitation treatment, year of decomposition, and their interactions. To take into account the effects of soil heterogeneity between plots, we included the scores of the first axis of PCA analyses of plot-specific soil characteristics as a covariable in model fitting (named *Soil characteristics*; see [Shihan and others \(2017\)](#) for further

274 details). These soil characteristics included texture, pH, cation exchange capacity (CEC), and
275 the concentrations of carbon, nitrogen, calcium, magnesium, sodium, potassium, iron,
276 manganese and aluminum. The full models were then simplified to determine the most
277 parsimonious models using the *stepAIC* function of the “MASS” package, an established model
278 selection procedure with both forward and backward selection algorithms, which ranks all
279 candidate models (all possible combinations of the initial explanatory variables included in the
280 full model) based on lowest AICs. The r^2 of the models were determined by using the
281 *r.squaredGLMM* function of the “MuMin” package, a function allowing to estimate the
282 marginal and conditional r^2 for mixed effects models. We present the r^2 and AIC values for both
283 the full model (with all initial explanatory variables) and the most parsimonious model.

284 For a more detailed understanding of how the diversity of leaf litter affected C and N
285 release during litter decomposition, we evaluated the effects of mean traits ($Trait_{CWM}$) and
286 functional trait dissimilarities ($Trait_{FD}$) of the 11 measured litter traits ([Supplementary Table](#)
287 [S1](#)). Firstly, we conducted a principal component analysis (PCA) using the CWM or the FD
288 values of the 11 measured litter traits. CWM1 and CWM2, and FD1 and FD2 represented the
289 two first components of the PCAs conducted using the CWM or the FD values across litter
290 mixtures. Secondly, we performed linear mixed-effects models (i.e. the same approach as for
291 the litter diversity effect) in order to decipher the relative contributions of $Trait_{CWM}$ and $Trait_{FD}$
292 tested conjointly. In this last model we tested the effects of the $Traits_{CWM}$ (i.e. CWM1 and
293 CWM2), $Traits_{FD}$ (i.e. FD1 and FD2), precipitation treatment, year of decomposition, and their
294 interactions on C and N release.

296 RESULTS

298 *Litter diversity effects on C and N release during decomposition*

Both C and N release from decomposing litter differed strongly between the first and second year of decomposition, regardless whether the model fitted litter species richness (Table 1) or litter species composition (Table 2). The C release was higher during the first year compared to the second year of decomposition (Figs. 1 and 2). In contrast, there was overall more N released during the second year compared to the first year of decomposition (Figs. 1 and 2).

In the statistical models including the effect of species richness, we found that litter C and N release were significantly affected by the interaction between year of decomposition and species richness (Table 1). This interaction resulted because the proportion of C and N release increased with species richness during the first year but not during the second year of decomposition (Fig. 1a and 1b). After one year of decomposition, the average C release increased from 31% in monospecific litters to 35% in 4-species mixtures, whereas the N release increased from 8% in monospecific litters to 17% in 4-species mixtures. The amount of variation explained by species richness was comparatively small, especially for C release. However, the positive relationship with species richness was robust, with similar or even higher variation explained when the 4-species level was excluded from the analysis ($R^2 = 0.14$ and 0.13 for C and N loss, respectively), or when the few microcosms showing apparent N immobilization (mostly at low species richness) were excluded from the analysis ($R^2 = 0.17$ for N loss).

In the complementary statistical models testing the importance of species composition (i.e. the 15 distinct litter treatments), we found that litter species composition strongly affected C and N release (Table 2; Supplementary Table S2). As indicated by the significant litter composition $\times$ year interaction, the differences between the 15 litter treatments depended on the year of decomposition (Table 2; Supplementary Table S2). Most of the litter treatments lost roughly twice to three times less C during the second year compared to the first year of

decomposition, but a few treatments lost much less (*Ulex* single species litter), or almost the identical amount of C (*Quercus* alone or mixed with *Cistus*) during the second year compared to the first year of decomposition. For N loss, the differences between one and two years of decomposition varied even more (Supplementary Table S2). The differences between the litter treatments increased during the second year compared to the first year of decomposition for C release (CV = 16% and 36% for one and two years of decomposition, respectively), but not for N release (CV = 53% and 45% for one and two years of decomposition, respectively) (Supplementary Table S2).

As litter composition significantly affected the C and N release, we further assessed the effects of the presence or absence of particular litter species (i.e. effects of litter species identity; Table 3; Fig. 3). The statistical model incorporating species identity effects (Table 3) showed that the presence of *Quercus* consistently led to higher C and N release (Fig. 3). The presence of *Quercus* litter enhanced C release to a larger extent during the second year than during the first year of decomposition (+51% and +17%, respectively) but enhanced N release to a larger extent during the second year (+240%) compared to the first year of decomposition (+69%) (Table 3; Fig. 3). The presence of *Cistus* increased the release of C (+13%) but not that of N, regardless of the year of decomposition (Fig. 3). The presence of *Ulex* generally decreased C and N release, and these effects were more pronounced during the second year (-37% and -30% for C and N release, respectively) compared to the first year of decomposition (-14% and -21% for C and N release, respectively) (Table 3; Fig. 3). Finally, the presence of *Rosmarinus* had the least consistent effects with a slight positive effect on C release during the first year of decomposition (+6%) that turned into a negative effect during the second year decomposition (-13%) (Table 3; Fig. 3). The neutral *Rosmarinus* effect on N release during the first year of decomposition turned into a strongly negative effect during the second year of decomposition (-33%) (Table 3; Fig. 3).

