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Functional Ecology

Associational decomposition: after-life traits and interactions among decomposing litters control during-life aggregation of plant species

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Authors' contributions

L. Barbe, V. Jung, C. Mony and A. Prinzing conceived the experiments. L. Barbe performed the experiments, with help of V. Jung, C. Mony, L. Uroy and A. Prinzing. L. Barbe analyzed the data, with help of L. Uroy. L. Barbe wrote the manuscript, with help of V. Jung, C. Mony, L. Uroy and A. Prinzing.

Data accessibility statement

All data are available at: <https://doi.org/10.5281/zenodo.3733785>

Accepted Article

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13

14 **Abstract**

15 1. While species may coexist at population level, their individuals may still segregate, at
16 least temporarily, and hence interact little. Aggregation among heterospecific
17 individuals may require particular during-life traits, for example traits limiting
18 competition for space or resources. But is aggregation also facilitated by after-life
19 traits accelerating decomposition of plant litters, notably through synergy among co-
20 decomposing litters?

21 2. We investigated the role of leaf traits, litter traits and litter-mixture decomposition in
22 the spatial aggregation of individuals of pairs of grassland species in a long-term
23 mesocosm experiment. We related aggregation between pairs of species to mean and
24 dissimilarity in (i) during-life traits related to resource-acquisition and clonal
25 dispersal, (ii) after-life traits related to decomposition, and (iii) rate and synergy of
26 decomposition.

27 3. In most years, aggregation of heterospecific individuals strongly increased with means
28 of (i) during-life clonal traits reducing competition for space, and (ii) after-life traits

29 increasing litter decomposition. Trait dissimilarities had comparatively less influence.
30 Interestingly, in the last year, synergistic decomposition among litters improved
31 species aggregation.

32 4. Overall, after-life interactions due to fast and synergistic decomposition appear to
33 increase during-life aggregation between individuals of different plant species. We
34 introduce the concept of “associational decomposition”, equivalent to associational
35 resistance, but mediated via decomposers rather than enemies.

36
37 **Key-words:** after-life traits, associational litter decomposition, ecosystem functioning,
38 grasslands, litter mixing, non-additive effects, plant aggregation

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43 **Introduction**

44 While plant species may coexist at the population level, their individuals may still segregate,
45 at least temporarily, and hence interact little. For instance, growth behaviour of plants can
46 lead to drastic changes of interspecific aggregation among heterospecific individuals within a
47 year (e.g. Saiz *et al.*, 2016). The mechanisms of coexistence of heterospecific populations
48 have been extensively studied and conceptualised (e.g. Tilman 1982), but aggregation
49 mechanisms of heterospecific individuals have clearly found less attention (e.g. Herben &
50 Hara, 2003; Benot *et al.*, 2013). So far, aggregation among heterospecific individuals has
51 been related to during-life processes, notably to competition for resources and space. Two
52 aggregated plant individuals should strongly compete with one another for a shared set of
53 resources, which should result in the exclusion of one of both individuals, ultimately
54 explaining competitive exclusion of species (Gause, 1934). Consistently, the theory of
55 limiting similarity states that if individuals occupy sufficiently different niches, competition
56 should be reduced and coexistence possible (Schwilk & Ackerly, 2005; Violle *et al.*, 2011;
57 Wilson & Stubbs, 2012). The theory has recently been expanded to the way by which species
58 occupy space rather than use resources (Benot *et al.*, 2013, Saiz *et al.*, 2016). However, we

59 often observe high aggregation in some environments or in some clades whose species have
60 functionally similar traits, for example in grassland environments and in the grass lineage in
61 general (Cahill *et al.*, 2008; Prinzing *et al.*, 2016; Linder *et al.*, 2018). In these cases, niche or
62 space partitioning is unlikely to explain species aggregation and alternative mechanisms have
63 to be considered. Such alternative mechanisms promoting species aggregation may include
64 facilitative interactions, equalizing mechanisms of competition or negative plant-soil
65 feedbacks (Kulmatiski *et al.*, 2008; van der Putten *et al.*, 2013; Kardol *et al.*, 2015; Jiang *et*
66 *al.*, 2017).

67 Here we suggest that, in perennial plants, aggregation among heterospecific individuals might
68 also depend on after-life recycling of resources. In perennial plants, organs die and
69 decompose while the plant is still alive, so decomposition impacts the fitness of the plant.
70 Faster litter decomposition should increase soil nutrient availability (Swift *et al.*, 1979,
71 Hobbie, 1992, 2015) which might compensate for nutrient exploitation through competitors
72 and hence facilitate their spatial aggregation. Faster decomposition may also reduce
73 competition between microbial decomposers and root-associated microorganisms (Lindahl &
74 Tunlid, 2015; Fernandez & Kennedy, 2016), which might ultimately benefit both of the
75 competitors and prevent exclusion of either. Furthermore, fast decomposition might increase
76 aggregation by reducing the thickness of the litter layer, facilitating immigration and
77 emergence of other plant species (Carson & Peterson, 1990; Jensen & Gutekunst, 2003;
78 Weltzin *et al.*, 2005). Such fast decomposition of a mixture of two aggregated individuals can
79 be either due to high quality of the mixture or to synergistic interactions between the
80 decomposition of the two litters (*i.e.* when litter-mixture decomposition is faster than
81 expected from the decomposition of single species; Wardle *et al.*, 2003; Gartner & Cardon,
82 2004; Makkonen *et al.*, 2013). High quality of litter mixture can be due to after-life traits
83 increasing litter decomposability, for example high water holding capacity (WHC, Makkonen
84 *et al.*, 2013), low C:N ratio (Quested *et al.*, 2007) or low phenolics content (Hättenschwiler &
85 Vitousek, 2000), because moist and nitrogen-rich litters containing few refractory molecules
86 will stimulate the growth and activity of many decomposers. High quality of litter mixture
87 may also depend on during-life traits related to resource acquisition and conservation, since
88 dead tissues often inherit the properties of living tissues (Aerts, 1996): in particular, high
89 during-life SLA and low LDMC generally correspond to more nutrient-rich litter tissues for
90 detritivores and decomposers (Quested *et al.*, 2007; Santiago, 2007; Pakeman, Eastwood &
91 Scobie, 2011). Last, the dissimilarity of after-life and during-life traits may trigger synergistic

92 interactions during litter-mixture decomposition: more dissimilar traits provide a larger range
93 of resources for generalist detritivores and decomposers or for a larger range of specialist
94 decomposers, which are more efficient than when they decompose monospecific litters
95 (Gessner *et al.*, 2010; Tardif & Shipley, 2011; Barbe *et al.*, 2018). Overall, heterospecific
96 individuals producing a litter mixture of high quality (due to physical or chemical traits
97 ensuring high decomposability) or a litter mixture which decomposes faster due to after-life
98 synergistic interactions, might aggregate spatially (Fig. 1).

