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Title: Drought inhibits synergistic interactions of native and exotic litter mixtures during decomposition in temperate grasslands

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1 **Abstract**

2 Background and Aims: Leaf litters commonly interact during decomposition in ways that can
3 synergistically increases rates of decay. These interactions have been linked to moisture
4 availability, suggesting that drought could slow decomposition rates by disrupting litter
5 interactions. Slowed decomposition may reduce competitive ability of exotic species that exploit
6 rapid decomposition rates as part of niche construction mechanisms. Here, we evaluated the
7 impacts of drought on interactions between native and exotic species' litter decomposition.

8

9 Methods: We considered litter mixtures of *Lupinus polyphyllus* (exotic N-fixing forb), *Trifolium*
10 *pratense* (native N-fixing forb), *Senecio inaequidens* (exotic non-N-fixing forb), and *Senecio*
11 *jacobaea* (native non-N-fixing forb) with the native grass *Alopecurus pratensis* and evaluated the
12 difference between the observed rate of decay and the one expected based on species
13 decomposing in monocultures. Litters were deployed in Belgium and Germany and exposed to a
14 56 day drought, which resembled local millennium drought (statistical recurrence of duration in
15 local precipitation series > 1000 years).

16

17 Results: Litter interactions reduced mass remaining by 81% in Belgium and 15% in Germany,
18 averaged across mixtures. Similarly, litter interactions reduced N remaining by 93% in Belgium
19 and 14% in Germany. Drought consistently removed these interactions and resulted in additive
20 litter decay. Litters of native and exotic species did not differ in their response to drought.

21

22 Conclusions: These findings support moisture availability as a key regulator of interactions
23 between litters during decomposition. Thus, increasing frequency of drought may slow nutrient
24 cycling to a greater extent than previously thought.

25

26 Keywords: non-additive effect, mixture, climate change, precipitation, litter, invasion, invasive,

27

28 **Introduction**

29 Increases in the frequency of extreme climatic events can significantly alter ecosystem structure
30 and function (Easterling et al. 2000; Smith 2011). As a result of rising temperatures, precipitation
31 regimes are expected to become more variable, increasing the frequency and magnitude of
32 drought in many regions (Stocker et al. 2014). Such increases in the prevalence of drought can
33 strongly affect biogeochemical cycling (Reichstein et al. 2013). Indeed, investigations into the
34 impact of drought on litter decomposition often find severely inhibited litter decomposition and
35 nutrient release (Liao et al. 2002; Schimel et al. 2007; Jentsch et al. 2011; Walter et al. 2013),
36 which are key regulators of soil fertility and nutrient cycling in many systems (Attiwill and
37 Adams 1993; Schimel 1995). However, investigations into the impacts of drought on
38 decomposition have almost wholly focused on the decomposition of individual species' litters
39 and have ignored the interactions between litters of different species that strongly affect decay
40 rates in diverse communities (Gartner and Cardon 2004).

41 Litter layers of natural systems are complex and are generally comprised of multiple
42 species, creating the opportunity for litter interactions. These interactions result in decomposition
43 and nutrient release of litter mixtures being non-additive, meaning that their decay rates cannot
44 be predicted based on the component species' identities alone (Gartner and Cardon 2004). Non-

45 additive effects are often observed as a result of differences in litter quality between litters within
46 the mixture (McTiernan et al. 1997; Liu et al. 2007; Ball et al. 2009), where nutrients from high-
47 quality (low C:N ratio) litters are transferred onto low-quality litters via leaching or microbial
48 processes. Nutrient transfer between litters has been hypothesized to give rise to synergistic
49 interactions that accelerate litter decay (Hättenschwiler et al. 2005) since nitrogen (N) is often
50 observed to limit microbial decomposition of cellulose-rich litters (Melillo et al. 1982; Knorr et
51 al. 2005). In accordance with this hypothesis, most non-additive effects are synergistic and
52 increase rates of decomposition and concurrent nutrient release (Gartner and Cardon 2004),
53 although antagonistic effects are not uncommon and may be tied to inhibitory effects of N
54 addition on some lignin-rich litters (Knorr et al. 2005). Thus, interactions between litters are an
55 important component of nutrient dynamics in many systems.

56 While differences in litter chemistry have received the most attention in explaining non-
57 additive effects, litter moisture has been shown to strongly regulate litter interactions of some
58 species (Makkonen et al. 2013). Litters with greater water holding capacity stimulate synergistic
59 interactions under limited moisture availability by providing a more hospitable microclimate for
60 decomposers (Wardle et al. 1997; Makkonen et al. 2013). Thus, changes in microclimate due to
61 drought may be particularly detrimental to litter interactions and inhibit synergism (Santonja et
62 al. 2015). Despite the demonstrated importance of litter interactions to decomposition and
63 nutrient release rates and the increasing likelihood of drought, the impact of drought on litter
64 interactions is still largely unknown.

65 Drought can directly reduce invasibility of some systems for plants (Jentsch et al. 2011),
66 but it may also have important consequences for invasion by affecting interactions between
67 native and exotic litters. A growing body of literature suggests that interactions between invasive

68 and native species' leaf litters contribute to the success of invasive species. According to a recent
69 meta-analysis (Liao et al. 2008), invasive species produce litter with 30% lower C:N ratio than
70 co-occurring native species on average, and decomposition rates in heavily invaded systems are
71 commonly twice those of intact systems. Because of these strong differences between native and
72 invasive species' litter, invasive species have been suggested to exploit non-additive effects as
73 part of niche construction mechanisms (Wright and Jones 2006; Schuster and Dukes 2014).
74 While few in number, most investigations into litter interactions between native and invasive
75 species find synergistic interactions (Ashton et al. 2005; Arthur et al. 2012; Chen et al. 2013),
76 supporting the hypothesis that invasive plants are able to increase nutrient availability to their
77 own benefit (Heneghan et al. 2002; Lee et al. 2012). Antagonistic interactions have also been
78 linked to improved competitive ability of other invasive plant species (Zhang et al. 2014;
79 Schuster and Dukes 2014). Therefore, drought may reduce the competitive ability of some
80 invasive plant species by inhibiting litter interactions.

81 Here, we report findings from a litter bag experiment conducted in Belgium and Germany
82 that considers litter mixtures containing species that are invasive throughout Europe and
83 compared them to mixtures containing functionally similar native species. *Lupinus polyphyllus*
84 Lindl. is a leguminous forb native to North America. It produces a large amount of leaf litter that
85 has been suggested to contribute to positive invasion feedbacks and suppress native plant species
86 (Vetter et al. submitted; Loydis et al. 2015). *Senecio inaequidens* DC. is native to South Africa
87 and readily invades disturbed areas (Ernst 1998), where it is often more stress-tolerant than
88 native species. In order to evaluate the impacts of these exotic species on the decay of native leaf
89 litter and how drought may alter these impacts, we considered three hypotheses: 1) drought
90 reduces interactions between litters in mixture, 2) litter mixtures containing exotic *L. polyphyllus*

91 or *S. inaequidens* exhibit greater synergistic non-additive effects than mixtures containing
92 functionally similar native species, and 3) differences in non-additive effects between litter
93 mixtures containing invasive species and those containing only native species are smaller under
94 drought than under wetter conditions.

95

96 **Methods**

97 **Study sites**

98 We considered two sites of the SIGNAL Experiment (Jentsch 2013): one in Belgium (51.25 °N,
99 4.67 °E) and another in Germany (49.92 °N, 11.58 °E). Both sites are located in semi-natural C₃
100 grasslands, meaning that they would eventually succeed into woodlands in the absence of
101 grazing or mowing, and are established on loamy sand soil (Germany) and sandy loam
102 (Belgium) soils. Mean annual temperature is 10.0 °C and 8.2 °C and mean annual precipitation is
103 787 mm and 654 mm in Belgium and Germany, respectively. Temperature and rainfall patterns
104 during the experimental period are presented in Online Resource Figs. S1 and S2.

105

106 **Litter bag experiment**

107 In order to evaluate differences between native and exotic species during decomposition, we
108 selected five species commonly found in the semi-natural grasslands of Central and Western
109 Europe of varying geographical origin and functional type. These species included *Alopecurus*
110 *pratensis* L. (native C₃ grass), *L. polyphyllus* (exotic N-fixing forb), *Trifolium pratense* L. (native
111 N-fixing forb), *S. inaequidens* (exotic non-N-fixing forb), and *Senecio jacobaea* L. (native non-
112 N-fixing forb). Leaf residue of each species was collected separately during November 2013
113 from the area surrounding each experimental site by cutting plants at ground level. Leaf residue

114 (henceforth “litter”) was then pooled by species and site and dried at 65 °C. Three subsamples of
115 each litter type for each site were set aside to test for initial tissue chemistry.

116 It is important to note that the residue used in this experiment was not truly litter, since it
117 was collected before it was fully senesced and therefore likely differed in chemical composition
118 from fully senesced litter. However, semi-natural grasslands in Europe are maintained by
119 mowing, drying the mown biomass on site, and finally removing the dried biomass with heavy
120 machinery, which leaves considerable portions of biomass to decay without being allowed to
121 senesce. Thus, the use of residue allowed us to more accurately describe the fate of plant biomass
122 in these systems.

123 We used litter to fill 10 cm x 10 cm bags made of 1-mm fiberglass mesh. Bags were
124 filled with either 2 g of a single species or a mixture of 1 g *A. pratensis* litter and 1 g of litter
125 from one of the forb species. In Germany, we were able to harvest sufficient material to produce
126 mixtures of *A. pratensis* and each of the forb species, but were only able to consider *S. jacobaea*
127 and *S. inaequidens* in Belgium. *A. pratensis* was included in each mixture because it is a major
128 component of many grasslands in Central Europe (Ellenberg and Leuschner 2010), and thus
129 represents one of the most likely litters with which sub-dominant species would be mixed.
130 Holding one species constant across all mixtures also allowed us to better elucidate the role of
131 the other four species in determining mixture decay rates. We filled enough bags of each
132 combination of species to allow for three collection points: prior to drought, at the end of
133 drought, and 3 months later (characterizing recovery after drought). With four replicates, this
134 resulted in a total of 216 bags in Germany ((5 species + 4 mixtures) x 2 rainfall treatments x 3
135 collection dates x 4 replicates) and 120 bags in Belgium ((3 species + 2 mixtures) x 2 rainfall
136 treatments x 3 collection dates x 4 replicates).