Consequences of reduced precipitation on C and N release during decomposition

The partial rain exclusion we applied to the experimental plots overall had weak effects on C and N release during decomposition. In fact, partial rain exclusion did not have a significant main effect in any of the statistical models we ran (Tables 1 to 4). The release of C, but not that of N, however, was distinctly affected by reduced rainfall among the different litter treatments (small but significant litter composition $\times$ partial rain exclusion effect, Table 2). With less rainfall, the monospecific *Rosmarinus* litter released less C (25% vs. 37%) whereas the monospecific *Ulex* litter released more C (23% vs. 20%). Overall, these differences were small and most of the litter treatments did not show any difference between plots with partial rain exclusion and control plots (data not shown). Partial rain exclusion interacted with the year of decomposition to influence N release, but not that of C (Table 2; Figs. 2a and 2b). Indeed, across all litter treatments there was 17% less N release with reduced precipitation compared to control plots during the first year of decomposition, while N release was similar in both treatments during the second year of decomposition (Fig. 2b).

CWM- versus FD-trait control over C and N release

Principal component analysis (PCA) of CWM traits showed that the first PCA axis (CWM1) explaining 61.7% variation was determined by high scores of P concentration and WHC, but low values of C concentration, and low ratios of C:P, N:P and lignin:P (Fig. 4a). High scores of the second PCA axis (CWM2) explaining 28.2% variation were related to high values of lignin concentration, and high ratios of C:N and lignin:N, while low scores were associated with high concentrations of N and phenolics. When considering the functional trait dissimilarity, the first PCA axis (FD1) explaining 43.3% variation separated litter mixtures according to increasing dissimilarity of WHC values, the concentrations in C and P, and N:P

and C:P ratios (Fig. 4b). High scores along the second axis (FD2) explaining 28.1% variation were largely determined by N-related traits with increasing dissimilarity in N concentration, and C:N and lignin:N ratios, while low scores were related to increasing dissimilarity in the concentrations of lignin and phenolics (Fig. 4b).

When evaluating conjointly the effects of CWM and FD traits in linear mixed-effects models, we found that C release was mostly controlled by the CWM2 and the interactions CWM1 $\times$ year and FD2 $\times$ year (Table 4). Increasing CWM1 scores (i.e. increasing values of WHC and P concentration) and decreasing CWM2 scores (i.e. increasing values of N and phenols concentration) were related to higher C release during both years of decomposition (Fig. 5). The interaction between CWM1 and year of decomposition resulted from a stronger relationship between C release and CWM1 during the second year compared to the first year of decomposition (Table 4; Fig. 5). Functional dissimilarity in trait values had no main effect on C release, but there was a significant interaction between FD2 and year of decomposition (Table 4), showing that increasing functional dissimilarity in initial N concentrations and C:N, and Lignin:N ratios stimulated C release during the second year of decomposition (Figs. 4 and 5).

The N release was significantly affected by CWM2, FD1 as well as the interactions CWM1 $\times$ year, FD2 $\times$ year and rainfall reduction $\times$ year (Table 4). Similar to what we observed for C release, increasing CWM1 scores and decreasing CWM2 scores were related to higher N release (Fig. 5), with a stronger impact of CWM1 during the second year of decomposition (significant CWM1 $\times$ year interaction, Table 4; Fig. 5). In contrast to C release, functional trait dissimilarity showed strong main effects on N release (Table 4). The FD1 effect indicates that decreasing functional dissimilarity in WHC and the concentrations of C and P, as well as the P stoichiometry led to higher N release (Figs. 4 and 5). On the other hand, increasing FD2 scores (i.e. increasing dissimilarity in N concentration, and C:N, and Lignin:N ratios) led to higher N

release only during the second year of decomposition (Fig. 5) as indicated by the FD2 × year interaction (Table 4).