99 Spatial aggregation due to during-life mechanisms has been shown to depend on "during-life"
100 traits controlling resource acquisition and clonality, and on their dissimilarity among species.
101 Resource-acquisition traits may be foliar traits such as high specific leaf area (SLA) or low
102 leaf dry matter content (LDMC), driving plant ability to uptake light and soil resources
103 (Santiago, 2007; Violle *et al.*, 2009). Plant species with dissimilar resource-acquisition traits
104 can occupy and exploit different niches, thereby permitting species aggregation (Schwilk &
105 Ackerly, 2005; Violle *et al.*, 2011; Wilson & Stubbs, 2012). Also, two plant species of low
106 resource acquisition capacities (for example, low SLA) might more easily aggregate than two
107 that have high capacities. Other during-life traits of importance are clonal-dispersal traits, for
108 instance the number of connections between ramets (*i.e.* branching degree) and the spacer
109 length, representing an important part of the strategy of plants for colonizing space (Lovett
110 Doust, 1981). Plant species having dissimilar clonal-dispersal traits, or values of clonal-
111 dispersal traits leading to a scattered or a sparse space colonization, may more easily
112 aggregate locally. In particular, plants having a low number of connections between ramets,
113 or a high spacer length may much more aggregate with other plants (Benot *et al.*, 2013; Saiz
114 *et al.*, 2016). Overall, the role of during-life traits for species aggregation has already been
115 widely documented.

116 In this study, we investigated the role of during-life traits, after-life traits and after-life
117 interactions during decomposition for the spatial aggregation of pairs of grassland species.
118 We tested the hypotheses that aggregation between two plant species may be favoured by: (i)
119 during-life traits that are dissimilar or that decrease competitiveness, (ii) during-life and after-
120 life traits favouring litter decomposition, and (iii) synergistic effects during litter-mixture
121 decomposition. We used a long-term mesocosm experiment to measure the aggregation of 27
122 pairs of grassland species, from a pool of the 8 more abundant perennial species of the
123 experiment. We focused on individual-scale aggregation driven by growth behaviour of plant
124 individuals: we recorded patterns at the spatiotemporal scale of individuals (5 x 5 cm),

125 repeated four times in 2-year intervals, and kept population-level processes constant as far as
126 possible by preventing seed production and immigration. We measured for each species pair
127 the trait mean and trait dissimilarity in number of connections between ramets, spacer length,
128 SLA, LDMC, WHC, and C:N ratio. We also accounted for the phylogenetic distance between
129 both species, as this distance may illustrate a dissimilarity in traits we did not measure
130 (Cadotte *et al.*, 2010; Pan *et al.*, 2015). We conceived a litter mixture for each pair of species,
131 and we measured litter-mixture decomposition per se as well as synergistic or antagonistic
132 effects during litter-mixture decomposition. We also explored which of the traits increase
133 decomposition of single-species or mixed litters and which render litter mixture
134 decomposition synergistic, and compared these traits to those influencing aggregation.

135 **Material and Methods**

136 *Study site and experimental design*

137 We used a long-term mesocosm experiment at the University of Rennes 1 (Western France,
138 48°06'58.6"N 1°38'15.5"W). This experiment was set up in 2009 and consists of mesocosms
139 (1.30 x 1.30 m, 0.25 m height) which contain seven different mixtures of grassland species,
140 each one replicated 10 times. Mixtures have three levels of species richness (4, 8, and 12)
141 with three distinct species compositions for each level (except for the 12-species level, which
142 has only one species composition: all the species involved in the experiment). The
143 composition and number of species sown in a given mesocosm in 2009 mimic natural
144 communities common to grassland ecosystems of our study region. However, we stress that
145 initial richnesses had often declined through time and that mesocosm-level composition and
146 richness are uninformative about the richness and composition in a given 5x5cm or 10x10cm
147 grid used further to analyse co-occurrence. For any level of mesocosm-level species richness,
148 local functional and phylogenetic dissimilarities within a given grid cell could be high and
149 low. Mesocosm soil was composed of 80% garden soil and 20% sand (i.e. a realistic
150 proportion compared to the soil of natural habitats of the study species, and a proportion
151 common to mesocosm experiments involving clonal plants, see Birch & Hutchings 1994),
152 and was homogenized before mesocosm construction. At the beginning of the experiment, we
153 applied a mineral fertilizer with slow release (N/P/K: 20/5/10) to fully homogenize the soil
154 nutrient content among mesocosms. Mean soil nutrient properties were initially: $[\text{NO}_3^-] =$
155 $34.4 \mu\text{g/g}$; $[\text{P}] = 0.37 \mu\text{g/g}$; $[\text{PO}_4^{2-}] = 40.3 \mu\text{g/g}$; C:N ratio = 7.21. Mesocosms were located
156 outside, in a microclimatically homogeneous garden, and were watered by rain during most

157 of the year, with an additional punctual artificial watering during dry summer weeks.
158 Mesocosms were mowed once a year and plant material was exported to mimic semi-natural
159 grasslands, but grassland species grow and produce litter rather continuously through the year
160 and so decomposition of a large quantity of litter took place in situ. We prevented sexual
161 reproduction (by cutting flowers) and immigration (by manual removal of immigrant species)
162 in mesocosms, to restrict as far as possible processes driving aggregation to individual clonal-
163 growth behaviour. We planted 12 perennial, clonal species with different growth behaviours
164 and clonal strategies: *Agrostis stolonifera* (L.), *Agrostis tenuis* (L.), *Brachypodium pinnatum*
165 (L.), *Centaurea nigra* (L.), *Chamaemelum nobile* (L.), *Dactylis glomerata* (L.), *Elytrigia*
166 *repens* (L.), *Festuca rubra* (L.), *Holcus lanatus* (L.), *Holcus mollis* (L.), *Lolium perenne* (L.)
167 and *Ranunculus repens* (M.). In each mixture, planted species were initially in equivalent
168 proportions and were randomly positionned. Among the 12 species, we selected the 8 most
169 abundant species: *Brachypodium pinnatum*, *Centaurea nigra*, *Dactylis glomerata*, *Elytrigia*
170 *repens*, *Festuca rubra*, *Holcus lanatus*, *Holcus mollis*, and *Ranunculus repens*. From these 8
171 species, we selected all possible pairs of species in order to obtain an important range of trait
172 means and trait dissimilarities among pairs. We excluded the pair *Centaurea nigra*-
173 *Ranunculus repens*, as it was the only pair of the experiment involving two dicotyledon
174 species, which would have hence generated an outlier in each analysis including phylogenetic
175 distance.