137 We deployed litter bags in May 2014 by pinning them to the soil surface. Bags were
138 placed after gently removing existing litter that was not attached to living plants. Bags destined
139 to experience drought were randomly placed in a 6 m x 7.5 m plot spaced at least 10 cm apart
140 and those serving as ambient controls were placed in the surrounding area no more than 5 m
141 away. Topography, plant cover, and soil conditions were similar throughout the experimental
142 area at each site. After 30 days of field incubation, the first set of litter bags was collected. Then,
143 the drought treatment was initiated by placing a rainout shelter over the drought plot. This was
144 constructed from hemispherical steel frames (6 m x 7.5 m in area) covered in clear plastic that
145 started 80 cm from the ground and extended upward (Online Resource Fig. S3). Drought was
146 imposed for 56 days (mean of 54 and 59 days, which correspond to a statistical return interval of
147 1000 years in Germany and Belgium, respectively), after which the rainout shelter was removed,
148 the second set of litter bags was collected, and the experimental area was irrigated with 10 mm of
149 ground water. Soil moisture data from Germany are presented in Fig. S4 (equipment failure
150 prevented the recording of data for Belgium). 180 days after deployment, we collected the third
151 and final set of litter bags. Once collected, litter was removed from bags, dried at 65 °C, gently
152 brushed to remove contaminants, and weighed. We then pulverized decomposed litter as well as
153 subsamples of undecomposed litter and analyzed them for C and N concentrations using an ECS
154 4010 element analyzer (Costech Analytical Technologies, Valencia, CA, USA).

155

156 **Statistical analyses**

157 In order to evaluate differences between species' litters' chemical composition, we conducted an
158 analysis of variance considering values from three samples of each species' undecomposed litter.
159 Litter chemistry (C:N), was analyzed by analysis of variance (ANOVA) as a function of species

160 identity and site, except when considering the N-fixers, *L. polyphyllus* and *T. pratense*, which
161 were analyzed as a function of species identity alone as they were only included in the Germany
162 trials. This approach was adopted for all other analyses considering N-fixers.

163 Mass loss and N loss of single species and mixed litter bags were calculated after
164 applying C correction (Robertson 1999) to account for any residual contamination in each litter
165 bag. Additionally, we used data from the first collection (after 30 days of incubation but prior to
166 any experimental manipulations) to control for differences between ambient and drought plots.
167 Therefore, mass and N loss were calculated relative to values after 30 days of decomposition
168 (henceforth “pre-treatment controls”) instead of relative to initial deployment values (e.g. mass
169 loss relative to 2 g; see Online Resource Fig. S5 for observed values). Using this method allowed
170 us to better isolate the effects of drought on litter decay than would otherwise be possible. The
171 remaining proportion of mass (g) and N (g) of single species’ litter relative to pre-treatment
172 controls were then evaluated with ANOVA as a function of species, collection date, drought, and
173 all possible interactions. N-fixers (*L. polyphyllus* and *T. pratense*) were analyzed separately from
174 *A. pratensis* and *Senecio* spp since N-fixers were only present in one of the two study locations.

175 We examined interactions between litters by comparing mass and N remaining relative to
176 pre-treatment controls to expected values based on single species litter bags. Expected values
177 were calculated as means of mass and N remaining relative to pre-treatment controls weighted by
178 each species’ initial contribution to the mixture’s mass or N. Expected mass remaining values
179 were calculated as $0.5M_A + 0.5M_F$, where M_A and M_F are the mean proportion mass remaining
180 of *A. pratensis* and forb single species litter bags relative to pre-treatment controls at the
181 corresponding collection date. Similarly, expected N remaining was calculated as $G_A N_A + G_F N_F$,
182 where G_A and G_F are the proportion of the mixture’s initial N (g) belonging to *A. pratensis* and a

183 forb species, respectively, and N_A and N_F are the mean proportion N (g) remaining of *A.*
184 *pratensis* and forb single species litter bags relative to pre-treatment controls at the
185 corresponding collection date. We then calculated non-additivity of each mixed litter bag as the
186 relative deviation from expected mass and N remaining values (Olson 1963): (observed–
187 expected)/expected. Thus, mass non-additivity was calculated as $(M_T - 0.5M_A + 0.5M_F)/(0.5M_A +$
188 $0.5M_F)$ where M_T is the observed total mass remaining of a litter bag relative to pre-treatment
189 controls. Similarly, N non-additivity was calculated as $(N_T - G_A N_A + G_F N_F)/(G_A N_A + G_F N_F)$
190 where N_T is the observed total N remaining of a litter bag relative to pre-treatment controls.

191 Negative non-additivity values indicate synergistic interactions (less mass or N remained
192 than expected) and positive non-additivity values indicate antagonistic interactions (more mass
193 or N remained than expected). Non-additive effects were determined to be significant if a 95%
194 confidence interval around the mean non-additivity value for a considered mixture did not
195 include zero. Decay of mixtures for which confidence intervals included zero was considered
196 additive.

197 We analyzed non-additivity separately for mixtures containing either non-N-fixing forbs
198 (*S. jacobaea* and *S. inaequidens*) or N-fixers (*L. polyphyllus* and *T. pratense*). For non-N-fixers,
199 we analyzed mass or nitrogen non-additivity with ANOVA as a function of collection date (86
200 days: drought, 180 days: post-drought), drought treatment (ambient or drought), forb species (*S.*
201 *jacobaea* or *S. inaequidens*), and site (Belgium or Germany) with all possible interactions. We
202 conducted similar analyses for N-fixers, but did not include the *site* term since *T. pratense* and *L.*
203 *polyphyllus* were only included in the Germany trials.

204 Data were rank-transformed for the analysis of variance to meet assumptions of
205 homoscedasticity. All analyses were conducted in R v.3.1.0 (R Core Team 2014). Tukey HSD
206 were performed to identify distinct groups within statistically significant effects.

207

208 **Results**

209 **Initial tissue chemistry and single species' decay**

210 Species' litters differed in their initial C:N (N-fixers, species: $p < 0.01$; *A. pratensis* and *Senecio*
211 spp., species: $p < 0.01$; Online Resource Table S1). Although *A. pratensis* had the highest C:N of
212 the five species considered, it varied greatly between the two countries (Fig. 1). In Germany, *A.*
213 *pratensis* litter had a mean C:N of $28.20 \pm 0.34 \text{ gC gN}^{-1}$, whereas it had a mean C:N of $17.58 \pm$
214 0.18 gC gN^{-1} in Belgium. *S. jacobaea* and *S. inaequidens* were more consistent between sites and
215 had intermediate C:N values between *A. pratensis* and the N-fixers. C:N of exotic *S. inaequidens*
216 was lower than that of native *S. jacobaea*, but exotic *L. polyphyllus* C:N was greater than that of
217 native *T. pratense*.

218 Decomposition of single species' litters was highly variable between treatments and
219 across sites. Litter decomposed in Germany, especially that of *A. pratensis* (site x species:
220 $p < 0.01$; Online Resource Fig. S6), generally had a greater proportion of mass remaining relative
221 to pre-treatment controls than litter decomposed in Belgium (site: $p < 0.01$). While drought
222 initially increased the proportion of mass remaining in *A. pratensis*, *S. jacobaea*, and *S.*
223 *inaequidens* by 29% across sites, these differences disappeared by the end of the experimental
224 period (Fig. 2a; collection x drought: $p = 0.01$). Drought also initially retarded the decomposition
225 of N-fixers and increased mass remaining by 43%. However, after the drought concluded,
226 droughted N-fixer litter had 37% less mass remaining than litter that had not been exposed to

227 drought (Fig. 2b; collection x drought: $p < 0.01$). N-fixers also differed in decomposition (species:
228 $p < 0.01$), and exotic *L. polyphyllus* retained 27% more mass relative to native *T. pratense* overall.
229 In contrast, none of the significant effects of species identity for *A. pratensis*, *S. jacobaea*, and *S.*
230 *inaequidens* on mass remaining (Table 1; Online Resource Fig. S7) were due to differences
231 between *S. inaequidens* and *S. jacobaea*.

232 Nitrogen release from single species' litters differed greatly between sites, but was not
233 strongly influenced by drought (Table 1). *A. pratensis* maintained the greatest proportion of N
234 remaining throughout the experimental period (species: $p < 0.01$). Exotic species' N loss did not
235 differ from that of their native counterparts for either N-fixers or non-N-fixers. At the German
236 site, litter retained 114% more N than in Belgium (site: $p < 0.01$). This difference in N loss rates
237 was also associated with differences in litters' responses to drought. In Belgium, *A. pratensis*, *S.*
238 *jacobaea*, and *S. inaequidens* retained 56% more N under drought than under ambient
239 conditions, but drought did not significantly affect N remaining within litter of these species in
240 Germany (Fig. 3a; drought x site: $p < 0.01$). Similarly, N loss from *T. pratense* and *L. polyphyllus*
241 exposed to drought did not differ significantly from that of litter decayed under ambient
242 conditions (Fig. 3b; drought: $p = 0.56$).