DISCUSSION

Litter C and N release in response to changing plant diversity

Leaf litter decomposition we measured here for the four plant species is similar to that reported for a range of other Mediterranean woody plant species (Fioretto and others 2003; Gallardo and Merino 2003; Castro and others 2010; Almagro and Martinez-Mena 2012). In agreement with our first hypothesis, we observed higher C and N release with increasing litter species richness only during the first year of decomposition (0-12 months) and not during the second year of decomposition (12-24 months). These positive richness effects remained comparatively small and accounted with 8% for C release and 15% for N release, which was only a minor part of the observed variability. However, the positive relationship between C and N loss and species richness was not a spurious statistical results. This relationship remain robust when we ran the analysis without the 4-species level or by excluding the N immobilizing litter at lower species levels. Relatively low variation accounted for by species richness is a common result in plant diversity experiments where composition effects (variability within richness levels) typically dominate over richness effects (Hooper and Vitousek 1997; Wardle and others 1997; Tilman and others 2014). This was also the case in our study with the statistical models based on species richness explaining respectively 2 and 5 times less variability in C and N release than the models based on litter species composition (Tables 1 and 2). Accordingly, changes in species richness will have only a small additional impact on C and N cycling in the studied Mediterranean shrubland compared to shifts in species composition or other spatially and temporally varying factors. These include for example fire or UV-radiation that both were

shown to critically control litter decomposition in drylands (e.g. [Austin and Vivanco 2006](#); [Throop and others 2017](#)), and which would have to be included for a more general assessment of how biogeochemical cycling is changing in response to climate and biodiversity change at relevant spatial and temporal scales.

A stimulation of litter decomposition with increasing species richness can occur due to a greater diversity of substrates varying in physico-chemical properties when more species are present in mixtures ([Hättenschwiler and others 2005](#); [Kominoski and others 2007](#); [Vos and others 2013](#)). This could enhance the available niches for soil biota, and thus lead to higher decomposer abundance and activity ([Hättenschwiler and others 2005](#)). Higher fungal and bacterial abundance with increasing litter species richness was indeed reported in the same litter samples we analyzed here after one year of exposure in the field ([Santonja and others 2017a](#)). Litter species richness effects are expected to occur mainly during the early stages of decomposition when litter characteristics differ the most among litter species. In contrast, richness effects may decrease in the later stages of decomposition as litter quality and chemical diversity converge ([Melillo and others 1989](#); [Chomel and others 2014](#); [Parsons and others 2014](#); but see [Wickings and others 2012](#)). However, the effects of litter composition persisted over time and became even stronger during the second year compared to the first year of decomposition for C release. These results rather support the “Initial Litter Quality Hypothesis” stating that initial litter trait effects persist during later stages of decomposition ([Berg and McClaugherty 2008](#)) and is in line with the recent findings of [Wickings and others \(2012\)](#) who reported that the chemistry of different litter types diverge during the decomposition process. This may also explain why the composition is generally more important than the richness of litter mixtures in the majority of litter diversity experiments (e.g. [Kominoski and others 2007](#); [Vivanco and Austin 2008](#); [Santonja and others 2015](#), and this study). Rather than the number of species, the specific litter traits represented by the different species composing the mixture

are driving the diversity effects. Indeed, we identified strong species identity effects on decomposition in our study. In general, *Cistus* and *Quercus* positively affected C and N release, whereas *Ulex* had negative effects. Differences in litter traits among these three species may explain their opposite effects. *Quercus* and *Cistus* had lower lignin and higher P concentrations compared to *Ulex*, two litter traits known to control litter decomposition. *Quercus* and *Cistus* also had a higher WHC than *Ulex*, a physical trait affecting litter humidity, which is important for decomposer activity (Hättenschwiler and others 2005; Makkonen and others 2013; Santonja and others 2015). However, the intensity of the effects of *Quercus* and *Ulex* varied depending on the year of decomposition. Moreover, *Rosmarinus* had a positive effect during the first year, while its effect became negative during the second year of decomposition. Collectively, these changing species identity effects over time highlight the fact that litter identity effects are not necessarily constant during the decomposition process.

Plant diversity can also affect decomposition independently from litter quality by modifying soil characteristics or microclimatic conditions (e.g. Joly et al. 2017). With a detailed assessment of soil parameters in each of the 92 experimental plots, we statistically accounted for an important part of environmental heterogeneity among plots and potential long-term effects of plant community composition beyond the quality of litter fall. These soil characteristics indeed had some rather minor effects on C release, but not on N release, in addition to our main treatment factors. However, by exposing the different litter treatments underneath the canopy of the same plant species composition, our data represent well the overall changes in C and N loss dynamics during decomposition in response to a shift in plant diversity, irrespective of the relative contribution of direct litter trait and indirect plant canopy effects.