176 *Plant mapping and measures of aggregation*

177 We mapped plant species distribution in each mesocosm in 2010, 2012, 2014 and 2016. We
178 used an 80 x 80 cm square grid of 5-cm cell size (256 cells per grid), and we noted for each
179 cell the presence of each rooted species. In each mesocosm, we calculated for each pair
180 present in this mesocosm the co-occurrence observed between both species (COobs), as the
181 number of cells where both species were present together. We also calculated the expected
182 co-occurrence between both species (COexp), as: $CO_{exp_{i,j}} = A_i \times A_j / 256$, where A_i is the
183 number of cells (*i.e.* abundance) occupied by species i and A_j is the number of cells occupied
184 by species j . Based on observed and expected co-occurrence, we calculated the spatial
185 aggregation between both species ($C_{i,j}$) using a spatial aggregation index, following Saiz *et*
186 *al.* (2016): $C_{i,j} = \frac{CO_{obs_{i,j}} - CO_{exp_{i,j}}}{CO_{exp_{i,j}}}$, where $CO_{obs_{i,j}}$ is the observed co-occurrence between
187 species i and j and $CO_{exp_{i,j}}$ the expected co-occurrence between species i and j . Negative
188 values of $C_{i,j}$ indicate that two species aggregate less than expected at random (*i.e.* spatial

189 segregation between species) while positive values indicate that two species aggregate more
190 than expected at random (*i.e.* spatial aggregation between species). Given that initial
191 plantation was at random, any later increases or decreases of co-occurrence are due to
192 aggregation or segregation among species. A pattern of co-occurrence is the outcome of a
193 process of co-occurrence in the previous year (we refrained from calculating differences
194 between years since this would basically be an index of indices and hence potentially little
195 robust). To avoid idiosyncrasies of individual mesocosms, we calculated for each species pair
196 the average aggregation across mesocosms (Fig. S1). We finally repeated the above
197 procedure pooling grids into 10 x 10 cm grids. Consistent with Saiz *et al.* (2016), we found
198 major year-to-year variation of aggregation between two species (Fig. S1).

199 *Functional trait measurements and calculations*

200 We measured for the 8 species two during-life traits related to clonal dispersal (number of
201 connections and spacer length), two during-life traits related to resource acquisition (SLA and
202 LDMC), and two after-life traits central to litter decomposition (WHC and C:N ratio). WHC
203 mainly determines the litter microclimate (Makkonen *et al.*, 2013) whereas C:N ratio is
204 related to the litter nutrient content (Pérez-Harguindeguy *et al.*, 2000; Qusted *et al.*, 2007).
205 SLA and LDMC, representing respectively the thinness of green leaves and their palatability,
206 are also involved in the litter decomposition process (Cornelissen & Thompson, 1997;
207 Qusted *et al.*, 2007; Santiago, 2007). We measured SLA, LDMC, WHC and C:N ratio on 5
208 different individuals, collected in different mesocosms, to obtain a representative mean value
209 for each species and each trait. We measured SLA and LDMC on green leaves according to
210 the protocols of Pérez-Harguindeguy *et al.* (2013). We measured WHC and C:N ratio on
211 naturally senesced leaves (note that WHC is simply the inverse of LDMC, for litter). 5-10
212 dead leaves were immersed in distilled water during 24h, drained and weight, then dried at
213 65°C during 48h and weighed again. WHC was calculated as water-saturated weight / dry
214 weight. We measured C:N ratio on 1-5 dead leaves for each of the 5 selected individuals,
215 using an elemental analyzer (FLASH EA 1112 Thermo Finnigan, Waltham, Massachusetts,
216 USA). We measured the number of connexions and the spacer length of each species on 10
217 different individuals grown in controlled pots (*i.e.* one individual per pot) to obtain a
218 representative mean value of these traits without competition (Benot *et al.*, 2013), since these
219 traits are particularly responsive to competition and clonality must be measured
220 independently from competition. Trait distributions are summarized in Table 1, and trait
221 correlations in Table S1. For each species pair, we calculated the dissimilarity in each of the 6

222 measured functional traits, as the absolute value of the difference between both per-species
223 means for the considered trait.

224 *Characterization of phylogenetic distance*

225 We measured phylogenetic distance for each species pair, using a dated tree of the Dutch
226 flora based on rbcL DNA sequences (see Hermant *et al.*, 2012) and containing all species of
227 our study. The authors used dated molecular phylogenies as a fixed age constraint for
228 estimating the age of the crown node of Angiospermae. For each species pair, we calculated
229 the patristic distance, which is the distance among both species (path-length distance) along
230 branches of tree from one species to the other one, divided by two. This distance represents
231 hence the age of the species' most recent common ancestor. Calculations were made using the
232 software Mesquite (Maddison & Maddison 2017).