243

244 **Litter Interactions**

245 Interactions between litters varied by species and across sites (Table 2). Mixing effects on
246 decomposition were always synergistic when present. Non-N-fixer mixtures decomposed in
247 Belgium were more synergistic than those decomposed in Germany (site: $p < 0.01$). This
248 difference was most evident at the end of the experimental period, when non-N-fixer mixtures
249 decomposed in Belgium exhibited synergistic effects 6.6 times greater than those detected in

250 Germany (92% and 14% reduction in mass remaining, respectively; collection x site: $p < 0.01$).
251 Mixtures in Belgium also exhibited more synergistic N loss than mixtures in Germany (site:
252 $p < 0.01$), although this was largely the result of strong synergy at the end of the experimental
253 period, when litter interactions led to a 93% reduction in N remaining (site x collection: $p < 0.01$;
254 Online Resource Fig. S8). Mixing effects on N loss in non-N-fixer mixtures became increasingly
255 synergistic over time, increasing 68% from the 86 day collection to the 180 day collection point
256 (collection: $p < 0.01$).

257 Decomposition of litter mixtures was consistently less synergistic in drought plots (Fig.
258 4). Whereas ambient conditions facilitated strong synergy between mixtures of non-N-fixers,
259 these interactions were fully negated directly following drought. However, differences in mass
260 non-additivity between drought and ambient mixtures had dissipated by the end of the
261 experiment and litter interactions reduced mass remaining by 53% across treatments (Fig. 5a;
262 drought x collection: $p < 0.01$). Although drought negated synergistic effects in both Belgium and
263 Germany (Fig. 4; drought: $p < 0.01$), the stronger synergy of mixtures in Belgium, which led to an
264 81% reduction in mass remaining, meant that the impact of drought was larger there than in
265 Germany (drought x site: $p < 0.01$; Online Resource Fig. S9). Differences in the decomposition of
266 non-N-fixer mixtures were greatest between the two sites directly following drought, when
267 drought resulted in more positive (antagonistic) non-additivity values in Belgium (drought x
268 collection x site: $p = 0.01$; Online Resource Fig. S10). Similar to non-N-fixer mixtures, N-fixer
269 mixtures exposed to ambient conditions decomposed synergistically and retained less mass than
270 expected, but mixtures decomposed under drought did not show signs of significant interactions
271 (Fig. 4; drought: $p < 0.01$). This effect was largely driven by mixtures collected at the end of the

272 experimental period, when drought eliminated synergistic litter interactions that would otherwise
273 reduce mass remaining by 24% in N-fixer mixtures (Fig. 5b; drought x collection: $p=0.03$).

274 Drought inhibited litter interactions on N release in most mixtures. For non-N-fixers,
275 drought negated synergistic effects on average (Fig. 4; drought: $p<0.01$), but this was almost
276 wholly the result of mixtures' responses in Belgium since neither ambient nor droughted
277 mixtures in Germany exhibited non-additive N loss (site x drought: $p<0.01$). Impacts of drought
278 on non-N-fixer mixtures were most pronounced directly following the drought, when a 58%
279 reduction in N remaining was negated (Fig. 5c; drought x collection: $p<0.01$). However, by the
280 end of the experimental period, mixtures under either treatment exhibited synergistic N loss.
281 Similar to non-N-fixers, drought completely negated synergistic N release in mixtures containing
282 N-fixers (Fig. 4; drought: $p<0.01$).

283 Although non-N-fixers were similarly capable of inducing non-additive effects in either
284 decomposition or N loss (mass, species: $p=0.40$; N, species: $p=0.65$), N-fixers differed
285 significantly. Across treatments, mixtures including exotic *L. polyphyllus* exhibited strong
286 synergistic effects on decomposition (18% reduction in mass remaining) by the end of the
287 experimental period, whereas mixtures including native *T. pratense* did not show signs of
288 consistent litter interactions (species x collection: $p=0.04$; Online Resource Fig. S11). Similarly,
289 the two mixtures that included N-fixers differed in their release of N (species: $p=0.04$). Mixtures
290 containing *L. polyphyllus* tended to release N synergistically and those containing *T. pratense*
291 tended to release N antagonistically, but neither interaction satisfied our criteria for significance
292 based on 95% confidence intervals.

293 The effect of drought on litter interactions did not differ consistently between mixtures
294 containing only natives and those that also contained exotic species (see species x drought effects

295 in Table 2). However, we did detect one instance of a native and an exotic differing in response
296 to drought. Drought led to antagonistic N release in mixtures containing either *T. pratense* or *L.*
297 *polyphyllus*, but did so at different points throughout the experimental period (Fig. 5d; species x
298 collection x drought: $p=0.02$). Although *L. polyphyllus* mixtures exposed to drought retained
299 17% more N than litters under ambient conditions, this difference dissipated by the end of the
300 experimental period and both droughted and ambient litter mixtures exhibited additive N release.
301 In contrast, *T. pratense* mixtures exposed to drought retained 68% more N than those exposed to
302 ambient conditions at the end of the experimental period.

303

304 **Discussion**

305 We examined the impact of drought on the decomposition of native and exotic litter mixtures in
306 semi-natural grasslands of Belgium and Germany. Under ambient conditions, we observed
307 synergistic non-additive effects in both decomposition and N release supporting earlier findings
308 of synergy within litter mixtures (Scherer-Lorenzen 2008; Poulette and Arthur 2012). Although
309 we had anticipated differences in the capacity of exotic species and their native counterparts to
310 induce non-additive effects, these differences were relatively small and only apparent between
311 the two N-fixing species (*L. polyphyllus* and *T. pratense*, Fig. 5d). Similarly, exotic species did
312 not consistently differ from their native counterparts in response to drought. However, we did
313 find drought to consistently inhibit synergistic interactions between litters on decomposition and
314 concurrent N release (Fig. 4).

315 Drought negated all synergistic effects that we observed and even promoted antagonism
316 in some instances (Fig. 5). Drought has been shown to slow decomposition and nutrient release
317 in the past (Liao et al. 2002; Walter et al. 2013), and our findings extend the impact of drought to

318 include reductions in litter mixing effects. Like all rainout shelters, ours altered light and
319 temperature as well as moisture availability in a way that may have impacted litter decay rates
320 (Vogel et al. 2013). However, Kreyling *et al.* (2016) found that light, moisture, and temperature
321 artifacts of identical rainout shelters were ecologically non-significant at the Germany site and
322 did not influence plant responses. Additionally, results similar to those presented here were
323 found in Mediterranean forests (Santonja et al. 2015), where drought resulted in a threefold
324 reduction in synergistic effects on decomposition, thus lending support to our findings.
325 Therefore, drought may have a more severe immediate impact on decomposition and nutrient
326 cycling than previously thought as a result of stifled litter interactions.

327 In contrast to findings of Santonja *et al.* (2015), non-additive effects were only
328 suppressed directly following drought for mixtures containing non-N-fixing forbs. By the end of
329 the experiment, non-N-fixer mixtures exhibited similar non-additive effects regardless of
330 previous moisture conditions (Fig. 5). In agreement with other studies of microbial (Austin et al.
331 2004) and plant (Dreesen et al. 2012) responses to drought, our findings suggest ecosystem
332 function may be restored quickly once moisture levels are no longer limiting. This effect was
333 consistent across our two sites. The difference in climate between the two countries may also
334 explain the larger mixing effects observed in Belgium, since microbial activity was likely
335 enhanced by the higher temperatures and greater moisture there (Aerts 1997; Zhang et al. 2008).
336 In contrast to mixtures containing non-N-fixers, mixtures containing N-fixers exhibited more
337 persistent effects of drought and non-additivity had not yet recovered by the end of the
338 experiment, particularly for mixtures including *T. pratense*. Therefore, drought impacts on non-
339 additivity may be transient-but-severe in wetter systems, although this effect is likely context-
340 and substrate-dependent.

341 Mixtures containing exotic species rarely differed from those containing only native
342 species. This was inconsistent with our hypothesis and contrary to findings of earlier studies that
343 compared exotic species to functionally distinct natives (e.g. Poulette and Arthur 2012; Chen et
344 al. 2013). Differences in tissue chemistry are often cited as the primary driver of litter
345 interactions (Hättenschwiler et al. 2005; Lummer et al. 2012; Tardif and Shipley 2014), and thus
346 the chemical similarity between *S. inaequidens* and *S. jacobaea* is a likely cause of their similar
347 decay rates and interactions with *A. pratensis*. Although we cannot compare non-N-fixers and N-
348 fixers directly due to the constraints of our statistical analyses, non-additivity of mixtures
349 containing either non-N-fixers or N-fixers in Germany were of similar magnitude, consistent
350 with the minor differences in tissue chemistry between litters (Fig.1). This finding reinforces the
351 notion that plant traits are the primary driver of litter interactions independent of species origin
352 (Finerty et al. 2016). Therefore, other observations of strong impacts of invasive plant litter on
353 non-additivity (Poulette and Arthur 2012; Chen et al. 2013; Schuster and Dukes 2014) may be
354 more trait-based than origin-based.

355 For the species considered here, native and exotic species' litters did not consistently
356 differ in response to drought: litter interactions of both were negated by drought. Thus, exotic
357 species that rely on litter-feedback mechanisms (Ehrenfeld 2010; Poulette and Arthur 2012;
358 Schuster and Dukes 2014) may be disadvantaged compared to more conservative native species.
359 Although drought can lower biotic resistance and promote invasion (particularly of annual
360 species: Pfeifer-Meister et al. 2016) in some systems, many exotic species adopt more resource-
361 intensive growth strategies and require ample moisture to succeed (Cavaleri and Sack 2010).
362 Thus, drought directly reduces invasibility in many water-limited systems (Kreyling et al. 2008;
363 Jentsch et al. 2011). The impacts of drought reported here may further reduce invasibility by

364 limiting the amount of nutrients available post drought (Austin et al. 2004), thus slowing exotic
365 plant recovery even under wet conditions (Kinugasa et al. 2012). Future increases in the
366 frequency of drought could therefore slow nutrient cycling by negating litter interactions with
367 consequences for ecosystem structure and function.

