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

3

4 **RUNNING HEAD:** Litter diversity effects on C and N dynamics

5

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21

22 **ABSTRACT**

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

42

43 **KEY WORDS**

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

46

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

## 52 INTRODUCTION

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

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

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

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

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

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

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

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

133

## 134 MATERIALS AND METHODS

135

### 136 *Study site*

137 The study was carried out in the Massif de l'Etoile located at the northern end of the  
138 Marseille city in southern France (43° 22' N, 5°25' E). The study site is at 275 m a.s.l. with a  
139 mean annual precipitation of 552 mm and a mean annual temperature of 14.6 °C (see Santonja  
140 and others (2017a) for further details). The soil is a shallow and stony rendzina (66 % of stones  
141 in the top 50 cm) on limestone, with a mean depth of <20 cm, mean pH of 7.9, mean C:N ratio  
142 of 18:1 and mean CEC of 36.8 cmol.kg<sup>-1</sup> (see Shihan and others (2017) for further details). The  
143 vegetation is a woody shrub-dominated “garrigue”, with shrub heights ranging between 0.2 and  
144 1.4 m and a total cover from 25% to 95% (Montès and others 2008). Four woody shrub species  
145 dominate the community and account for 73% of total vegetation cover: *Quercus coccifera* L.  
146 (*Quercus*, with an average cover across all plots of 36%), *Cistus albidus* L. (*Cistus*, 18%), *Ulex*  
147 *parviflorus* Pourr. (*Ulex*, 10%), and *Rosmarinus officinalis* L. (*Rosmarinus*, 9%) (see  
148 Rodriguez-Ramirez and others (2017) for further details).

149

### 150 *Experimental setup*



151           The experimental setup has been described previously ([Santonja and others 2017a](#)), but  
152 briefly we selected plots of different combinations of the four dominant woody shrub species.  
153 Those included all 15 possible combinations of single-, two-, three- and four-species mixtures  
154 with a replication of six plots per species combination (except for the four-species mixture that  
155 was replicated eight times), yielding a total of 92 plots. Half of the plots of each species  
156 combination were randomly assigned to a control treatment, and the other half to a partial rain  
157 exclusion treatment. The plots were equipped with a 4 m × 4 m solid aluminum frame, held 2  
158 m above the ground by aluminum posts at the outer border of the 16 m<sup>2</sup> plot area and fixed to  
159 the ground with reinforcing bars in October 2011. We mounted stainless steel gutters on top of  
160 the aluminum frame. Contrary to the partial rain exclusion plots, the gutters in the control plots  
161 were mounted upside down, which means they did not intercept rainfall but affected other  
162 microclimatic variables in the same way (e.g. light interception, wind patterns). The rainwater  
163 was channeled away from the plots via a supplementary PVC gutter and a pipe fixed at the  
164 border of the frame. The density of the gutters was chosen to target an average exclusion of 25  
165 to 30% of the total annual rainfall. This corresponds to the mean of predicted changes during  
166 the dry season in the Mediterranean area at the end of the 21st century (i.e. 20-30% decrease,  
167 [Giorgi 2006](#); [Giorgi and Lionello 2008](#); [Mariotti and others 2008](#); [Dubrovsky and others 2014](#)).  
168 However, it would be more than the average change predicted for an entire year. For example,  
169 by using CMIP3 multi-model simulations, [Mariotti and others \(2008\)](#) reported that climate  
170 projections for the Mediterranean basin predict a 15% decrease per year, with a higher decrease  
171 during the dry season (-23%) compared to the wet season (-10%) when most of the total rainfall  
172 occurs.

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

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

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

201 of the different litter species in the 11 multi-species litter mixtures. We installed seven cylinders  
202 in the central 4 m<sup>2</sup> part of each of the 92 plots, yielding a total of 644 cylinders. To place the  
203 cylinders we avoided rock outcrops and very shallow soil.

