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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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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⁻¹ (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 m² 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 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 m² 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 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 R² 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 ² (AIC)		0.31 (4234.2)		0.12 (4390.8)	
MPM R ² (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^2 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	
		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)	

813

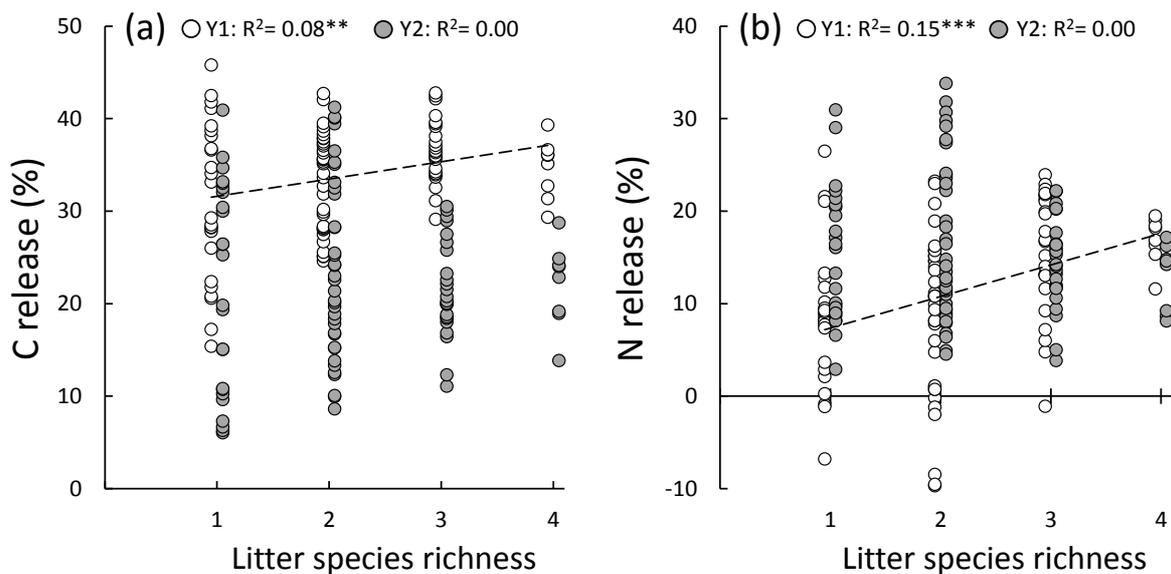
814

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 ** $P < 0.01$, *** $P < 0.001$).

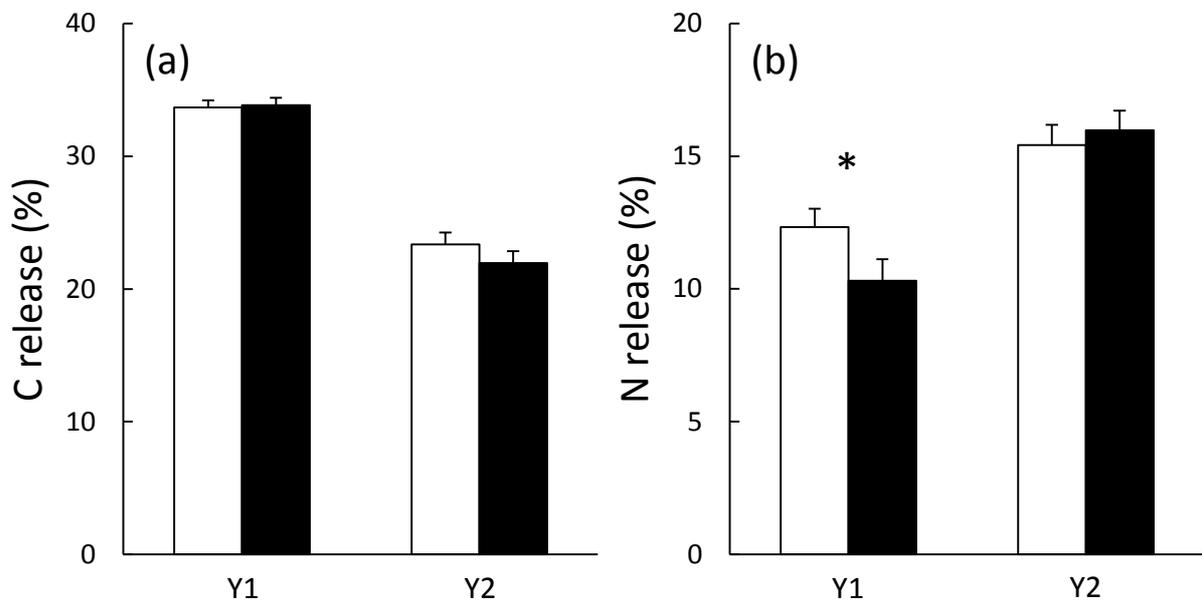
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827