Litter C and N release in response to reduced precipitation

Previous litter decomposition studies conducted in Mediterranean systems found a significant reduction in decomposition with reduced water availability (e.g. [Saura-Mas and others 2012](#); [Almagro and others 2015](#); [Santonja and others 2015](#); [Santonja and others 2017b](#)). Based on these studies, we predicted that partial rain exclusion would slow down the C and N release from the litter, and more so during the second year compared to the first year of decomposition, as [Garcia-Palacios and others \(2016\)](#) showed an increase in the relative importance of abiotic controls in later compared to earlier decomposition stages. In contrast to these previous studies and our second hypothesis, reduced precipitation did not have large effects on C release. The extent of negative effects of reduced rainfall may depend on the type of ecosystem, but also on the amount of rainfall that is removed. The small effects we observed are likely due to the comparatively small change in precipitation with an average of 12% less annual rainfall, which is lower than in most partial rain exclusion experiments, but close to the average predictions for the Mediterranean Basin (-15%, [Mariotti and others 2008](#)) and for southern France (-10%, [Polade and others 2014](#)). In addition, Mediterranean decomposer communities are rather drought tolerant and might be less affected by slight changes in the amount of rainfall compared to other ecosystem types. In fact, the strong seasonal shifts in both temperature and soil water availability and the rather extreme environmental conditions during summer typical for the Mediterranean climate could represent a strong selection pressure for microbial decomposer communities, resulting in high drought tolerance ([Curiel-Yuste and others 2014](#)). Moreover, decomposition might be under stronger direct control of solar UV-radiation, which can have a strong impact on surface litter decomposition in drylands ([Austin and Vivanco 2006](#); [Almagro and others 2015](#); [Almagro and others 2017](#)), overriding the indirect effects of the small difference in precipitation we simulated here.

The N release was more sensitive to our precipitation treatment compared to C release, probably because the release of N differs in its dynamics compared to that of C ([Moore and](#)

others 2005; Garcia-Palacios and others 2016; Santonja and others 2017a). The relative contribution of N immobilization vs. N release during litter decomposition depends on environmental conditions and initial litter quality (Parton and others 2007). Contrary to what we hypothesized, the 17% lower N release with less precipitation occurred during the first year rather than the second year of decomposition. This contrasts the earlier findings by Garcia-Palacios and others (2016), who reported an increase in abiotic control (predominantly soil moisture) over C and N release with proceeding decomposition. The different results may be related to an overall shorter time span of decomposition in Garcia-Palacios and others' (2016) study covering only the early stage decomposition according to the definition used in our study, different experimental setups (mass loss classes vs. specific time span) and different ecosystem types (forests vs. shrubland) that may respond distinctly to changes in abiotic conditions.

CWM- versus FD-trait control over C and N release

The functional trait-based metrics CWM (community weighted mean) and FD (functional dissimilarity) allow to distinguish between mass-ratio and niche differentiation as two key mechanisms of diversity effects. Actually, the models incorporating CWM and FD explained more variation than the models based on species richness alone and a similar amount of variation as the models based on species composition and species identity (Tables 1 to 3). The metrics CWM and FD both predicted C and N loss, with FD being more important overall for N release compared to C release. Carbon release was most strongly related to the first component of the CWM-trait PCA, indicating that increasing total litter P concentration and water holding capacity (WHC) stimulate C release. Accordingly, when the two relatively P-rich litter species *Cistus* and *Quercus*, which also had comparatively high WHC, were present in litter mixtures, C release was higher compared to when they were absent. Along with N, P is a frequently limiting nutrient in Mediterranean ecosystems (McMaster and others 1982; Henkin

and others 1998; Sardans and Peñuelas 2013), but P appears more important than N in driving C release during litter decomposition in the studied shrubland. Maintaining litter humidity for a longer time-period by high WHC may be particularly important for decomposer activity in Mediterranean ecosystems (Hättenschwiler and others 2005; Makkonen and others 2013; Santonja and others 2015). N release was determined more by the second component of the CWM-trait PCA, identifying N concentration, N-based stoichiometric ratios, and phenolics as the main drivers of N dynamics. It has previously been shown that the relative contribution of N immobilization vs. N release is strongly determined by the initial litter N concentration (Aber and Melillo 1982; Parton and others 2007). In strong contrast to C release, both axes of the FD-trait PCA were associated to N release patterns. In line with the niche complementarity hypothesis, litter mixtures with contrasting litter quality may improve the availability of different resources for decomposers (Schimel and Hättenschwiler 2007; Handa and others 2014). Similar to our results, Garcia-Palacios and others (2017) also identified litter CWM traits to be important drivers of both, litter C and N release, and FD traits as additional major drivers of litter N release. Collectively, these findings suggest that different aspects of litter trait diversity control C and N dynamics during litter decomposition.

An important result reported here was that the first PCA axis of CWM-traits (i.e. increasing total litter P concentration and WHC) and the second PCA axis of FD-traits (i.e. increasing dissimilarity in N-related traits) accounted for more of the variance in the later than the initial stage of decomposition for both C and N release. This pattern indicates that initial litter traits maintain their importance in controlling elemental cycling in later stages of decomposition, and that legacy effects of initial litter traits actually increase during the course of litter decomposition (Garcia-Palacios and others 2016). Interestingly, legacy effects of litter traits were not only expressed *via* mean traits of litter mixtures, but also by their dissimilarity in N-related traits among litter species present in the mixture. In other words, C and N release

in later stage decomposition increased in litter mixtures with increasing differences in initial N concentrations among component species rather than with overall high litter N content. It has previously been suggested that fungi- or leaching-driven N transfer among litter species varying in their initial N concentration may contribute to litter mixture effects (Schimel and Hättenschwiler 2007; Vos and others 2013; Barantal and others 2014; Handa and others 2014). In our study, however, this response may also result at least in part from species identity effects. Indeed, the presence of the N-rich species *Ulex* in litter mixtures (i.e. mixtures with rather high overall N content) had a negative effect on C and N release. This *Ulex* presence effect may also originate from low WHC and low P concentration, the two traits driving the CWM trait response.