233 *Litter decomposition and non-additive effects*

234 For the 8 species, we collected in autumn 2009 naturally-senesced leaf litter from one to
235 several spatially-proximate individuals in the mesocosm experiment. Litter was air-dried, and
236 we conceived from one to three litter mixtures for each of the 27 species pairs. Combinations
237 of individual species pairs were replicated two or three times (and averages calculated across
238 replications), except for those involving the two species for which the amount of litter
239 material collected was too low (*Brachypodium pinnatum* and *Festuca rubra*). Both species
240 composing litter mixtures were in roughly equivalent proportions (50-50) and we noted their
241 precise proportion. Litter mixtures were placed into 8 x 8 cm litterbags, which had 5 mm
242 mesh on their lower side to allow decomposers to freely access the litter, and 2 mm mesh on
243 their upper side to avoid contamination by allochthonous litter. Each litterbag contained 1g of
244 litter (oven-dried equivalent). We exposed each litter mixture in a randomly chosen
245 mesocosm in February 2015 (fresh litter is available during that period in the study region),
246 and started the decomposition experiment. Exposing at random ensured that observed
247 decomposition was not biased by either the effect of the species providing the litter mixture
248 on soil conditions, nor particular adaptations of decomposers to local litter (Austin *et al.*,
249 2014). To calculate synergistic or antagonistic effects during litter-mixture decomposition,
250 we also exposed near each litter mixture two litterbags containing the corresponding mono-
251 specific litters alone. These litterbags also contained 1g of litter (oven-dried equivalent) and
252 had meshes identical to those of litter-mixture litterbags. We collected all litterbags 6 weeks
253 later, when they reached 30-60% mass loss. Such high mass loss results from the humid and

254 relatively warm, frost-free winter in the study region which increases decomposer activity
255 (Barbe *et al.*, 2018). We cleaned the litter by hand and oven-dried it for three days at 65°C.
256 We calculated mass loss (%) as $1 - (m_1/m_0) \times 100$, where m_1 is the oven-dry weight at
257 collection and m_0 the initial oven-dry equivalent dry weight. Thereafter we refer to
258 decomposition as this mass loss after 6 weeks of exposition. We calculated the expected
259 decomposition of litter mixtures, as the mean decomposition between the two mono-specific
260 litters weighted by the precise proportion of the two species in the litter mixture.

261 We calculated non-additive effects (NAE) of litter-mixture decomposition as $NAE = (O - E) /$
262 E , where O is the observed decomposition of litter mixture and E is the expected
263 decomposition of litter mixture. Litter-mixture decomposition could be additive ($NAE = 0$,
264 *i.e.* no significant difference between observed and expected decomposition), synergistic
265 ($NAE > 0$, *i.e.* observed decomposition higher than expected), or antagonistic ($NAE < 0$, *i.e.*
266 observed decomposition lower than expected; see Wardle *et al.*, 1997). See Fig. S2 for mean
267 observed decomposition of species pairs, and Fig. S3 for mean decomposition of species
268 alone.

269 *Statistical analyses*

270 Prior to analyses, we centered-reduced all independent and dependent variables (that is, for
271 each value of the variable, subtracting the mean of the variable and dividing the difference by
272 the standard-deviation of the variable) to permit comparisons of regression coefficients
273 among and within models. We used multiple ordinary least square (OLS) regression models
274 to evaluate the role of during and after-life traits, as well as phylogenetic distance, on the
275 decomposition of single litters, litter mixtures and on non-additive effects during
276 decomposition. We built all possible models (*i.e.* all possible combinations of independent
277 variables) and we performed a best subset search to select the best model based on AIC and
278 BIC (Burnham & Anderson 2002; Chen & Chen 2008). We graphically explored residuals
279 using QQ and predicted-vs-residual plots and we excluded two residual outliers (caused by
280 extreme positive values of non-additive effects) to fulfill the assumption of normality and
281 homogeneity of residuals.

282 We used multiple OLS regression models to explain aggregation of species pairs by the
283 means and dissimilarities in their traits related to (i) during-life clonal dispersal, (ii) during-
284 life resource acquisition, (iii) after-life litter decomposition, and by (iv) after-life non-additive
285 effects during decomposition and (v) phylogenetic distance between both species. This

286 approach was applied separately for each of the four dates (2010, 2012, 2014, 2016). For
287 each date, we constructed and selected the best model, according to the method explained
288 above. We also tested aggregation against each of (i), (ii) and (iii+iv) separately (Table S2),
289 and found the power and robustness of these models to be always lower than those of the full
290 model. We hence only present the full model in the body of the manuscript. We did these
291 analyses for aggregations based on both 5x5 and 10x10cm grids. Both analyses led to similar
292 major conclusions (Fig. S1 and Table S3), and therefore we only present the results based on
293 the finer grained grid in the Results. All statistical analyses were performed with R 3.0.3
294 software (R Development Core Team, 2016).

295 **Results**

296 *Effect of during-life and after-life traits on the litter decomposition and decomposition* 297 *synergy*

298 Mean decomposition of single litters (*i.e.* that had decomposed in isolation) and
299 decomposition of litter mixtures were strongly explained by during-life and after-life traits
300 (Table 2). In particular, decomposition of single litters was positively related to WHC,
301 LDMC and C:N ratio, and negatively related to SLA (Table 2, Fig. S4). Litter-mixture
302 decomposition was positively related to WHC and negatively related to SLA, and also
303 positively related to phylogenetic distance (Table 2). Non-additive effects during
304 decomposition were less explained (Table 2), and decomposition was synergistic among
305 species of low WHC. Overall, trait means had a strong influence on the decomposition
306 process, whereas dissimilarities had no effect. Non-additive effects were mainly positive,
307 from -8.7% (antagonism) to +64.8% (synergy). The mean of non-additive effects was overall
308 +14% synergy and was significantly larger than 0 ($t = 3.8$, $P = 8.10^{-4}$, $df = 24$).

309 *Effect of during-life traits on plant aggregation*

310 Aggregation was strongly related to functional traits of species pairs across the four dates of
311 the experiment (adjusted- R^2 ranging from 0.46 to 0.61, see Table 3). In particular, means of
312 during-life traits related to clonal dispersal and resource acquisition impacted aggregation.
313 Specifically, aggregation was positively related to mean number of connections per ramet in
314 2010 and 2012 and spacer mean length in 2010 and 2014 (Table. 3). High SLA was
315 negatively related to aggregation in 2010, 2012, and 2014, while high LDMC was in relation
316 to high aggregation at these dates. Interestingly, these traits related to resource acquisition
317 and retention were also associated to litter decomposition (Table 2). Similar to

318 decomposition, we observed that trait dissimilarities had a much smaller effect on
319 aggregation than trait means: only dissimilarity in LDMC was negatively related to
320 aggregation in 2014 and dissimilarity in number of connections per ramet were found to
321 positively relate to aggregation in 2014 (Table 3).