204

### 205 *Litter traits and decomposition*

206 Initial litter quality was determined from four subsamples of each species-specific litter  
207 batch ([Supplementary Table S1](#)). Carbon and N concentrations were determined by thermal  
208 combustion using a Flash EA 1112 series C/N elemental analyzer (Thermo Scientific, USA).  
209 Phosphorus (P) concentrations were measured colorimetrically using the molybdenum blue  
210 method according to the protocol of [Santonja and others \(2015\)](#). Lignin concentration was  
211 determined according to the van Soest extraction protocol ([van Soest and Wine 1967](#)) using a  
212 fiber analyzer (Fibersac 24; Ankom, Macedon, NJ, USA). The concentration of phenolics was  
213 measured colorimetrically using the method of [Peñuelas and others \(1996\)](#) with gallic acid as a  
214 standard. To determine the water holding capacity (WHC), intact leaf litter samples were  
215 soaked in distilled water for 24 h, drained and weighed. The dry weight was determined after  
216 drying samples at 60 °C for 48 h. WHC was calculated according to the formula:

$$217 \text{moist weight / dry weight} \times 100\%.$$

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

$$220 \text{Trait}_{\text{CWM}} = \sum_{i=1}^n p_i \times \text{trait}_i$$

221 where  $p_i$  is the relative abundance for species  $i$  and  $\text{trait}_i$  is the trait value for species  $i$ .

222 Functional dissimilarity (FD) was calculated according to Rao's quadratic entropy ([Botta Dukat](#)  
223 [2005](#); [Epps and others 2007](#)) for each litter mixture as:

$$224 \text{Trait}_{\text{FD}} = \sum_{i=1}^n \sum_{j=1}^n p_i p_j * d_{ij}$$

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

229         The remaining leaf litter was collected from the plastic cylinders after one and two years  
230 of field exposure. We considered the first year as an initial stage of decomposition that is  
231 dominated by leaching losses, which can account for up to 30% of initial mass depending on  
232 litter species (Berg and McClaugherty 2008). Mass loss in our study varied between 18.6%  
233 (*Ulex*) and 36.5% (*Cistus*) in the single species treatments after one year. The advanced  
234 decomposition during the second year, we defined here as a later stage of decomposition with  
235 a range of mass loss between 27.0% (*Ulex*) and 53.0% (*Quercus*) in the single species  
236 treatments after two years. We retrieved four replicates in December 2012 (368 mesocosms)  
237 and three replicates in December 2013 (276 mesocosms) of plot-specific leaf litter. The  
238 remaining leaf litter was put in plastic bags, and immediately transferred to the laboratory. Leaf  
239 litter was separated into species, thoroughly brushed to remove adhering soil particles, freeze-  
240 dried (Lyovac GT2®) and weighed to obtain litter dry mass data of each species in each  
241 mesocosm. After weighing the component litter species, all litter from an individual field  
242 mesocosm was again put together and then ground using a ball mill to a fine powder before  
243 chemical analyses. Carbon and N concentrations were measured from remaining litter material  
244 using the same procedure as described for initial concentrations. Initial and final concentrations  
245 of C and N after one year, and after two years of decomposition were multiplied with initial,  
246 and final litter mass after one and two years, respectively, for the calculation of the amount of  
247 C and N loss. The difference between initial amounts and those remaining after one year was  
248 used to calculate total C and N release during the first year of decomposition. The difference

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

251

### 252 *Statistical analyses*

253 Statistical analyses were performed with the R software ([R Core Team 2016](#)) with  
254 significance levels indicated as \* for  $P < 0.05$ , \*\* for  $P < 0.01$  and \*\*\* for  $P < 0.001$ . We used  
255 a linear mixed-effects model approach (“nlme” package) to test the effect of diversity, partial  
256 rain exclusion, and decomposition (initial and later) stage and their interactions on C and N  
257 release. To take into account the fact that we had four and three replicate mesocosms per plot  
258 for first year and second year, respectively, the random part of the model indicated that the  
259 mesocosms were nested within plots with the following R syntax (“random =  
260 ~1|plot/mesocosm”). Due to the large number of potentially important predictors, we carried  
261 out three distinct statistical models to test for the litter diversity effect on C and N release. The  
262 first model tested the impact of litter species richness (i.e. the number of litter species  
263 decomposing together (1 to 4 species)), precipitation treatment, year of decomposition, and  
264 their interactions. As the “nlme” package does not permit inclusion of species richness and  
265 species composition simultaneously in the model because mixtures containing all four litter  
266 types also represent the same community composition, a second complementary model was  
267 used to test the impact of litter species composition (i.e. the specific combinations of litter  
268 species, corresponding to 15 litter treatments), precipitation treatment, year of decomposition,  
269 and their interactions. In a third model we tested the impact of litter species identity (i.e. the  
270 presence/ absence of a particular species), precipitation treatment, year of decomposition, and  
271 their interactions. To take into account the effects of soil heterogeneity between plots, we  
272 included the scores of the first axis of PCA analyses of plot-specific soil characteristics as a  
273 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.