368

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516 Table 1: Statistical output of single species analyses for *A. pratensis* and *Senecio* spp. (top) and
 517 N-fixers (bottom) mass remaining and N remaining relative to pre-treatment controls. Significant
 518 parameter values ($p < 0.05$) are bolded.
 519

Group	Parameter	df	Mass		N	
			F	p	F	p
<i>A. pratensis</i> and <i>Senecio</i> spp.	Species (S)	2,68	10.32	<0.01	27.11	<0.01
	Collection (C)	1,68	130.91	<0.01	145.22	<0.01
	Drought (D)	1,68	1.79	0.19	0.85	0.36
	Site (T)	1,68	49.45	<0.01	59.77	<0.01
	S x C	1,68	3.37	0.04	0.95	0.39
	S x D	2,68	1.05	0.36	0.95	0.39
	C x D	1,68	6.33	0.01	2.87	0.10
	S x T	2,68	10.14	<0.01	5.66	0.01
	C x T	1,68	2.89	0.09	1.22	0.27
	D x T	1,68	2.58	0.11	8.78	<0.01
	S x C x D	2,68	0.69	0.51	0.82	0.44
	S x C x T	2,68	2.18	0.12	2.11	0.13
	S x D x T	2,68	0.89	0.42	0.35	0.70
	C x D x T	1,68	0.95	0.33	1.78	0.19
	S x C x D x T	2,68	1.20	0.31	0.90	0.41
N-fixers	Species (S)	1,24	8.06	<0.01	0.04	0.85
	Collection (C)	1,24	259.69	<0.01	23.31	<0.01
	Drought (D)	1,24	4.04	0.06	0.34	0.56
	S x C	1,24	0.02	0.88	1.49	0.23
	S x D	1,24	0.10	0.75	0.04	0.85
	C x D	1,24	25.84	<0.01	2.29	0.14
	S x C x D	1,24	1.39	0.25	0.40	0.53

520
 521

522 Table 2: Statistical output of litter mixture analyses for Non-N-fixers (top) and N-fixers (bottom)
 523 non-additivity values of mass remaining and N remaining. Significant parameter values ($p < 0.05$)
 524 are bolded.
 525

Group	Parameter	df	<u>Mass</u>		<u>N</u>	
			F	p	F	p
Non-N-fixers	Species (S)	2,68	0.71	0.40	0.21	0.65
	Collection (C)	1,68	31.23	<0.01	9.78	<0.01
	Drought (D)	1,68	24.29	<0.01	20.64	<0.01
	Site (T)	1,68	58.63	<0.01	77.41	<0.01
	S x C	1,68	0.44	0.51	1.45	0.24
	S x D	2,68	0.85	0.36	1.63	0.21
	C x D	1,68	15.00	<0.01	18.75	<0.01
	S x T	2,68	0.44	0.51	2.97	0.09
	C x T	1,68	33.39	<0.01	65.68	<0.01
	D x T	1,68	8.59	0.01	10.42	<0.01
	S x C x D	2,68	2.08	0.16	0.42	0.52
	S x C x T	2,68	1.59	0.21	1.23	0.27
	S x D x T	2,68	0.40	0.53	0.10	0.74
	C x D x T	1,68	7.07	0.01	2.78	0.10
	S x C x D x T	2,68	2.25	0.14	1.84	0.18
N-fixers	Species (S)	1,24	2.33	0.14	4.97	0.04
	Collection (C)	1,24	264.00	0.12	0.19	0.67
	Drought (D)	1,24	9.01	0.01	12.85	<0.01
	S x C	1,24	4.27	0.05	1.03	0.32
	S x D	1,24	1.39	0.25	0.34	0.57
	C x D	1,24	5.12	0.03	1.24	0.28
	S x C x D	1,24	4.07	0.06	6.59	0.02

526
 527

528 Figure 1. Mean C:N ratios (\pm SE) of litter collected at each site by species (Ap: *A. pratensis*, Si:
529 *S. inaequidens*, Sj: *S. jacobaea*, Lp: *L. polyphyllus*, and Tp: *T. pratense*). Species identity is
530 categorized as native (open) or exotic (hashed). N-fixers are indicated by shading. Statistically
531 similar groups based on rank-transformed data are indicated by letters (a-c for Ap, Sj, and Si, and
532 x-y for Lp and Tp).

533

534 Figure 2. Mean mass remaining (\pm SE) of single species litter bags comprised of *A. pratensis*, *S.*
535 *inaequidens*, and *S. jacobaea*. (a; present in Germany and Belgium) or *L. polyphyllus* and *T.*
536 *pratense* (b; present in Germany only) under ambient (open) or drought (hashed) conditions after
537 86 and 180 days of decay. Values are relative to mean mass remaining in pre-treatment controls.
538 ** indicates $p < 0.01$. Non-significant interactions are not shown. Statistically similar groups
539 based on rank-transformed data are indicated by letters

540

541

542 Figure 3. Mean N remaining (\pm SE) of single species litter bags comprised of *A. pratensis* and
543 *Senecio* spp. (a; present in Germany and Belgium) or N-fixers (b; present in Germany only)
544 under ambient (open) or drought (hashed) conditions averaged over the experimental period.
545 Values are relative to mean N remaining in pre-treatment controls. NS indicates $p > 0.05$, **
546 indicates $p < 0.01$. Non-significant interactions are not shown. Statistically similar groups based
547 on rank-transformed data are indicated by letters

548

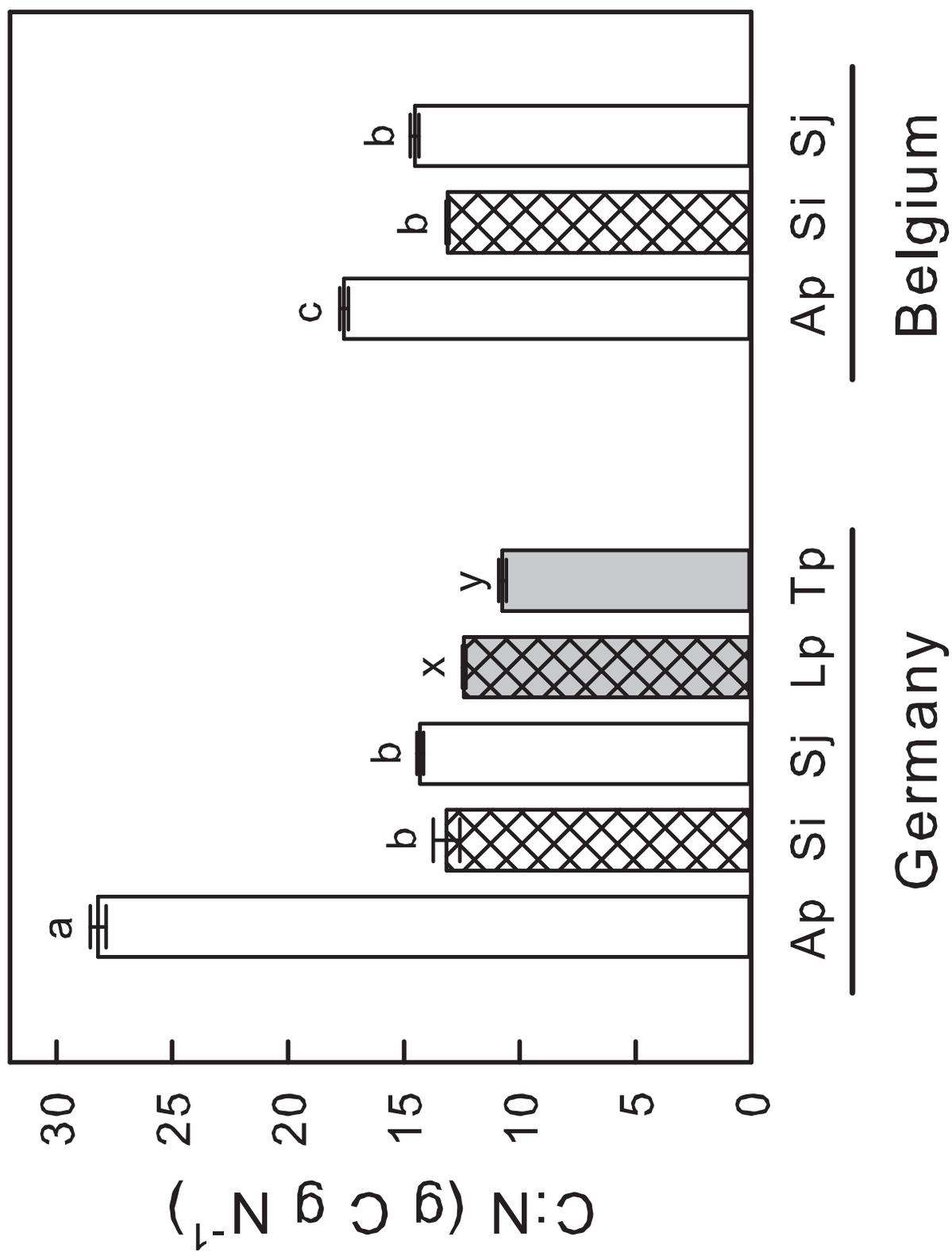
549 Figure 4. Mean non-additivity values (\pm 95% confidence interval) over the experimental period
550 for litter mixtures containing non-N-fixers (a,c) in Germany and Belgium or N-fixers (b,d) in