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

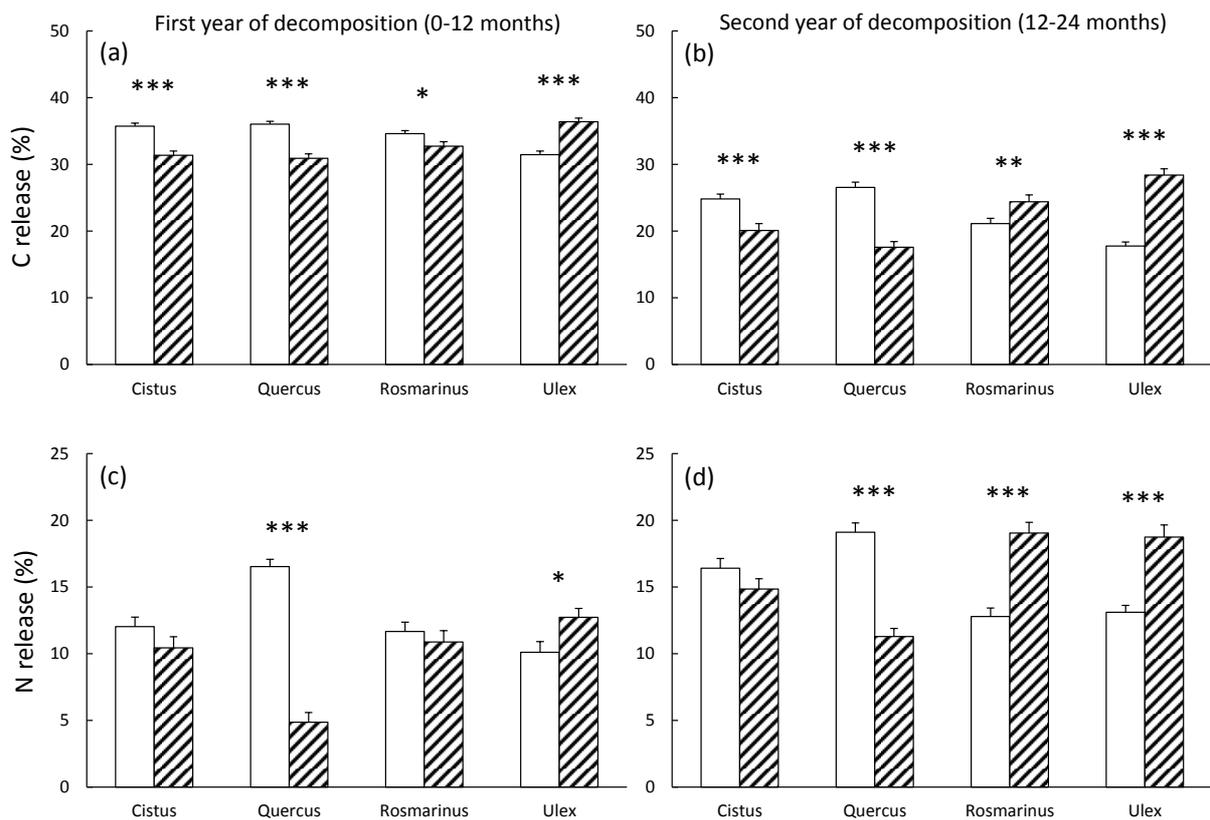


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838

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 (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

848

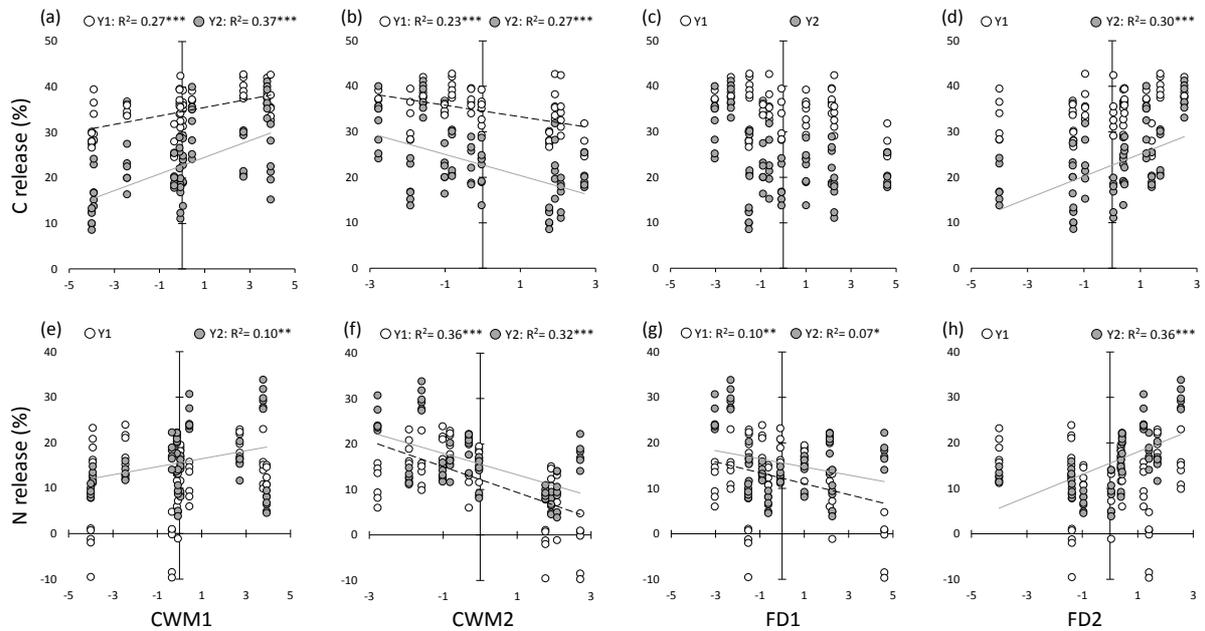


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850

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



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