CONCLUSION

Changes in the diversity of dominant shrub species in the studied Mediterranean shrubland had stronger effects on C and N release during litter decomposition than a moderate reduction in precipitation. Litter species richness had a small positive effect on C and N release only during the first year of decomposition. The litter diversity effects were mostly driven by the presence of particular litter species and their litter traits. Surprisingly, these effects were stronger during the second than the first year of decomposition, suggesting increasing legacy effects of initial litter traits with ongoing decomposition. Furthermore, our analyses of litter functional trait-based metrics support the view that both mass-ratio and niche complementarity are important in understanding plant diversity control over elemental cycling in decomposing plant litter.

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AUTHOR'S CONTRIBUTIONS

M.S., V.B., A.R., and S.H. designed the study; M.S. and A.R. conducted the experiment and collected the data; M.S., A.M. and S.H. analyzed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Tables

Table 1. Effects of litter species richness, change in precipitation, and year of decomposition on carbon and nitrogen release. Only the variables retained in the most parsimonious models are reported (d.f. = degrees of freedom, P -values = * $P < 0.05$, *** for $P < 0.001$). At the bottom of the table the values of R^2 and AIC weight of the general model including all factors (All) and of the most parsimonious model (MPM) are shown. Soil characteristics and precipitation treatment were initially included in the models based on litter species richness, but they were not retained in the most parsimonious models.

	d.f.	Carbon release		Nitrogen release	
		F -value	P -value	F -value	P -value
(Intercept)	1	1837.8	***	459.9	***
Richness (R)	1	1.7		4.6	*
Year (Y)	1	213.4	***	21.9	***
$R \times Y$	1	5.4	*	17.2	***
All R^2 (AIC)		0.31 (4234.2)		0.12 (4390.8)	
MPM R^2 (AIC)		0.30 (4226.7)		0.11 (4383.9)	

Table 2. Effects of litter species composition, change in precipitation and year of decomposition on carbon and nitrogen release. Only the variables retained in the most parsimonious models are reported (d.f. = degrees of freedom, P -values = * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$). The term “soil characteristics” refers to plot-specific soil parameters that were systematically included in all models as co-variable. At the bottom of the table the values of R^2 and AIC weight of the general model including all factors (All) and of the most parsimonious model (MPM) are shown.

	d.f.	Carbon release		Nitrogen release	
		F -value	P -value	F -value	P -value
(Intercept)	1	12089.2	***	2143.6	***
Soil characteristics	1	8.1	**		
Composition (C)	14	33.6	***	27.1	***
Precipitation (P)	1	0.5		3.0	
Year (Y)	1	435.9	***	55.7	***
C $\times$ P	14	2.1	*		
C $\times$ Y	14	7.0	***	13.7	***
P $\times$ Y	1			5.3	*
All R^2 (AIC)		0.68 (4078.6)		0.56 (4223.7)	
MPM R^2 (AIC)		0.67 (4065.1)		0.54 (4191.1)	

Table 3. Effects of the presence of litter species, change in precipitation, and year of decomposition on carbon and nitrogen release. Only the variables retained in the most parsimonious models are reported (d.f. = degrees of freedom, P -values = * $P < 0.05$, ** $P < 0.01$, *** for $P < 0.001$). The term “soil characteristics” refers to plot-specific soil parameters that were systematically included in all models as co-variable. At the bottom of the table the values of R^2 and AIC weight of the general model including all factors (All) and of the most parsimonious model (MPM) are shown.

	d.f.	Carbon release		Nitrogen release	
		F -value	P -value	F -value	P -value
(Intercept)	1	6653.8	***	1254.1	***
Soil characteristics	1	4.4	*		
Cistus (C)	1	42.6	***	4.3	*
Quercus (Q)	1	100.2	***	178.2	***
Rosmarinus (R)	1	0.7		5.9	*
Ulex (U)	1	90.9	***	21.6	***
Precipitation (P)	1			1.6	
Year (Y)	1	280.5	***	32.3	***
$Q \times Y$	1	7.3	**	6.9	**
$R \times Y$	1	13.9	***	24.0	***
$U \times Y$	1	16.9	***	5.0	*
$P \times Y$	1			3.9	*
All R^2 (AIC)		0.59 (4104.4)		0.42 (4271.4)	
MPM R^2 (AIC)		0.59 (4095.7)		0.41 (4263.2)	

Table 4. Effects of community weighted mean traits (CWM), functional trait dissimilarity (FD), change in precipitation, and year of decomposition on carbon and nitrogen release. CWM1 and CWM2, and FD1 and FD2 represented the two first components of the PCAs conducted using the CWM or the FD values in Fig. 4. Only the variables retained in the most parsimonious models are reported (d.f. = degrees of freedom, P -values = * $P < 0.05$, ** $P < 0.01$, *** for $P < 0.001$). At the bottom of the table the values of R^2 and AIC weight of the general model including all factors (All) and of the most parsimonious model (MPM) are shown.