295

## 296 **RESULTS**

297

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

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

305 In the statistical models including the effect of species richness, we found that litter C  
306 and N release were significantly affected by the interaction between year of decomposition and  
307 species richness (Table 1). This interaction resulted because the proportion of C and N release  
308 increased with species richness during the first year but not during the second year of  
309 decomposition (Fig. 1a and 1b). After one year of decomposition, the average C release  
310 increased from 31% in monospecific litters to 35% in 4-species mixtures, whereas the N release  
311 increased from 8% in monospecific litters to 17% in 4-species mixtures. The amount of  
312 variation explained by species richness was comparatively small, especially for C release.  
313 However, the positive relationship with species richness was robust, with similar or even higher  
314 variation explained when the 4-species level was excluded from the analysis ( $R^2 = 0.14$  and  
315  $0.13$  for C and N loss, respectively), or when the few microcosms showing apparent N  
316 immobilization (mostly at low species richness) were excluded from the analysis ( $R^2 = 0.17$  for  
317 N loss).

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

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

332 As litter composition significantly affected the C and N release, we further assessed the  
333 effects of the presence or absence of particular litter species (i.e. effects of litter species identity;  
334 Table 3; Fig. 3). The statistical model incorporating species identity effects (Table 3) showed  
335 that the presence of *Quercus* consistently led to higher C and N release (Fig. 3). The presence  
336 of *Quercus* litter enhanced C release to a larger extent during the second year than during the  
337 first year of decomposition (+51% and +17%, respectively) but enhanced N release to a larger  
338 extent during the second year (+240%) compared to the first year of decomposition (+69%)  
339 (Table 3; Fig. 3). The presence of *Cistus* increased the release of C (+13%) but not that of N,  
340 regardless of the year of decomposition (Fig. 3). The presence of *Ulex* generally decreased C  
341 and N release, and these effects were more pronounced during the second year (-37% and -30%  
342 for C and N release, respectively) compared to the first year of decomposition (-14% and -21%  
343 for C and N release, respectively) (Table 3; Fig. 3). Finally, the presence of *Rosmarinus* had  
344 the least consistent effects with a slight positive effect on C release during the first year of  
345 decomposition (+6%) that turned into a negative effect during the second year decomposition  
346 (-13%) (Table 3; Fig. 3). The neutral *Rosmarinus* effect on N release during the first year of  
347 decomposition turned into a strongly negative effect during the second year of decomposition  
348 (-33%) (Table 3; Fig. 3).



349

350 ***Consequences of reduced precipitation on C and N release during decomposition***