322 *Effects of after-life traits and synergistic decomposition on plant aggregation*

323 After-life traits strongly explained aggregation at most dates of the experiment. Similar to the
324 effects of during-life traits on aggregation, aggregation was related to after-life trait means
325 rather than dissimilarities (Table 3). Specifically, a high WHC and high C:N of litter mixtures
326 were positively related to aggregation in 2010, 2012 and 2014 (Fig. 2). These traits also
327 favoured litter decomposition (Table 2, Fig. S4). Moreover, non-additive effects of litter-
328 mixture decomposition were associated with aggregation at the most recent date of the
329 experiment: in 2016, aggregation increased with synergistic litter-mixture decomposition
330 (Table 3, Fig. 3). Besides, aggregation increased with phylogenetic distance between species
331 in 2012 (Table 3).

332 **Discussion**

333 We observed that plant aggregation was associated to both during-life and after-life traits,
334 validating our initial hypotheses. However, trait means rather than trait dissimilarities
335 explained aggregation, partially contradicting our expectations. Overall, we observed that the
336 after-life traits that were positively related to aggregation were also positively related to litter
337 decomposition. Interestingly, plant aggregation was also positively related to synergistic
338 litter-mixture decomposition in the last year of the experiment.

339 *Trait means that affect resource and space use also affect plant aggregation*

340 Means of during-life traits known to affect resource acquisition and clonal dispersal were
341 strong drivers of species aggregation during most years of the experiment. Consistent with
342 our expectation, high aggregation was correlated with low mean SLA and with high mean
343 LDMC, *i.e.* traits which might reduce resource acquisition and hence competition between
344 species (Violle *et al.*, 2009). Moreover, aggregation increased with high spacer mean length
345 and high number of connections between ramets. These trait means facilitate mixing of
346 ramets among individuals and hence species without competitive replacement, contrary to a
347 more densely-aggregated growth (Benot *et al.*, 2013). Compared to trait means, dissimilarity
348 in traits were only little associated to aggregation: aggregation increased with dissimilarity in

349 LDMC and in number of connections in 2014, and phylogenetic distance in 2012. This
350 relatively weak effect of trait (or phylogenetic) dissimilarities suggests that in the present
351 experiment aggregation was less due to niche differentiation, and more due to competitive
352 hierarchies (Kraft *et al.*, 2014).

353 *Trait means accelerating litter decomposition promote plant aggregation*

354 All traits that we found to increase decomposition were also positively related to aggregation
355 between species. Specifically, in the decomposition experiment, high decomposition was
356 associated to low SLA and high LDMC during life, and high WHC and high C:N after life -
357 and high aggregation was associated to each of these trait values in most years of the
358 experiment. Here again, trait means rather than dissimilarities were important, and we can
359 speculate that this indicates that detritivores and decomposers might have required particular
360 nutrient contents and abiotic conditions controlled by these traits, rather than diverse
361 resources and habitats. Litters with high WHC probably maintained favourable microclimate
362 for decomposers, thereby increasing litter decomposition (consistent with Makkonen *et al.*,
363 2012, 2013). In contrast, the increase of decomposition with decrease in SLA, and increase in
364 LDMC or C:N is surprising as these trait states are considered to relate to decreased
365 nutritional quality of litter for detritivores (Quested *et al.*, 2007; Santiago, 2007). Possibly,
366 poor litter quality triggered compensatory feeding of detritivores to ensure sufficient resource
367 assimilation (Gessner *et al.*, 2010) and thereby increased decomposition. Such compensatory
368 feeding would be particularly plausible in our system where annual mowing and export of
369 biomass gradually depleted nutrient availability for soil organisms, resulting in higher
370 consumption of litter with low nutritional quality. Regardless of the mechanisms involved,
371 traits related to fast litter decomposition promoted during-life aggregation between the plant
372 species that continuously produce this litter. In sessile, perennial organisms like plants, after-
373 life traits affecting after-life processes can hence strongly impact during-life interactions, via
374 an extended decomposition phenotype: decomposition of a plant litter ultimately driven by
375 the plant's genotype and contributing to the plant's fitness through modification of above- and
376 below-ground conditions (Terhorst & Zee, 2016). Future studies might scale up to population
377 level and test whether after-life decomposition also favours coexistence at that level.

378 *Associational decomposition, a mechanism of coexistence of heterospecific individuals*

379 Between years we observed major changes to aggregation among species, and the
380 aggregation in the last year could be explained by after-life synergistic decomposition of

381 coexisting litters. Our results demonstrated a new kind of biotic interaction promoting plant
382 aggregation through synergistic decomposition. These after-life interactions among plants
383 could represent a dynamic "litter-interaction filter" for plant community assembly, equivalent
384 to other biotic interaction filters that control assembly (Dwyer & Laughlin, 2017; Loughnan
385 & Gilbert, 2017). Barbe *et al.* (2018) recently found that decomposition synergy is strongest
386 among grasses. Combined with the present result of facilitation of aggregation through
387 synergistic interactions during decomposition, this might contribute to explaining why
388 grasses coexist so often despite being functionally similar, and why their evolutionary
389 success was particularly strong (Cahill *et al.*, 2008; Linder *et al.*, 2018). We could name this
390 facilitation of decomposition an "associational decomposition", similar to associational
391 resistance of plants against herbivores (Barbosa *et al.*, 2009). Plants might benefit from
392 reciprocal acceleration in their litter decomposition, which might overall improve soil
393 fertility. The benefits might be symmetric, so that associational decomposition could
394 represent a mutualistic process between two cooperative species. Alternatively, higher soil
395 nutrient availability might benefit only to the most resource-acquisitive species, and
396 associational decomposition could rather represent an indirect competitive strategy. Overall,
397 we argue that in terrestrial ecosystems, litter decomposition should not be considered as a
398 collateral consequence of species traits, which "only" impacts overall ecosystem functions
399 such as elemental fluxes or primary production (Garnier *et al.*, 2004; Cornwell *et al.*, 2008).
400 Litter decomposition might rather be considered a unique part of the ecological strategy of
401 species, and a unique mechanism of coexistence among individuals and ultimately among
402 populations. Coexistence of populations permitting synergistic decomposition might relax
403 major trade-offs in plant life, for instance by maintaining nutrient recycling even in plants
404 that produce low-SLA leaves to reduce herbivory or desiccation (Fine *et al.*, 2006; Lind *et*
405 *al.*, 2013).