	d.f.	Carbon release		Nitrogen release	
		F -value	P -value	F -value	P -value
(Intercept)	1	10596.3	***	1474.7	***
CWM1	1	145.3	***	6.4	*
CWM2	1	98.9	***	135.3	***
FD1	1			54.9	***
FD2	1	0.0		18.8	***
Precipitation (P)	1	0.0		2.8	
Year (Y)	1	430.4	***	26.0	***
CWM1 $\times$ Y	1	9.1	**	29.1	***
FD2 $\times$ Y	1	29.3	***	47.9	***
P $\times$ S	1	2.8		4.2	*
All R^2 (AIC)		0.62 (2981.7)		0.43 (3165.8)	
MPM R^2 (AIC)		0.62 (2970.2)		0.43 (3159.8)	

Figures

Fig. 1. Carbon (a) and nitrogen (b) release as a function of litter species richness during the first year (Y1 = white symbol) and the second year of decomposition (Y2 = grey symbol). Each symbol represents the mean value (n = 4 microcosms per plot for Y1, and n = 3 microcosms for Y2) of each of the 92 experimental plots. The C and N release values are indicated in percent loss relative to the initial amount (for the first year of decomposition) or relative to the amount remaining after 12 months of decomposition (for the second year of decomposition). Significant linear relationships after one year of decomposition are indicated with dotted lines, adjusted R^2 and associated P -values ** $P < 0.01$, *** $P < 0.001$).

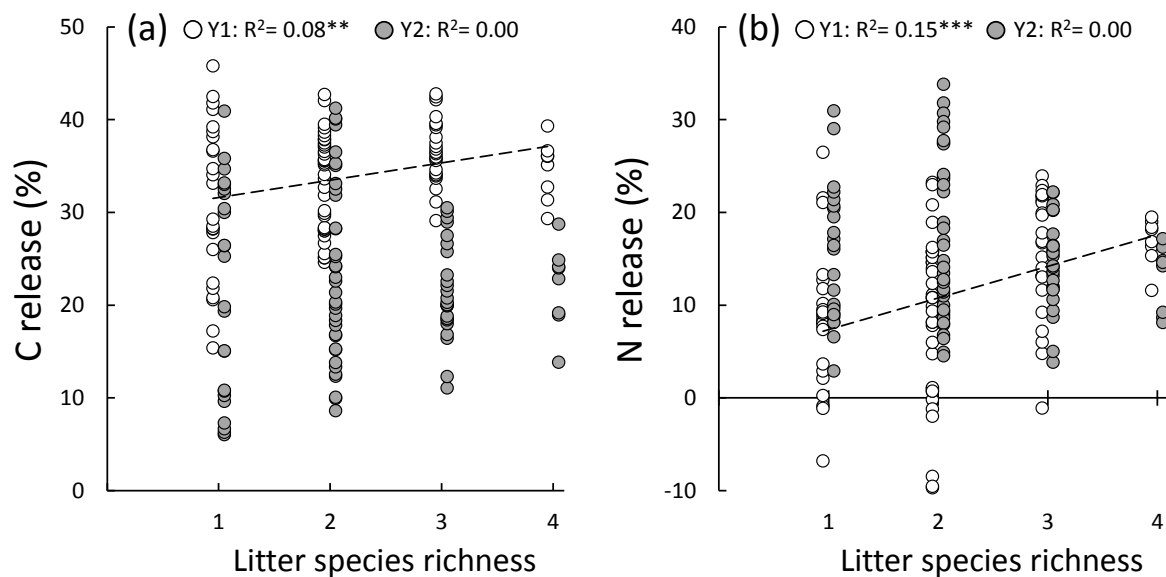


Fig. 2. Carbon (a) and nitrogen (b) release in control plots (white bars) and plots with partial rain exclusion (black bars) during the first year (Y1 = 0 to 12 months) and the second year of decomposition (Y2 = 12 to 24 months). Each bar represents the mean value $\pm$ SE per precipitation treatment; $n = 184$ and 138 microcosms for the first and the second year of decomposition, respectively. The C and N release values are indicated in percent loss relative to the initial amount (for the first year of decomposition) or relative to the amount remaining after 12 months of decomposition (for the second year of decomposition). Stars denote significant differences between control plots and plots with partial rain exclusion ($* P < 0.05$).