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

364

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

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

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

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

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

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

400

## 401 **DISCUSSION**

402

### 403 *Litter C and N release in response to changing plant diversity*

404 Leaf litter decomposition we measured here for the four plant species is similar to that  
405 reported for a range of other Mediterranean woody plant species (Fioretto and others 2003;  
406 Gallardo and Merino 2003; Castro and others 2010; Almagro and Martinez-Mena 2012). In  
407 agreement with our first hypothesis, we observed higher C and N release with increasing litter  
408 species richness only during the first year of decomposition (0-12 months) and not during the  
409 second year of decomposition (12-24 months). These positive richness effects remained  
410 comparatively small and accounted with 8% for C release and 15% for N release, which was  
411 only a minor part of the observed variability. However, the positive relationship between C and  
412 N loss and species richness was not a spurious statistical results. This relationship remain robust  
413 when we ran the analysis without the 4-species level or by excluding the N immobilizing litter  
414 at lower species levels. Relatively low variation accounted for by species richness is a common  
415 result in plant diversity experiments where composition effects (variability within richness  
416 levels) typically dominate over richness effects (Hooper and Vitousek 1997; Wardle and others  
417 1997; Tilman and others 2014). This was also the case in our study with the statistical models  
418 based on species richness explaining respectively 2 and 5 times less variability in C and N  
419 release than the models based on litter species composition (Tables 1 and 2). Accordingly,  
420 changes in species richness will have only a small additional impact on C and N cycling in the  
421 studied Mediterranean shrubland compared to shifts in species composition or other spatially  
422 and temporally varying factors. These include for example fire or UV-radiation that both were

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

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

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

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

470

471 ***Litter C and N release in response to reduced precipitation***

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

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

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

508

#### 509 *CWM- versus FD-trait control over C and N release*

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

522 [and others 1998](#); [Sardans and Peñuelas 2013](#)), but P appears more important than N in driving  
523 C release during litter decomposition in the studied shrubland. Maintaining litter humidity for  
524 a longer time-period by high WHC may be particularly important for decomposer activity in  
525 Mediterranean ecosystems ([Hättenschwiler and others 2005](#); [Makkonen and others 2013](#);  
526 [Santonja and others 2015](#)). N release was determined more by the second component of the  
527 CWM-trait PCA, identifying N concentration, N-based stoichiometric ratios, and phenolics as  
528 the main drivers of N dynamics. It has previously been shown that the relative contribution of  
529 N immobilization vs. N release is strongly determined by the initial litter N concentration ([Aber  
530 and Melillo 1982](#); [Parton and others 2007](#)). In strong contrast to C release, both axes of the FD-  
531 trait PCA were associated to N release patterns. In line with the niche complementarity  
532 hypothesis, litter mixtures with contrasting litter quality may improve the availability of  
533 different resources for decomposers ([Schimel and Hättenschwiler 2007](#); [Handa and others  
534 2014](#)). Similar to our results, [Garcia-Palacios and others \(2017\)](#) also identified litter CWM traits  
535 to be important drivers of both, litter C and N release, and FD traits as additional major drivers  
536 of litter N release. Collectively, these findings suggest that different aspects of litter trait  
537 diversity control C and N dynamics during litter decomposition.

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



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

557

## 558 CONCLUSION

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

569

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579

#### 580 **AUTHOR'S CONTRIBUTIONS**

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

585

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772

773 **Tables**

774

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

782

	d.f.	Carbon release		Nitrogen release	
		<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value
(Intercept)	1	1837.8	***	459.9	***
Richness (R)	1	1.7		4.6	*
Year (Y)	1	213.4	***	21.9	***
R × Y	1	5.4	*	17.2	***
All R <sup>2</sup> (AIC)		0.31 (4234.2)		0.12 (4390.8)	
MPM R <sup>2</sup> (AIC)		0.30 (4226.7)		0.11 (4383.9)	

783

784

785 **Table 2.** Effects of litter species composition, change in precipitation and year of  
786 decomposition on carbon and nitrogen release. Only the variables retained in the most  
787 parsimonious models are reported (d.f. = degrees of freedom,  $P$ -values = \*  $P < 0.05$ , \*\*  $P <$   
788  $0.01$ , and \*\*\*  $P < 0.001$ ). The term “soil characteristics” refers to plot-specific soil parameters  
789 that were systematically included in all models as co-variable. At the bottom of the table the  
790 values of  $R^2$  and AIC weight of the general model including all factors (All) and of the most  
791 parsimonious model (MPM) are shown.

792

	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 × P	14	2.1	*		
C × Y	14	7.0	***	13.7	***
P × 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)	

793

794

795 **Table 3.** Effects of the presence of litter species, change in precipitation, and year of  
796 decomposition on carbon and nitrogen release. Only the variables retained in the most  
797 parsimonious models are reported (d.f. = degrees of freedom,  $P$ -values = \*  $P < 0.05$ , \*\*  $P <$   
798  $0.01$ , \*\*\* for  $P < 0.001$ ). The term “soil characteristics” refers to plot-specific soil parameters  
799 that were systematically included in all models as co-variable. At the bottom of the table the  
800 values of  $R^2$  and AIC weight of the general model including all factors (All) and of the most  
801 parsimonious model (MPM) are shown.