406 *Does the positive feedback of faster decomposition equalize or stabilize plant coexistence?*

407 We observed in our study that high plant aggregation was related to faster decomposition of
408 mixtures of species. At the population level, this might suggest a possible positive feedback
409 between coexisting plants, which produce litter mixtures that in turn facilitate coexistence.
410 This beneficial feedback might be due to improved nutrient recycling and nutrient availability
411 (Hobbie, 1992, 2015), improved microclimatic conditions, facilitation of ramet to penetrate a
412 thin litter layer, or increased activity of mutualist organisms in the local soil (Eisenhauer *et*
413 *al.*, 2009, 2012; Kardol *et al.*, 2015; Fernandez & Kennedy, 2016). If we try to rank this

414 coexistence feedback between stabilizing and equalizing (Wilson, 2011) we could opt for
415 both. We could opt for stabilizing as the advantage of mixing litters of two species gets lost if
416 one of the species gets overly abundant and takes over. In this case, positive litter mixing
417 effects would become more rare (mass-ratio hypothesis, Grime, 1998), to the disadvantage of
418 the dominant species. However, we could also opt for equalizing if the recycled nutrients are
419 equally available for both con- and heterospecifics, hence equalizing relative fitness
420 differences between coexisting species, and finally delaying the competitive exclusion. Thus,
421 our results suggest a novel perspective on litter decomposition: litter decomposition might
422 mediate plant-plant interactions and change the outcome of competition among species. Litter
423 decomposition studies might therefore bring novel insights for how plant species persist or
424 disappear through time.

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601 **Supporting Information**

602 Table S1. Correlations between functional traits of study species

603 Table S2. Models explaining interspecific spatial aggregation separately with different types
604 of traits

605 Table S3. Models explaining interspecific spatial aggregation at the 10x10cm scale

606 Figure S1. Changes to interspecific spatial aggregation over time

607 Figure S2. Means of litter-mixture decomposition of species pairs

608 Figure S3. Means of single-litter decomposition of species

609 Figure S4. Effects of after-life traits on litter decomposability

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622 **Tables**

623 **Table 1.** Means and standard deviations of species functional traits (specific leaf area, SLA;
 624 leaf dry matter content, LDMC; number of connections; spacer length; water holding
 625 capacity, WHC; carbon:nitrogen ratio, C:N). SLA is in mm²/mg, LDMC in mg/g, spacer
 626 length in cm, and number of connections, WHC and C:N have no unit. n=10 for the number
 627 of connections and for spacer length, n=5 for the other traits.

Species	SLA		LDMC		Number of connections		Spacer length		WHC		C:N ratio	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<i>Ranunculus repens</i>	28.5	3.0	177.0	13.4	27.6	15.8	14.98	0.70	3.30	0.21	21.7	5.3
<i>Centaurea nigra</i>	19.5	2.2	207.3	17.5	1.4	0.5	0.44	0.04	3.16	0.29	43.5	1.7
<i>Holcus lanatus</i>	32.1	5.1	257.5	29.8	33.5	15.6	0.28	0.02	4.33	0.25	33.6	8.8
<i>Elytrigia repens</i>	21.2	1.7	356.0	12.6	57.0	35.2	2.78	0.11	2.68	0.29	19.5	4.1
<i>Dactylis glomerata</i>	23.0	1.5	283.9	16.0	4.5	3.9	0.51	0.04	3.73	0.41	19.7	1.0
<i>Festuca rubra</i>	19.0	2.6	284.8	48.2	7.8	21.6	0.87	0.09	3.73	0.20	22.2	0.9
<i>Holcus mollis</i>	23.4	3.6	495.2	15.7	19.5	6.8	4.03	0.23	2.43	0.38	21.3	2.3
<i>Brachypodium pinnatum</i>	23.3	5.1	380.1	47.3	28.3	14.7	1.03	0.05	2.22	0.34	46.4	1.9

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643 **Table 2.** Explaining decomposition of the mixtures of litter species, of the corresponding
644 single-species litters, and of the corresponding non-additive effect of mixing decomposition
645 (i.e. whether the observed litter-mixture decomposition exceeds or falls below the mean
646 single decomposition of mixed species; synergy or antagonism, respectively). Explanatory
647 variables are means and dissimilarities in after-life litter traits (Water Holding Capacity
648 [WHC] and C:N ratio) and in during-life resource-acquisition traits (also related to litter
649 decomposition, Specific Leaf Area [SLA] and Leaf Dry Matter Content [LDMC]). Moreover,
650 to take into account unmeasured traits influencing decomposition, we also accounted for
651 phylogeny: in models explaining mean single decomposition of mixed species, we included
652 the presence or absence of a dicot among the two mixed species; in models explaining litter-
653 mixture decomposition and non-additive effects we included phylogenetic distance among
654 decomposing plants (related to but more informative than the presence of dicots).
655 Explanatory variables are presented with their standardized regression coefficient and its
656 significance (***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$). Model parameters are the adjusted R^2
657 (Adj- R^2), the F statistic (F) with the degree of freedom (df) and the probability of the model
658 (P -value).

		Variables	Mean single decomposition of mixed species	Litter-mixture decomposition	Non-additive effects
During-life traits	Resource-acquisition traits	Mean SLA	-0.43***	-0.46***	
		Mean LDMC	0.44**		
		Dissimilarity SLA			
		Dissimilarity LDMC			
After-life traits	Litter traits	Mean C:N	0.17*		
		Mean WHC	0.63***	0.32**	-0.46*
		Dissimilarity C:N			
		Dissimilarity WHC			
		Presence of dicots	2.17***		
Model parameters		Phylogenetic distance		0.80***	-0.28
		Adj-R ²	0.90	0.79	0.24
		F _{df}	50.0 ₂₁	34.2 ₂₃	4.84 ₂₂
		P-value	6.10 ⁻¹¹	1.10 ⁻⁸	2.10 ⁻²

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662 **Table 3.** Pairwise spatial aggregation among species at each date of the experiment explained
663 by the combination of trait means and dissimilarities in (i) during-life traits related to
664 resource-acquisition (SLA, Specific Leaf Area; LDMC, Leaf Dry Matter Content), (ii)
665 during-life traits related to clonal-dispersion, (iii) after-life litter traits (C:N, carbon:nitrogen
666 ratio; WHC, Water Holding Capacity), (iv) non-additive effects of litter decomposition and
667 (v) phylogenetic distance. Variables selected in the model are presented by their standardized
668 regression coefficients and significances (***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$). Model
669 parameters are the adjusted R² (Adj-R²), the F statistic (F) with the degree of freedom (df)
670 and the probability of the model (P-value). See Appendix Tab. S2 for analyses based on
671 subsets of variables, giving consistent results, and see Table S3 for similar results obtained at
672 a scale of 10x10cm. See Methods for model construction.