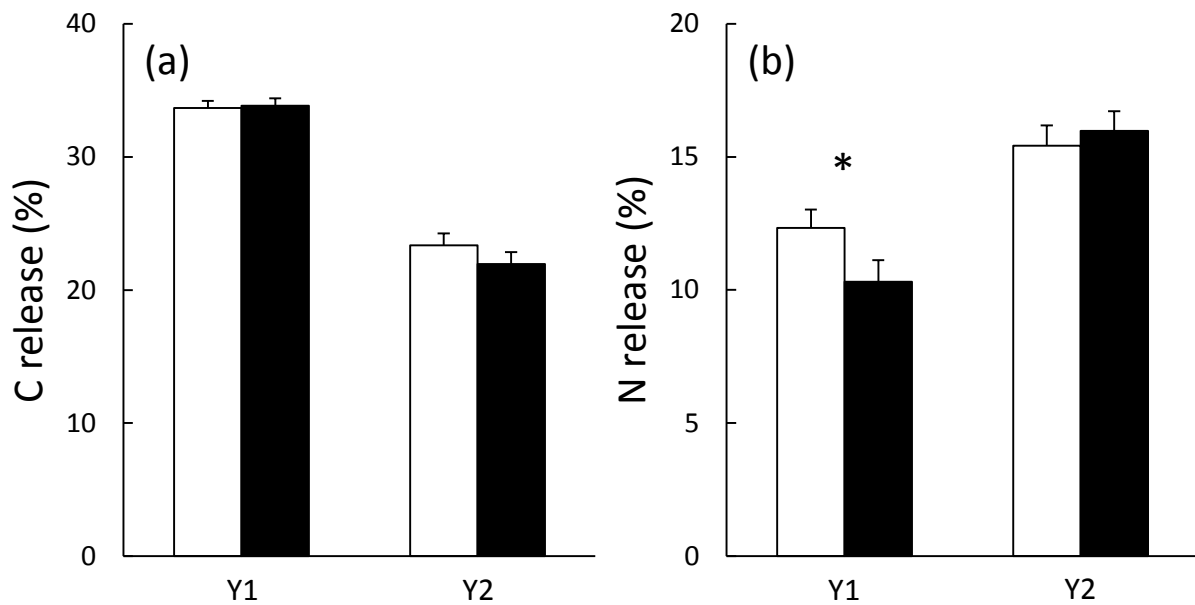


Fig. 3. Carbon (panels a and b) and nitrogen (panels c and d) release in the presence of a particular litter species (presence = white bar and absence = striped bar) during the first year (panels a and c) and the second year of decomposition (panels b and d). Each bar represents the mean value $\pm$ SE. In (a) and (c), $n = 200$ and 168 microcosms for species presence and absence, respectively. In (b) and (d), $n = 150$ and 126 microcosms for species presence and absence, respectively. The values are indicated in percent loss relative to the initial amount (for the first year of decomposition) or relative to the amount remaining after 12 months of decomposition (for the second year of decomposition). Stars denote significant differences between mixtures containing the specific species and in its absence (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