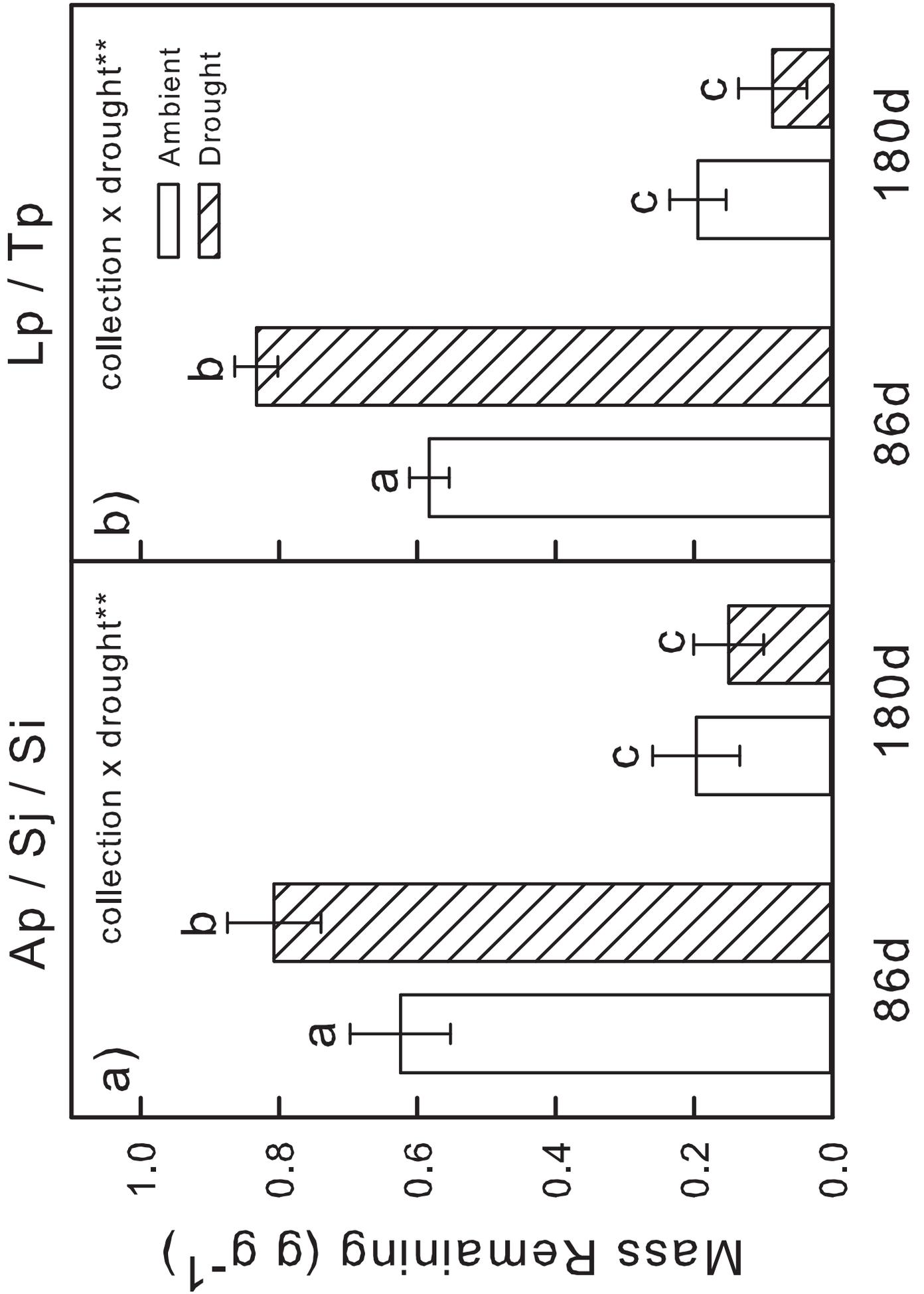
551 Germany that were decomposed under ambient conditions (open) or drought (closed). Values are
552 provided for mass remaining (a,b) and N remaining (c,d). Non-additive effects are significant if
553 their associated confidence interval does not include zero (dashed line). ** indicates $p < 0.01$.
554 Non-significant interactions are not shown.

555

556 Figure 5. Mean non-additivity values ($\pm 95\%$ confidence interval) for litter mixtures containing
557 non-N-fixers (a,c) in Germany and Belgium or N-fixers (b,d) in Germany that were decomposed
558 under ambient conditions (open) or drought (closed). Values are provided for mass remaining
559 (a,b) and N remaining (c,d) across all species of the relevant group (circles; a,b,c) or by species
560 (downward triangle: *L. polyphyllus*, upward triangle: *T. pratense*; d). Non-additive effects are
561 significant if their associated confidence interval does not include zero (dashed line). * indicates
562 $0.01 \leq p \leq 0.05$, ** indicates $p < 0.01$. Non-significant interactions are not shown. Statistically
563 similar groups based on rank-transformed data are indicated by letters