802

	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)	

803

804

805 **Table 4.** Effects of community weighted mean traits (CWM), functional trait dissimilarity (FD),  
806 change in precipitation, and year of decomposition on carbon and nitrogen release. CWM1 and  
807 CWM2, and FD1 and FD2 represented the two first components of the PCAs conducted using  
808 the CWM or the FD values in Fig. 4. Only the variables retained in the most parsimonious  
809 models are reported (d.f. = degrees of freedom, *P*-values = \* *P* < 0.05, \*\* *P* < 0.01, \*\*\* for *P*  
810 < 0.001). At the bottom of the table the values of R<sup>2</sup> and AIC weight of the general model  
811 including all factors (All) and of the most parsimonious model (MPM) are shown.

812

	d.f.	Carbon release		Nitrogen release	
		<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -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 × Y	1	9.1	**	29.1	***
FD2 × Y	1	29.3	***	47.9	***
P × S	1	2.8		4.2	*
All R <sup>2</sup> (AIC)		0.62 (2981.7)		0.43 (3165.8)	
MPM R <sup>2</sup> (AIC)		0.62 (2970.2)		0.43 (3159.8)	

813

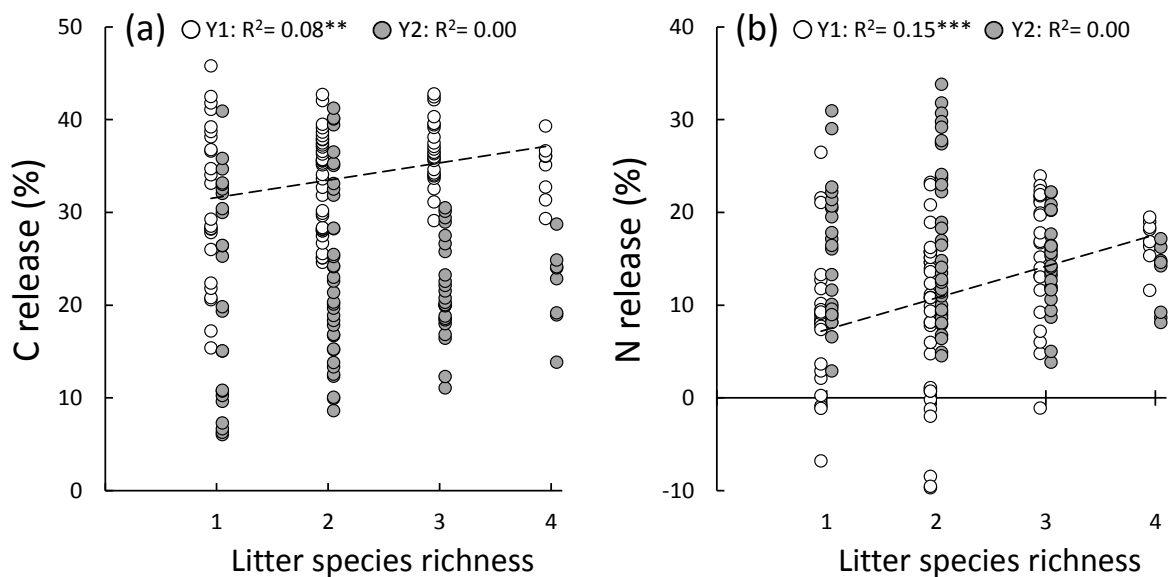
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815 **Figures**