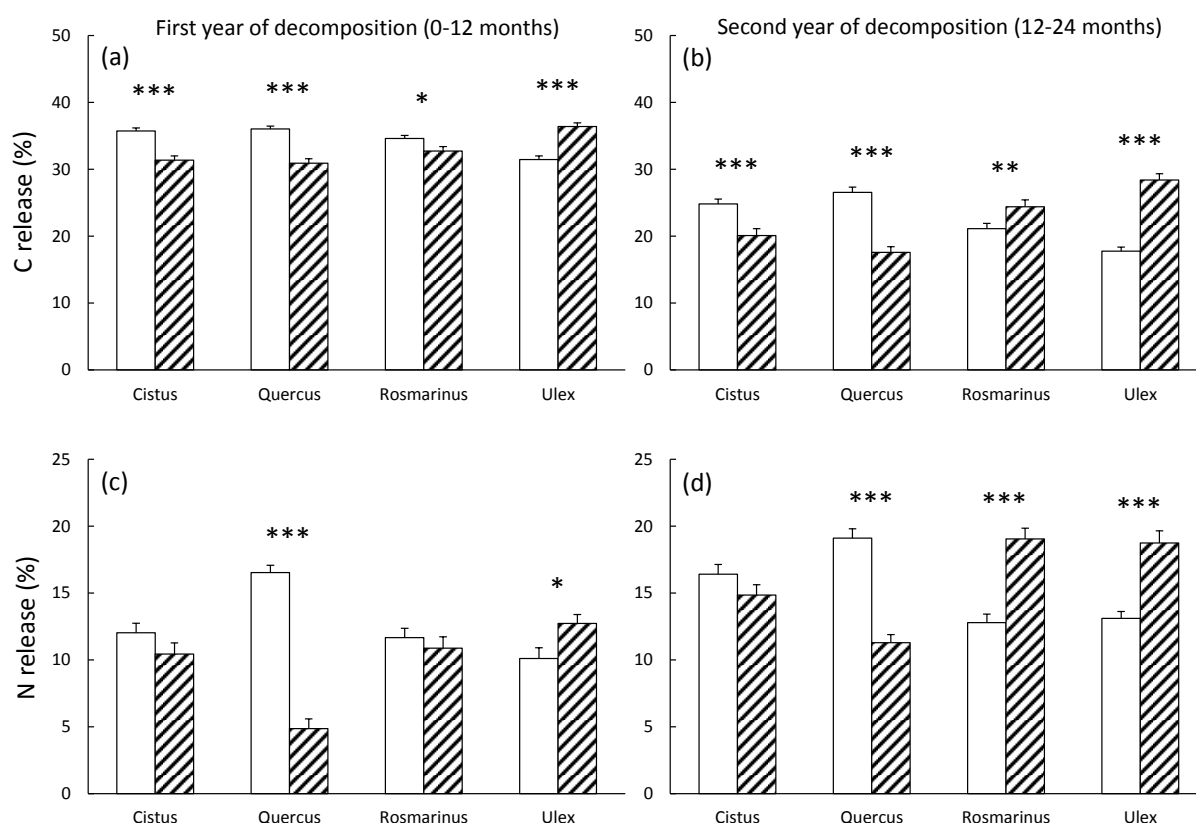


Fig. 4. Principal component analysis (PCA) of community-weighted mean traits (a) and functional trait dissimilarity (b). Variance explained by each principal component and associated eigenvalues are shown in brackets. CWM = Community-Weighted Mean Trait, FD = Functional Trait Dissimilarity. C = Cistus, Q = Quercus, R = Rosmarinus, U = Ulex. Combinations of capital letters correspond to combinations of plant species in litter mixtures.

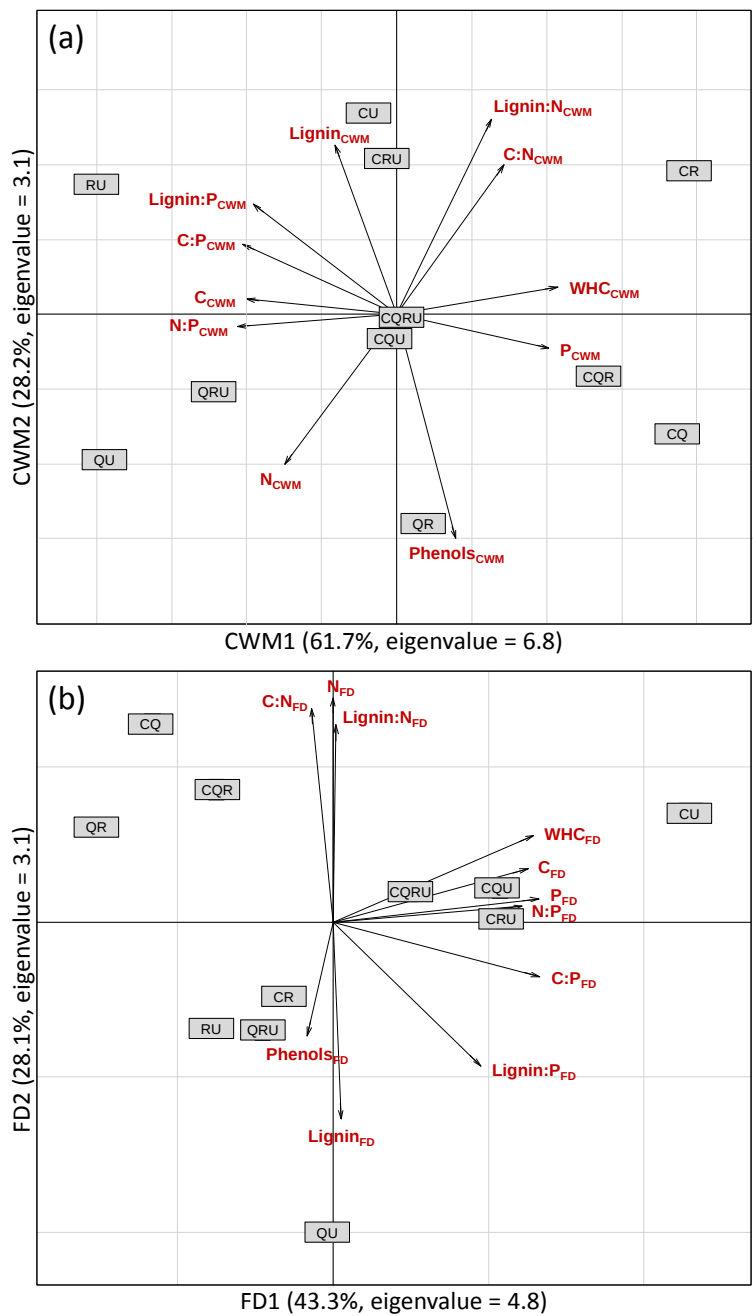


Fig. 5. Carbon (panels a, b, c and d) and nitrogen (panels e, f, g and h) release as a function of community weighted mean traits (CWM1 and CWM2; panels a, b, e and f) and functional trait dissimilarities (FD1 and FD2; panels c, d, g and h) during the first year (Y1 = white symbol) and the second year of decomposition (Y2 = grey symbol). Each symbol represents the mean value ($n = 4$ and 3 microcosms per plot for Y1 and Y2, respectively) of each of the 68 multi-species experimental plots. Significant relationships retained in the linear mixed-effects model (Table 4) are indicated with dotted (Y1) or grey lines (Y2), adjusted R^2 and associated P -values (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