564





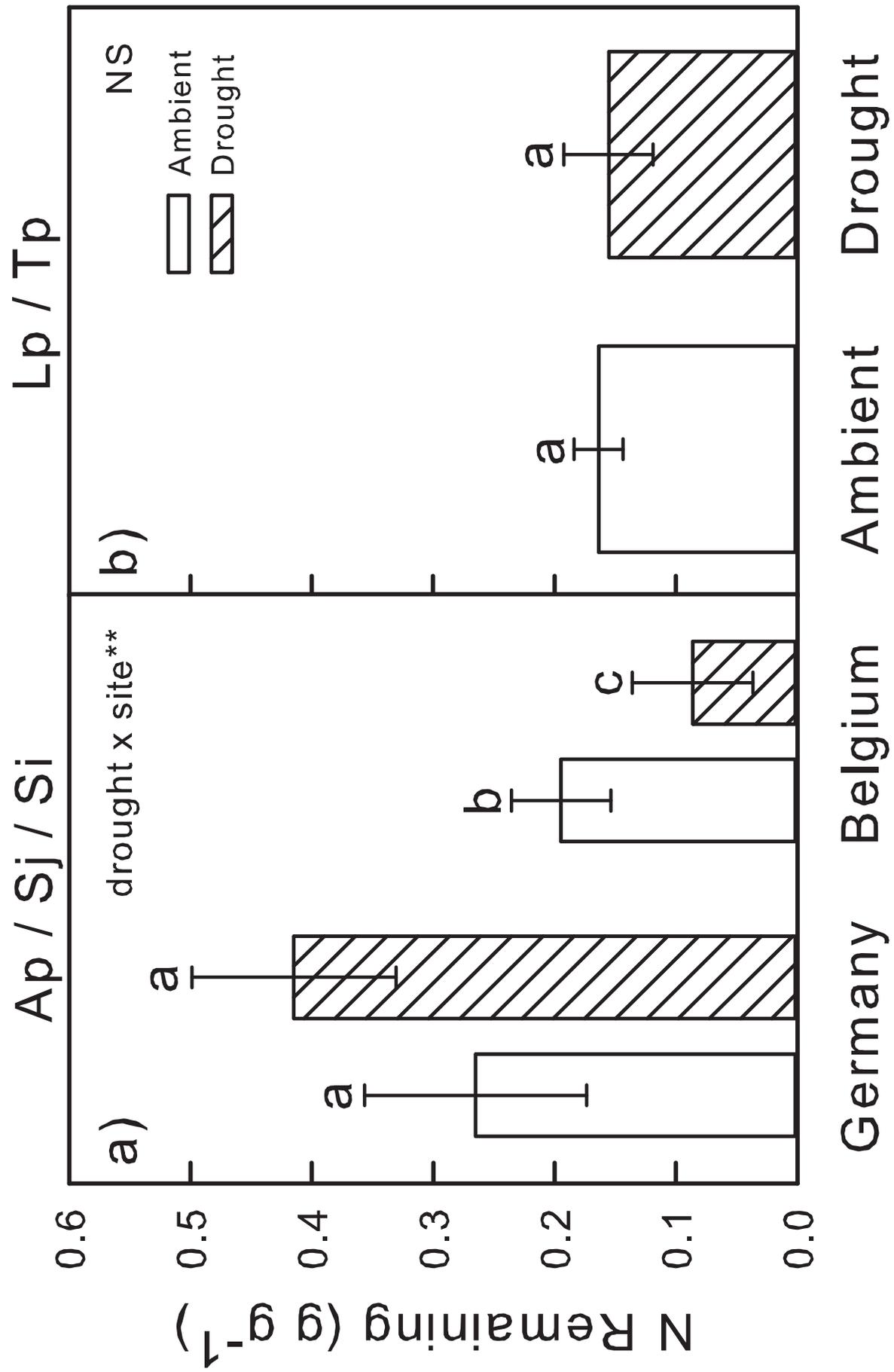
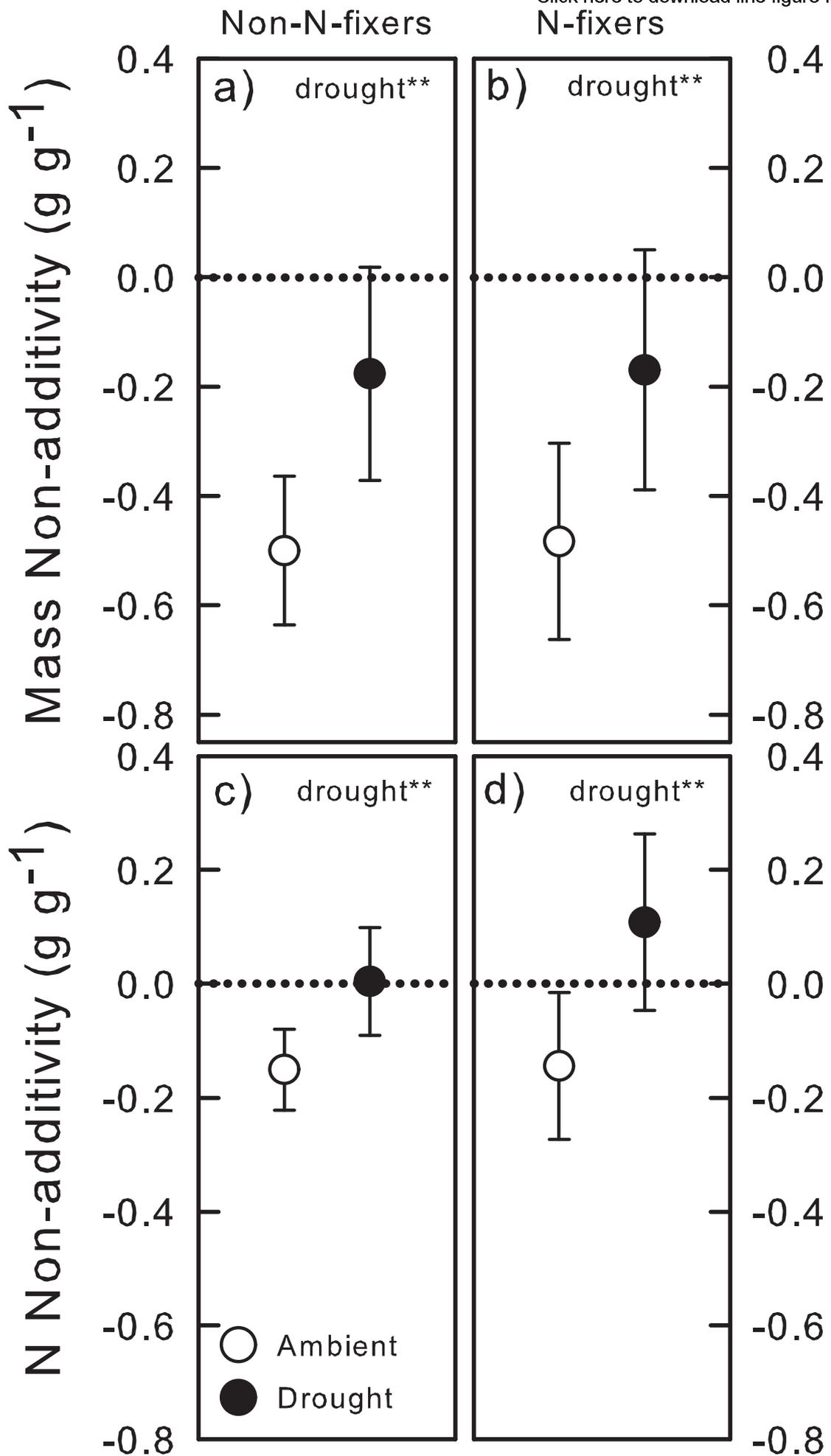
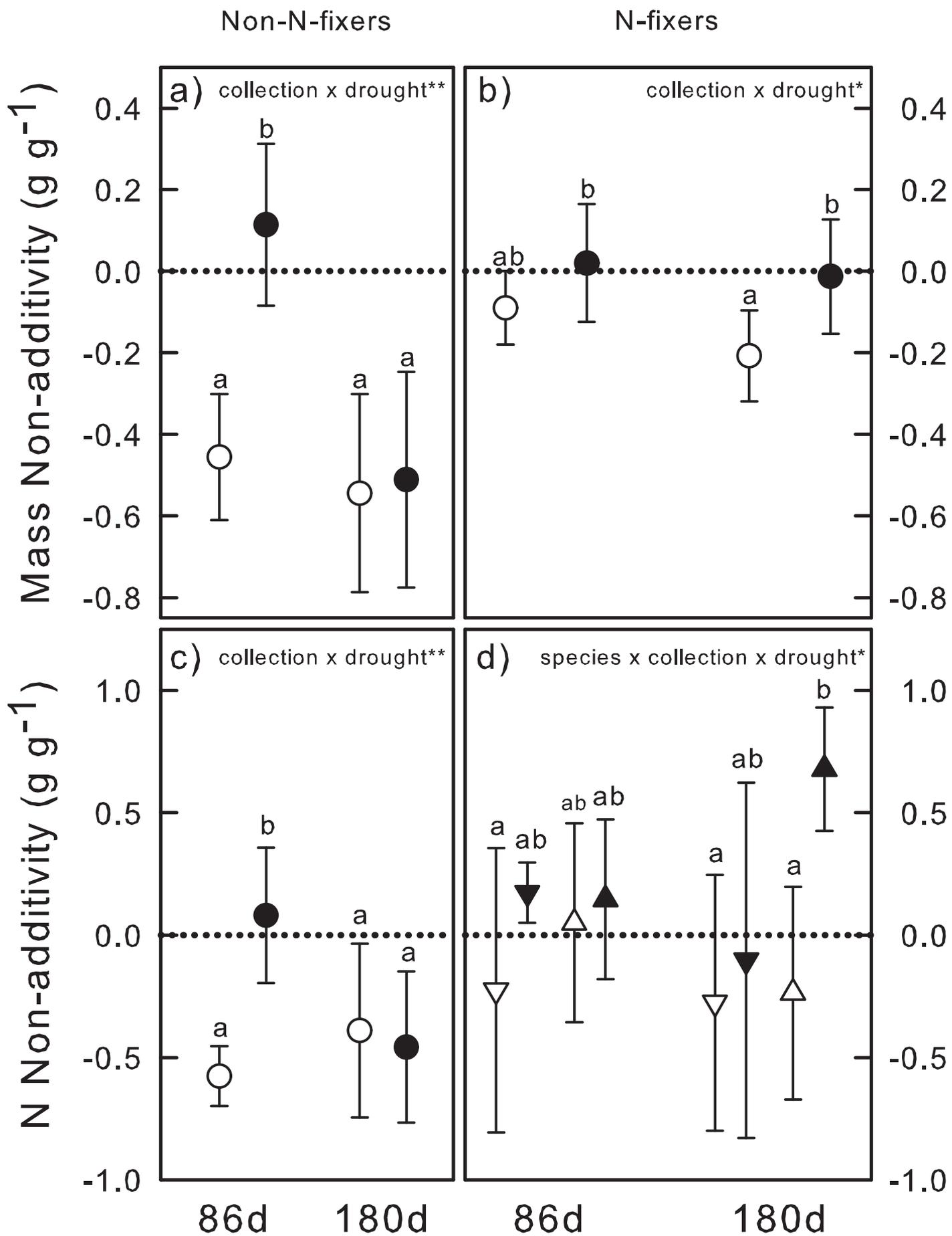


Fig. 4

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Online Resource

Title: Drought inhibits synergistic interactions of native and exotic litter mixtures during decomposition in temperate grasslands

Journal: Plant and Soil

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Table S1. Statistical output of initial litter C:N analyses for *A. pratensis* and *Senecio* spp. (top) and N-fixers (bottom). Differences between litters are tested among groups.

Group	Parameter	df	F	p
<i>A. pratensis</i> and <i>Senecio</i> spp.	Species (S)	2,15	28.24	<0.01
	Site (T)	1,15	0.47	0.50
	S x T	2,15	0.57	0.58
N-fixers	Species (S)	1,4	82.97	<0.01

Figure S2. Cumulative rainfall at each site (Germany: red, Belgium: green) over the experimental period. Equipment failure resulted in missing data for Germany during June and August.

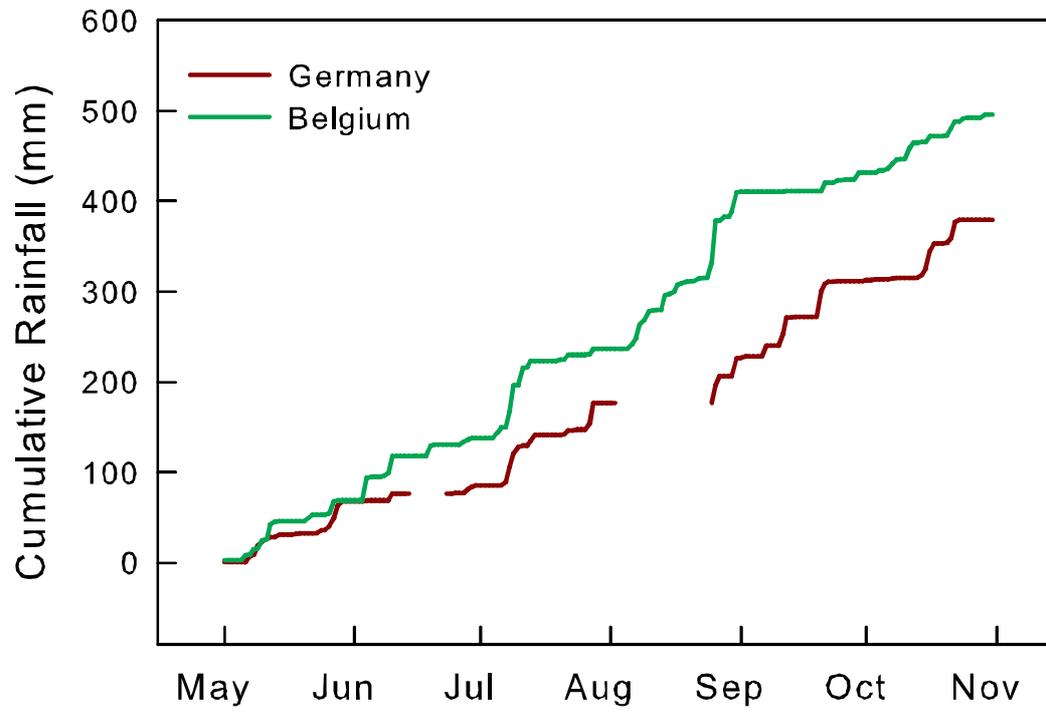


Figure S3. Example of a rainout shelter used at the SIGNAL experiment in Bayreuth, Germany.



Figure S4. Mean Volumetric Water Content of the top 5 cm of soil in ambient control (blue, n=3) and droughted areas (orange, n=2) at the Germany site based on EC-5 soil moisture probes (Decagon Devices, Pullman, WA, USA). Rainout shelters effectively reduced soil moisture over the course of the drought period (June and July), after which soil moisture levels equaled those of ambient control areas. Late-season soil moisture in droughted plots was lower than that in ambient control areas, consistent with depletion of deep soil moisture.