		Variables	Spatial aggregation			
			2010	2012	2014	2016
During-life traits	Resource-acquisition traits	Mean SLA	-1.33**	-1.15**	-0.69*	
		Mean LDMC	0.93	1.96**	1.30*	
		Dissimilarity SLA				
		Dissimilarity LDMC			-0.47*	
	Clonal-dispersal traits	Mean number of connections	1.00***	0.78**		
		Mean spacer length	1.52**		2.18***	
After-life traits	Litter traits	Dissimilarity number of connections			0.38*	
		Dissimilarity spacer length		1.06*		
		Mean C:N	1.05*	0.79*	1.32**	
		Mean WHC	1.59*	1.66*	1.31*	-0.44**
	Dissimilarity C:N					
	Dissimilarity WHC					
	Non-additive effects during decomposition				0.39*	
Model parameters	Phylogenetic distance		0.96**		0.15	
	Adj-R ²	0.56	0.50	0.61	0.46	
	F _{df}	6.4 ₂₀	4.7 ₁₉	6.8 ₁₉	7.9 ₂₁	
	P-value	7.10 ⁻⁴	4.10 ⁻³	4.10 ⁻⁴	1.10 ⁻³	

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679 **Figure captions**

680 Figure 1. After-life interactions during litter-mixture decomposition might determine plant
681 species aggregation. At t_n , a first contact between litters from two heterospecific individuals
682 generates antagonistic or synergistic effects during decomposition. At t_{n+1} , synergistic effects
683 benefit to the fitness of both individuals that provided the litter (e.g. through soil enrichment),
684 such as these individuals aggregate spatially. Meanwhile, antagonistic effects are detrimental
685 to the fitness of both individuals that provided the litter (e.g. through thickening of the litter
686 layer that limits emergence of their ramets) such as these individuals segregate spatially.

687 Figure 2. Relationship between spatial aggregation among species pairs and means of litter-
688 mixture traits (Water Holding Capacity, WHC; C:N ratio), at each of the four dates of the

689 experiment. Significant relationships are indicated with the *P*-value, and non-significant ones
690 with NS. Each graph presents partial residuals, i.e. accounts for the remaining independent
691 variables. See Methods for model construction and Table. 3 for model parameters. See Table
692 S3 for similar results obtained at a scale of 10x10cm.

693 Figure 3. Relationship between spatial aggregation among species pairs and non-additive
694 effects during litter-mixture decomposition, at each of the four dates of the experiment.
695 Significant relationships are indicated with the *P*-value, and non-significant ones with NS.
696 Each graph presents partial residuals. See Methods for model construction and Table. 3 for
697 model parameters. See Table S3 for similar results obtained at a scale of 10x10cm.

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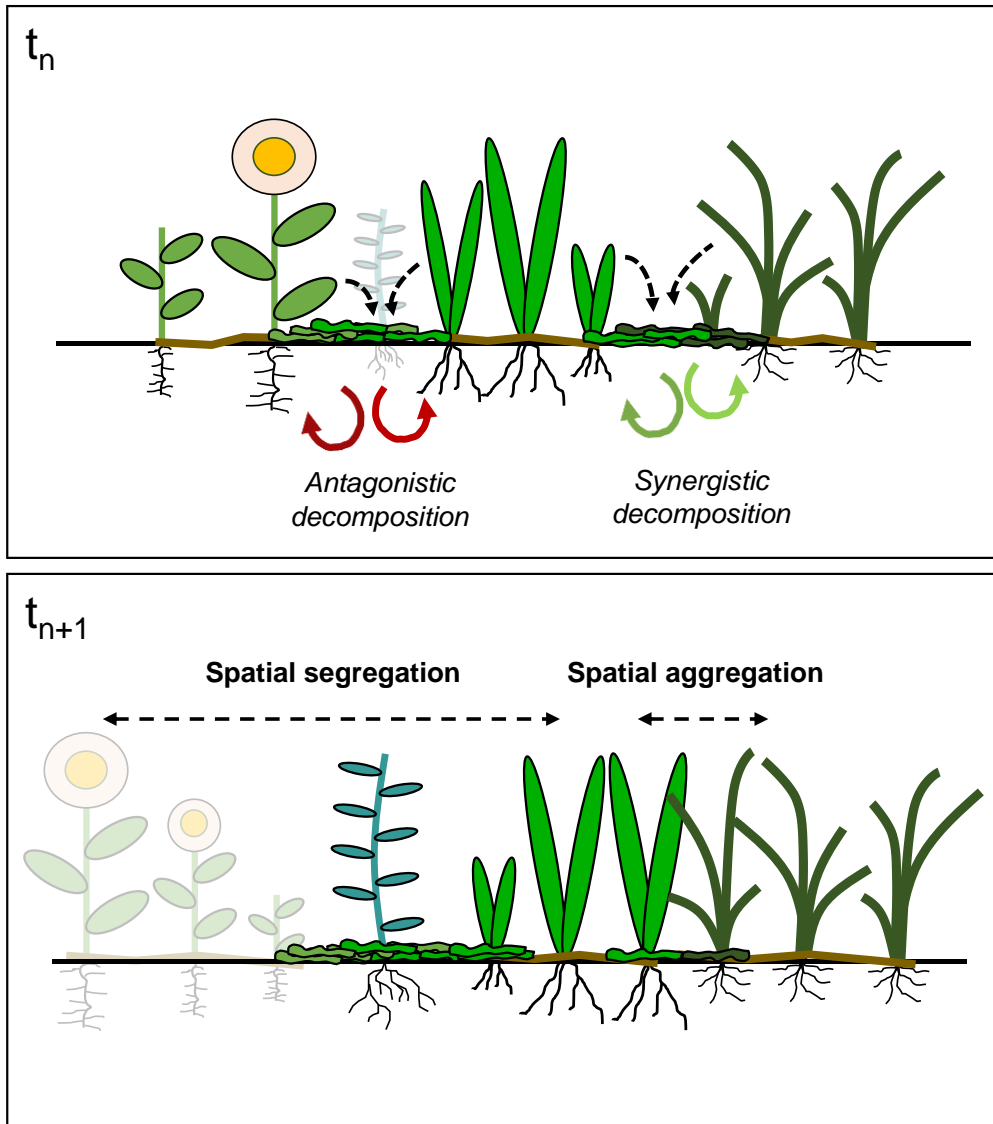
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707 **Figures**