816

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

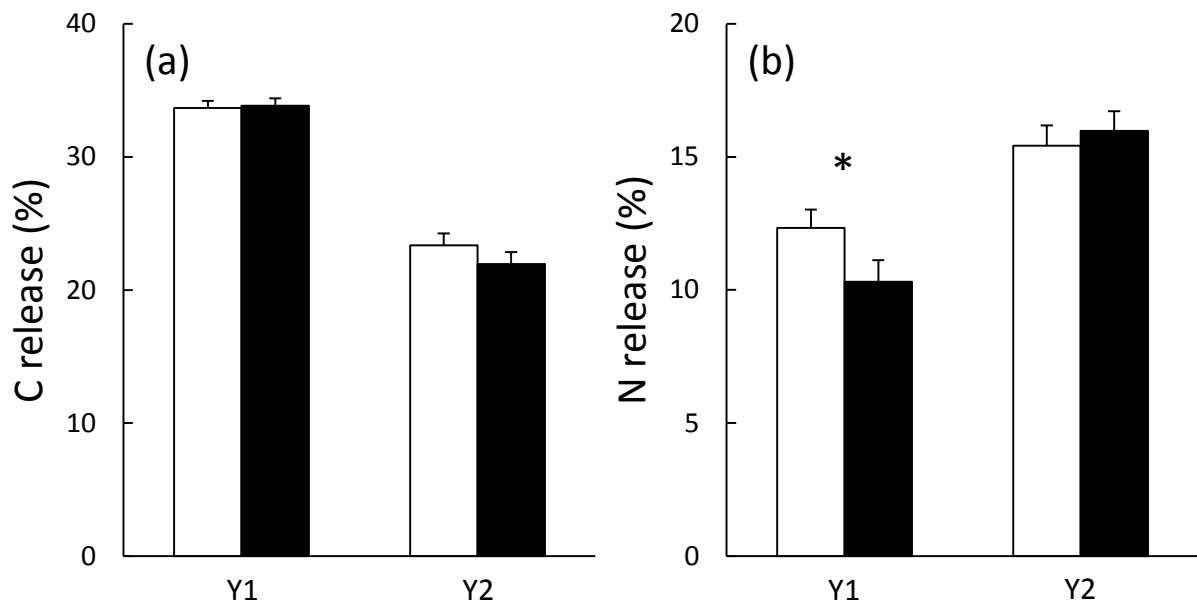
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827

828 **Fig. 2.** Carbon (a) and nitrogen (b) release in control plots (white bars) and plots with partial  
829 rain exclusion (black bars) during the first year (Y1 = 0 to 12 months) and the second year of  
830 decomposition (Y2 = 12 to 24 months). Each bar represents the mean value  $\pm$  SE per  
831 precipitation treatment; n = 184 and 138 microcosms for the first and the second year of  
832 decomposition, respectively. The C and N release values are indicated in percent loss relative  
833 to the initial amount (for the first year of decomposition) or relative to the amount remaining  
834 after 12 months of decomposition (for the second year of decomposition). Stars denote  
835 significant differences between control plots and plots with partial rain exclusion (\*  $P < 0.05$ ).  
836