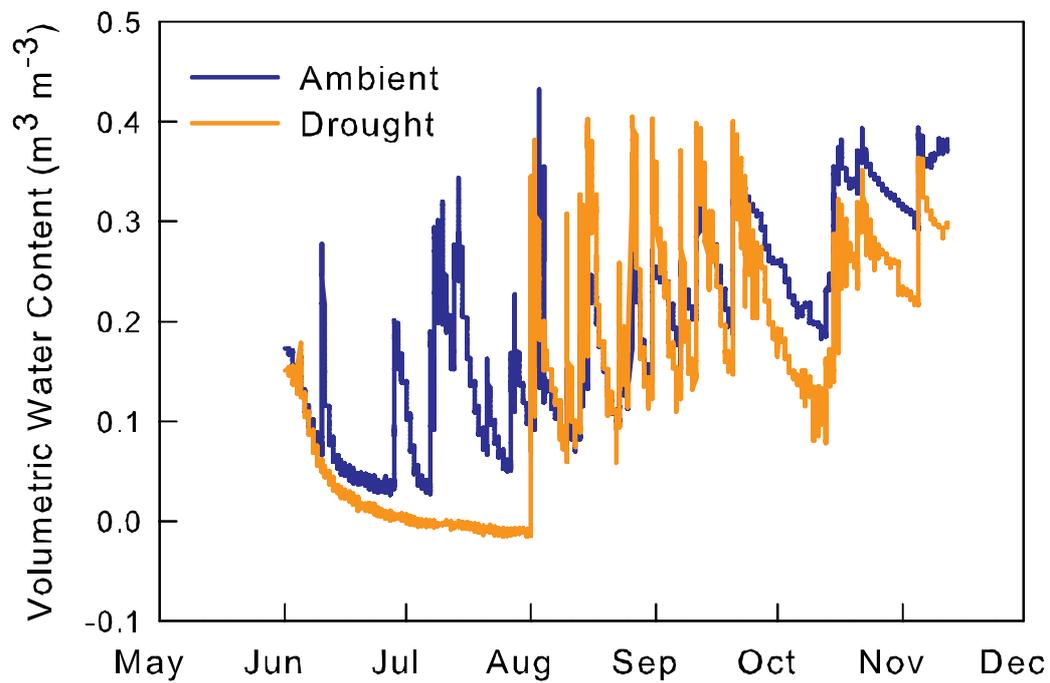


Figure S5. Observed mass and N remaining of single species litter bags deployed in Germany or Belgium. Values are means ($n=4$, \pm SE) for each species (Ap: *A. pratensis*, Si: *S. inaequidens*, Sj: *S. polyphyllus*, and Tp: *T. pratense*) either under ambient conditions (abbreviated “amb”); open or in plots that experienced an extreme mid-season drought (abbreviated “drght”; hashed).

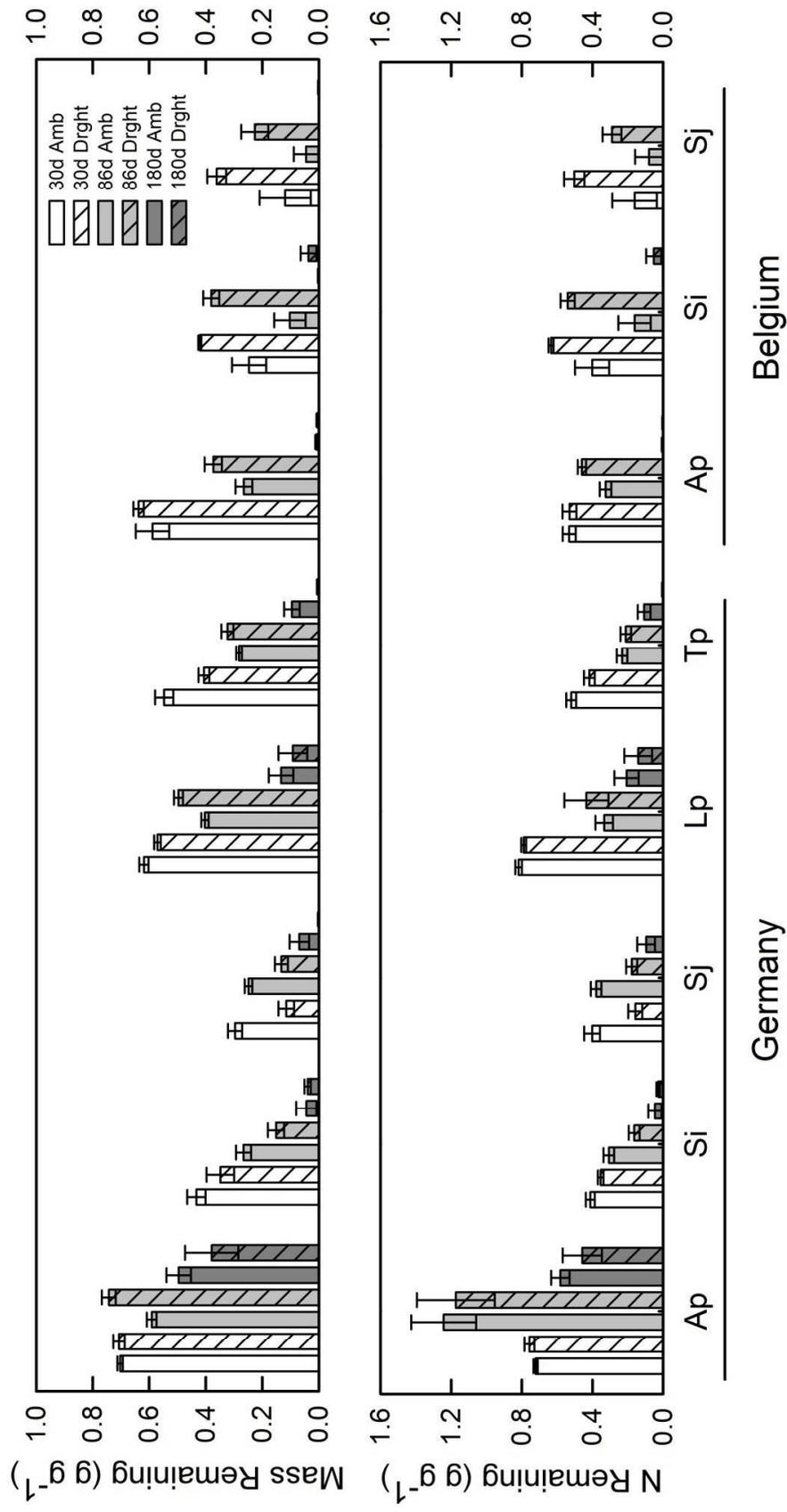


Figure S6. Mean(\pm SE) mass (a) and N (b) remaining in *A. pratensis*, *S. inaequidens*, and *S. jacobaea* single species litter bags relative to pre-treatment controls averaged across collections in Belgium and Germany. * indicates $0.01 < p \leq 0.05$, and ** indicates $p \leq 0.01$.

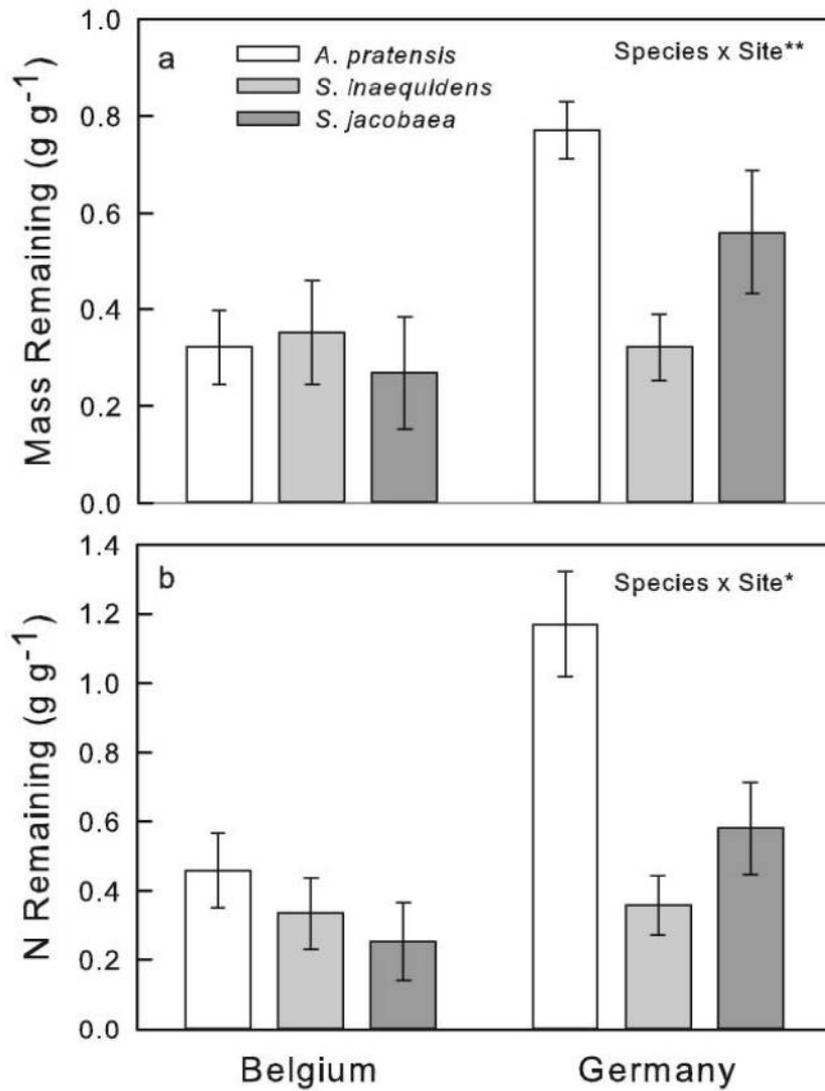


Figure S7. Mean (\pm SE) mass remaining of single species litter bags relative to pre-treatment controls at 86 and 180 days for *A. pratensis*, *S. inaequidens*, and *S. jacobaea*. * indicates $0.01 < p \leq 0.05$.