708

709 Figure 1.

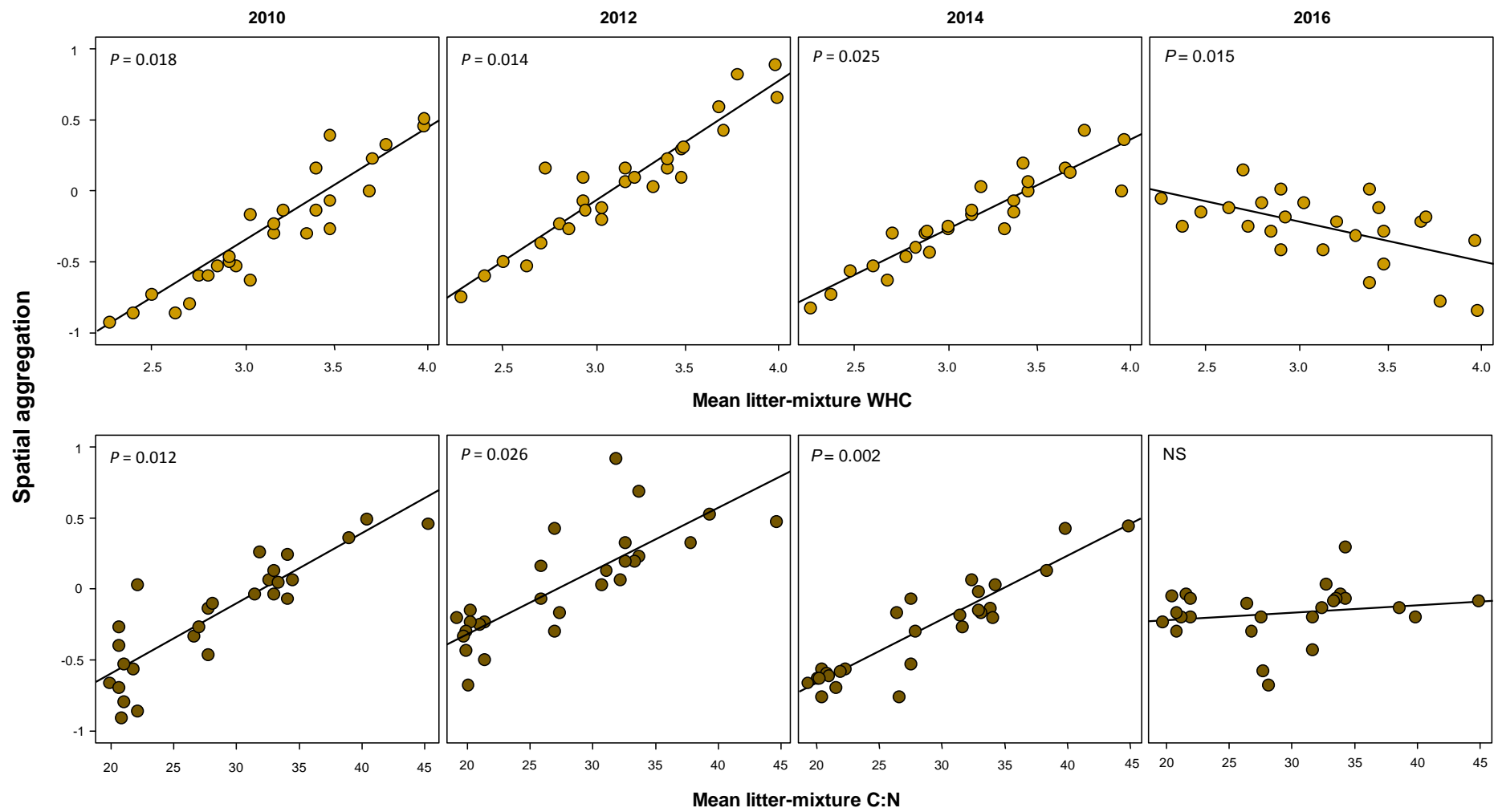


Figure 2.

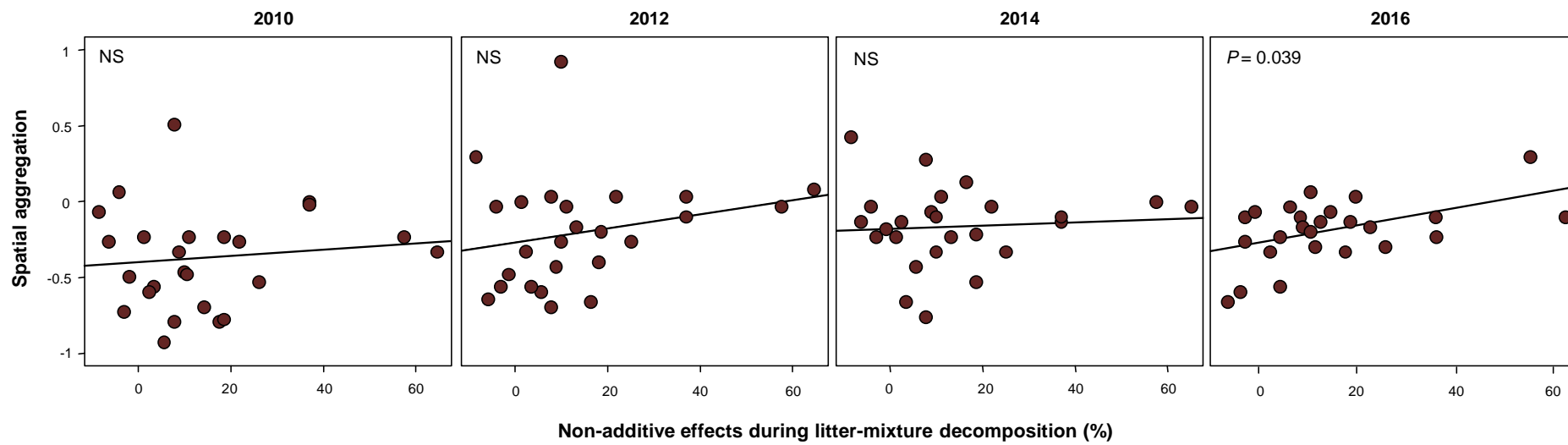


Figure 3.