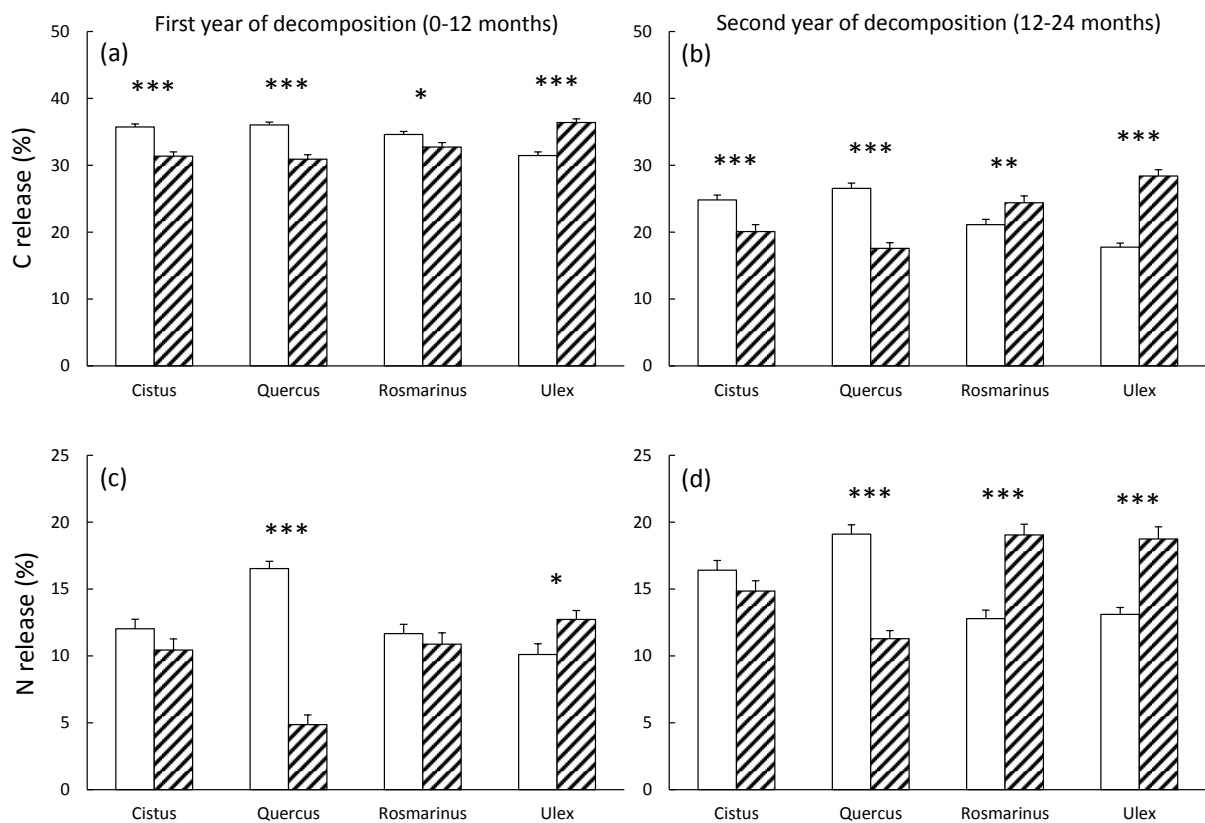
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838



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

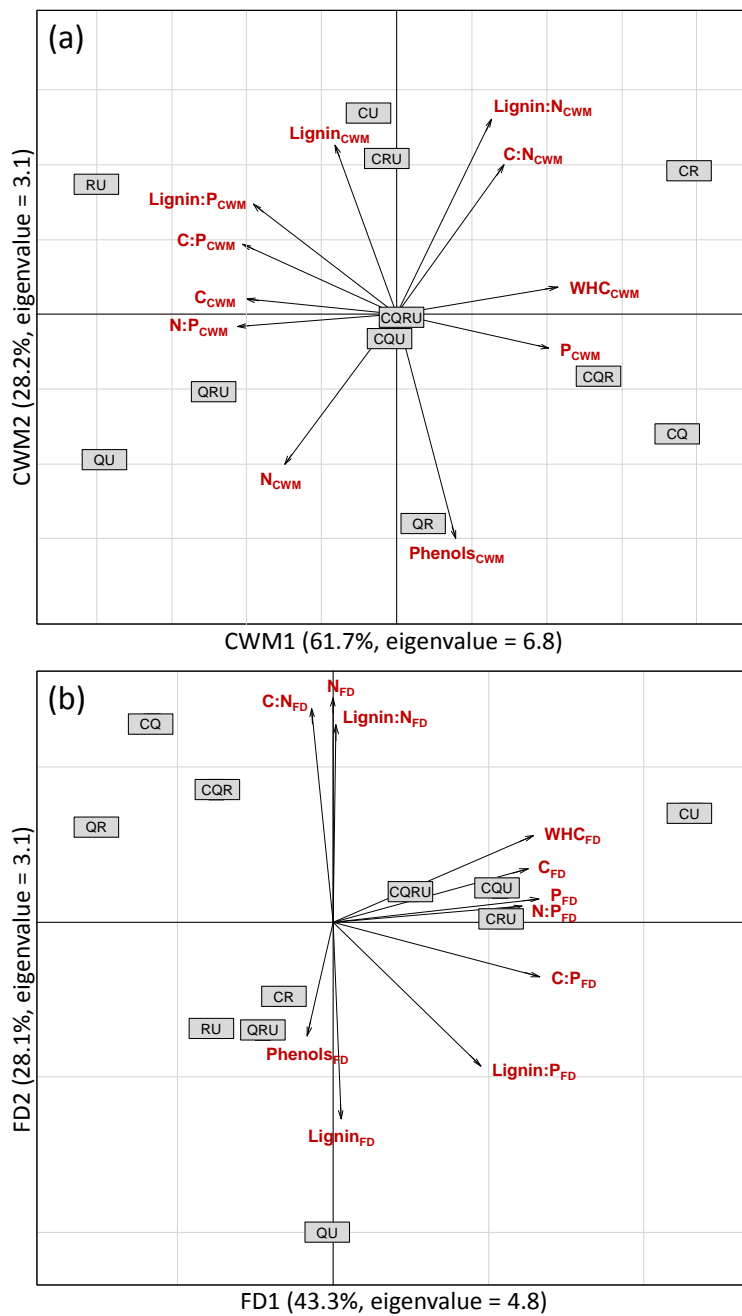
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850