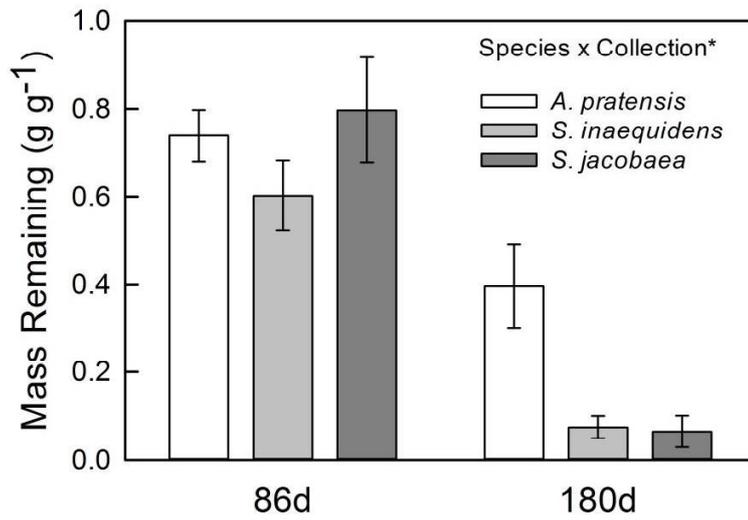


Figure S8. Mean non-additivity values (\pm 95% confidence interval) at 86 days (circles) and 180 days (squares) for litter mixtures containing non-N-fixers decomposed in Germany or Belgium. Values are provided for mass remaining (a) and N remaining (b). Non-additive effects are significant if their associated confidence interval does not include zero (dashed line). ** indicates $p \leq 0.01$.

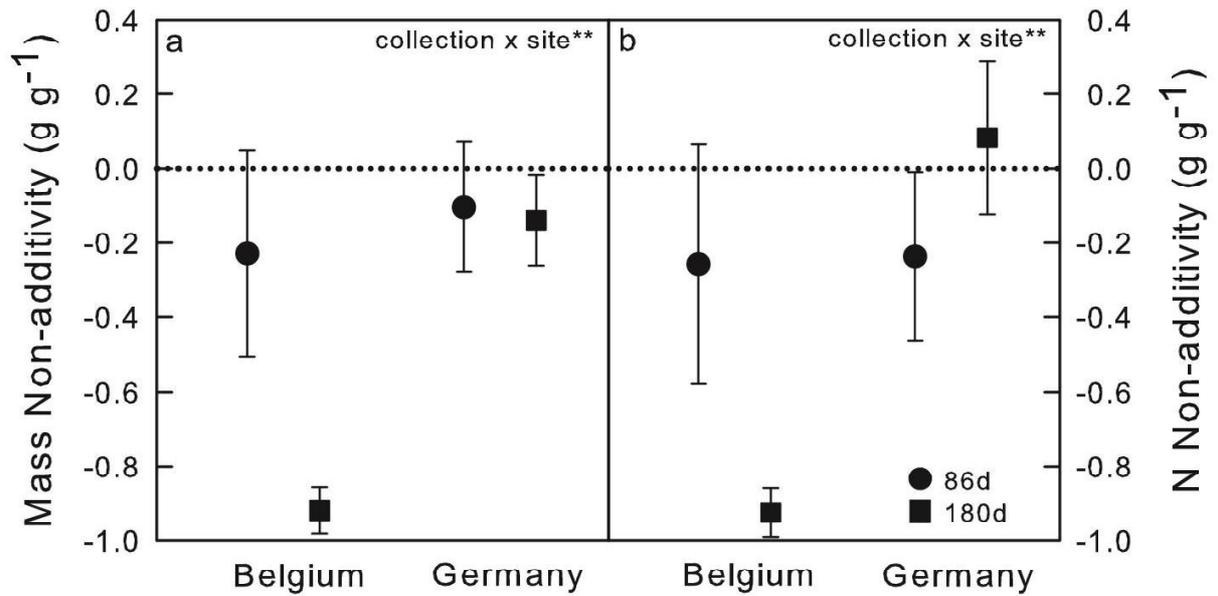


Figure S9. Mean non-additivity values (\pm 95% confidence interval) over the experimental period for litter mixtures containing non-N-fixers decomposed in Germany or Belgium under ambient conditions (open) or drought (closed). Values are provided for mass remaining (a) and N remaining (b). Non-additive effects are significant if their associated confidence interval does not include zero (dashed line). ** indicates $p \leq 0.01$.

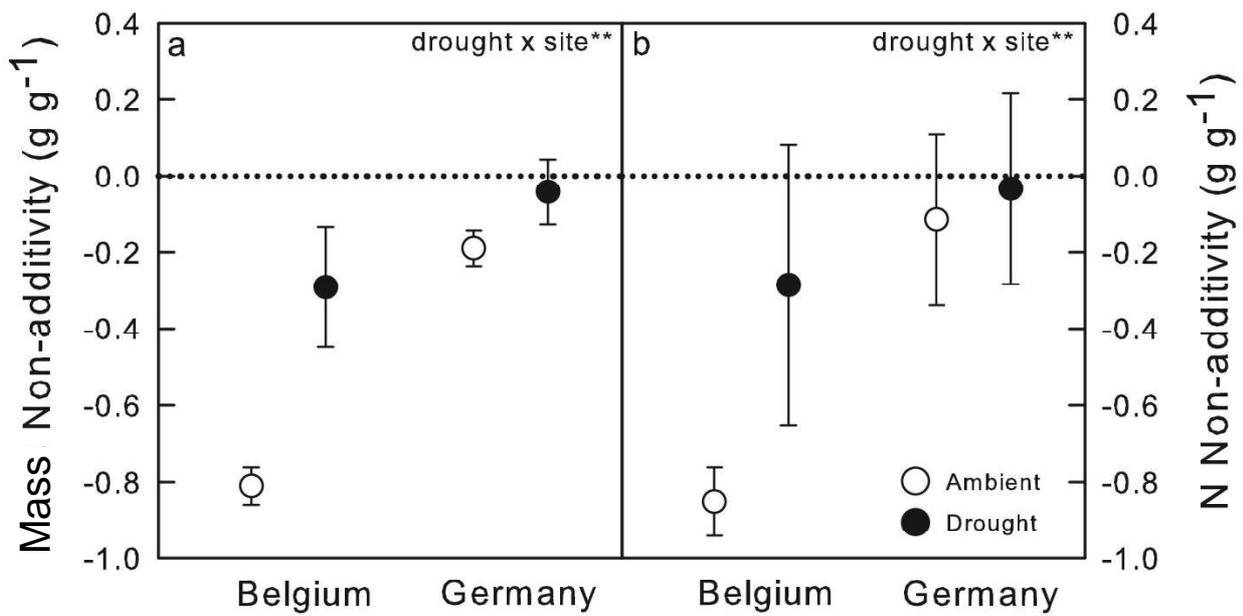


Figure S10. Mean mass non-additivity values (\pm 95% confidence interval) at 86 days (circles) and 180 days (squares) for litter mixtures containing non-N-fixers decomposed in Germany or Belgium under ambient conditions (open) or drought (closed). Non-additive effects are significant if their associated confidence interval does not include zero (dashed line). * indicates $0.01 < p \leq 0.05$.

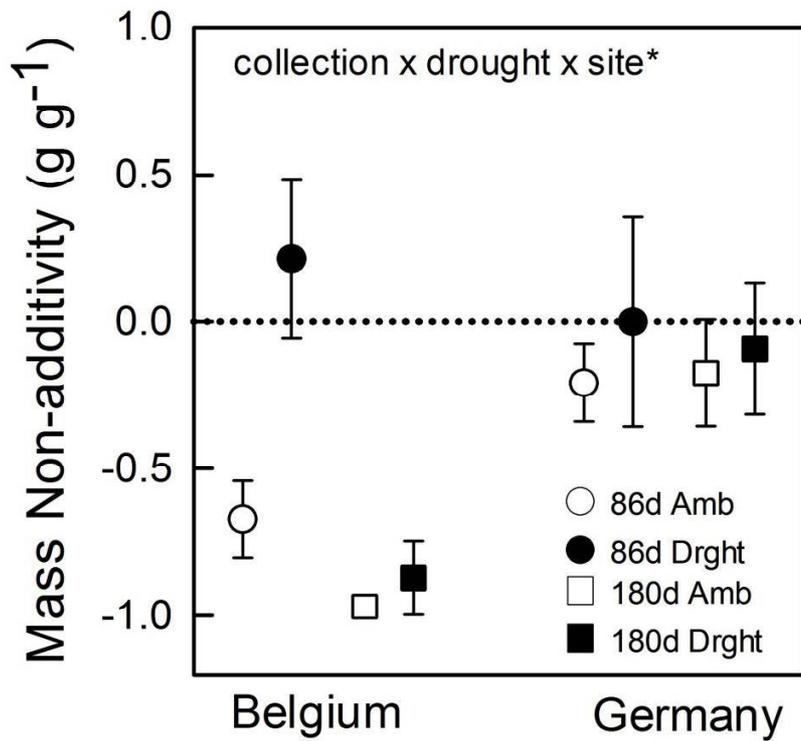


Figure S11. Mean mass non-additivity values (\pm 95% confidence interval) at 86 and 180 days for litter mixtures containing either *L. polyphyllus* or *T. pratense* decomposed in Germany. ** indicates $p \leq 0.01$.