851 **Fig. 4.** Principal component analysis (PCA) of community-weighted mean traits (a) and  
 852 functional trait dissimilarity (b). Variance explained by each principal component and  
 853 associated eigenvalues are shown in brackets. CWM = Community-Weighted Mean Trait, FD  
 854 = Functional Trait Dissimilarity. C = Cistus, Q = Quercus, R = Rosmarinus, U = Ulex.  
 855 Combinations of capital letters correspond to combinations of plant species in litter mixtures.  
 856

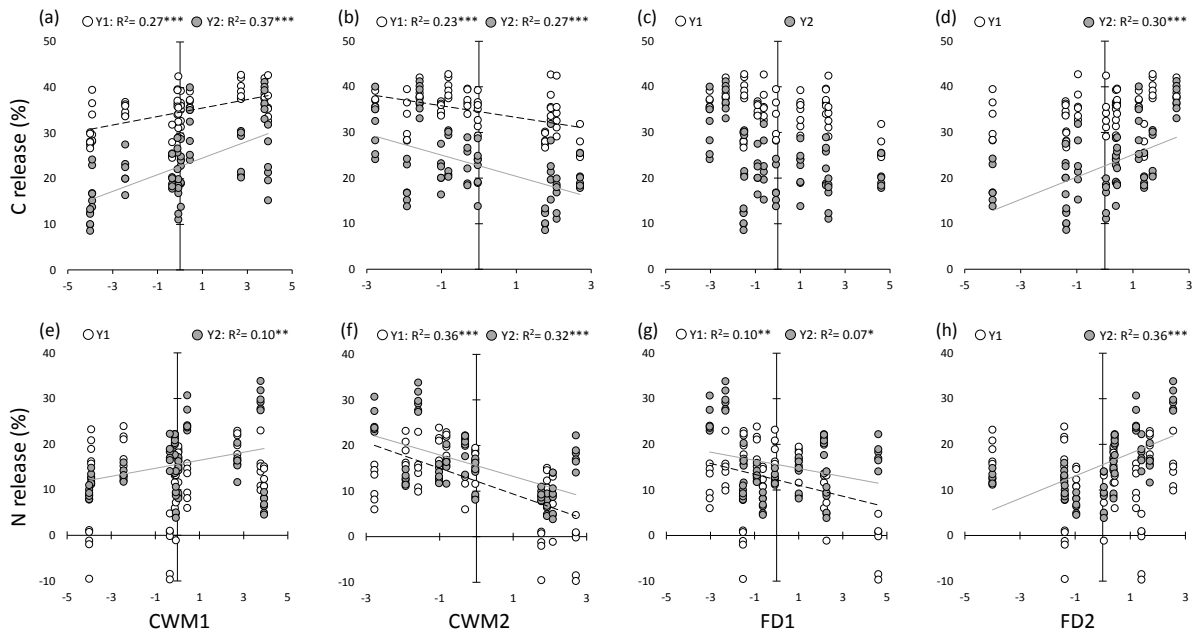


857

858

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

867



868

869