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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>

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## Abstract

1. While species may coexist at population level, their individuals may still segregate, at least temporarily, and hence interact little. Aggregation among heterospecific individuals may require particular during-life traits, for example traits limiting competition for space or resources. But is aggregation also facilitated by after-life traits accelerating decomposition of plant litters, notably through synergy among co-decomposing litters?
2. We investigated the role of leaf traits, litter traits and litter-mixture decomposition in the spatial aggregation of individuals of pairs of grassland species in a long-term mesocosm experiment. We related aggregation between pairs of species to mean and dissimilarity in (i) during-life traits related to resource-acquisition and clonal dispersal, (ii) after-life traits related to decomposition, and (iii) rate and synergy of decomposition.
3. In most years, aggregation of heterospecific individuals strongly increased with means of (i) during-life clonal traits reducing competition for space, and (ii) after-life traits

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

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

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

## Introduction

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

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

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

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

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

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

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

## Material and Methods

### *Study site and experimental design*

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



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

#### Plant mapping and measures of aggregation

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

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

#### *Functional trait measurements and calculations*

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

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

## Results

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

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

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

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

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

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

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

## **Discussion**

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

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

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

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

#### *Trait means accelerating litter decomposition promote plant aggregation*

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

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

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

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

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

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



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

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## Supporting Information

- Table S1. Correlations between functional traits of study species
- Table S2. Models explaining interspecific spatial aggregation separately with different types of traits
- Table S3. Models explaining interspecific spatial aggregation at the 10x10cm scale
- Figure S1. Changes to interspecific spatial aggregation over time
- Figure S2. Means of litter-mixture decomposition of species pairs
- Figure S3. Means of single-litter decomposition of species
- Figure S4. Effects of after-life traits on litter decomposability

## Tables

**Table 1.** Means and standard deviations of species functional traits (specific leaf area, SLA; leaf dry matter content, LDMC; number of connections; spacer length; water holding capacity, WHC; carbon:nitrogen ratio, C:N). SLA is in mm<sup>2</sup>/mg, LDMC in mg/g, spacer length in cm, and number of connections, WHC and C:N have no unit. n=10 for the number 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



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

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

**Table 3.** Pairwise spatial aggregation among species at each date of the experiment explained by the combination of trait means and dissimilarities in (i) during-life traits related to resource-acquisition (SLA, Specific Leaf Area; LDMC, Leaf Dry Matter Content), (ii) during-life traits related to clonal-dispersion, (iii) after-life litter traits (C:N, carbon:nitrogen ratio; WHC, Water Holding Capacity), (iv) non-additive effects of litter decomposition and (v) phylogenetic distance. Variables selected in the model are presented by their standardized regression coefficients and significances (\*\*\*:  $P < 0.001$ ; \*\*:  $P < 0.01$ ; \*:  $P < 0.05$ ). Model parameters are the adjusted  $R^2$  (Adj- $R^2$ ), the F statistic (F) with the degree of freedom (df) and the probability of the model ( $P$ -value). See Appendix Tab. S2 for analyses based on subsets of variables, giving consistent results, and see Table S3 for similar results obtained at a scale of 10x10cm. See Methods for model construction.

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

## Figure captions

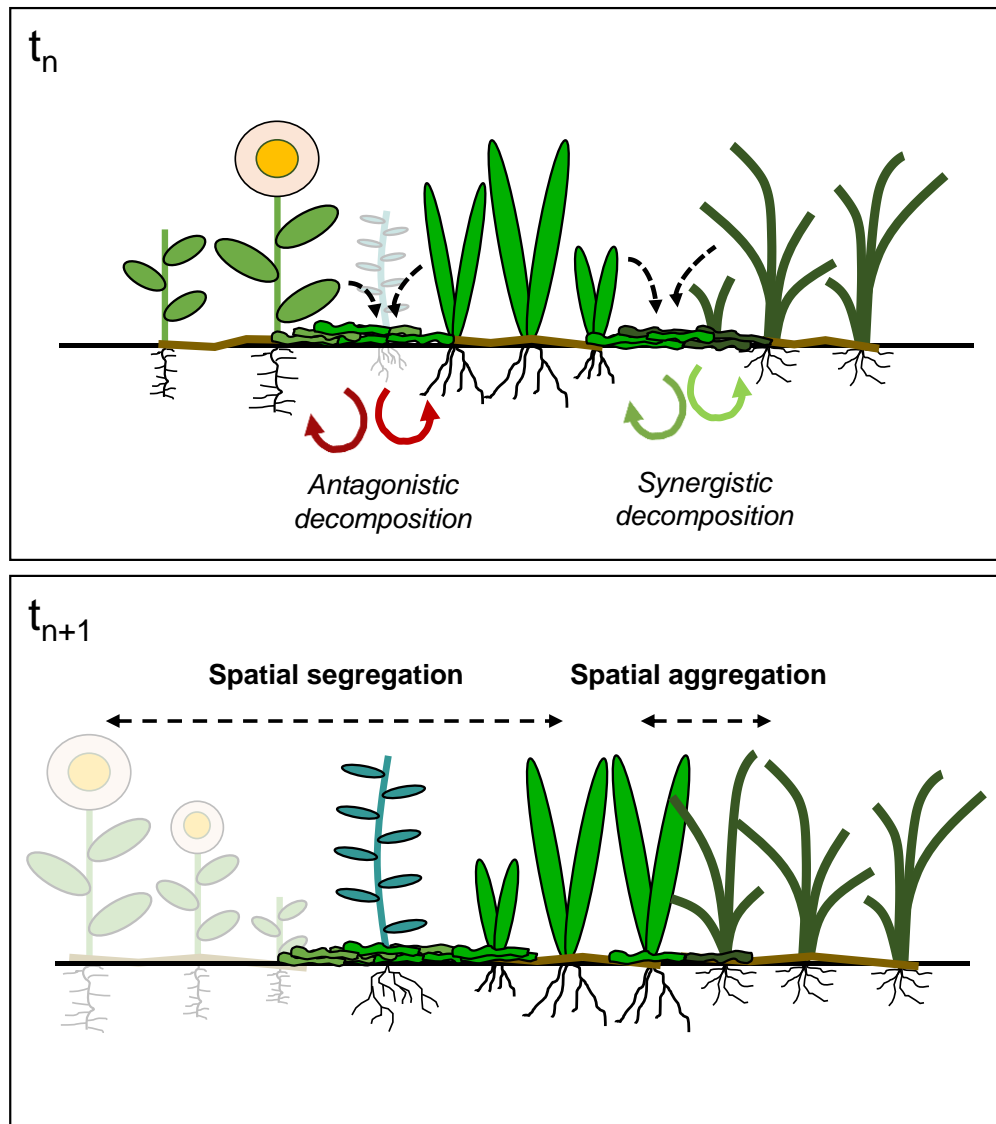
Figure 1. After-life interactions during litter-mixture decomposition might determine plant species aggregation. At  $t_n$ , a first contact between litters from two heterospecific individuals generates antagonistic or synergistic effects during decomposition. At  $t_{n+1}$ , synergistic effects benefit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

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

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

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

**Figures**



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709 Figure 1.

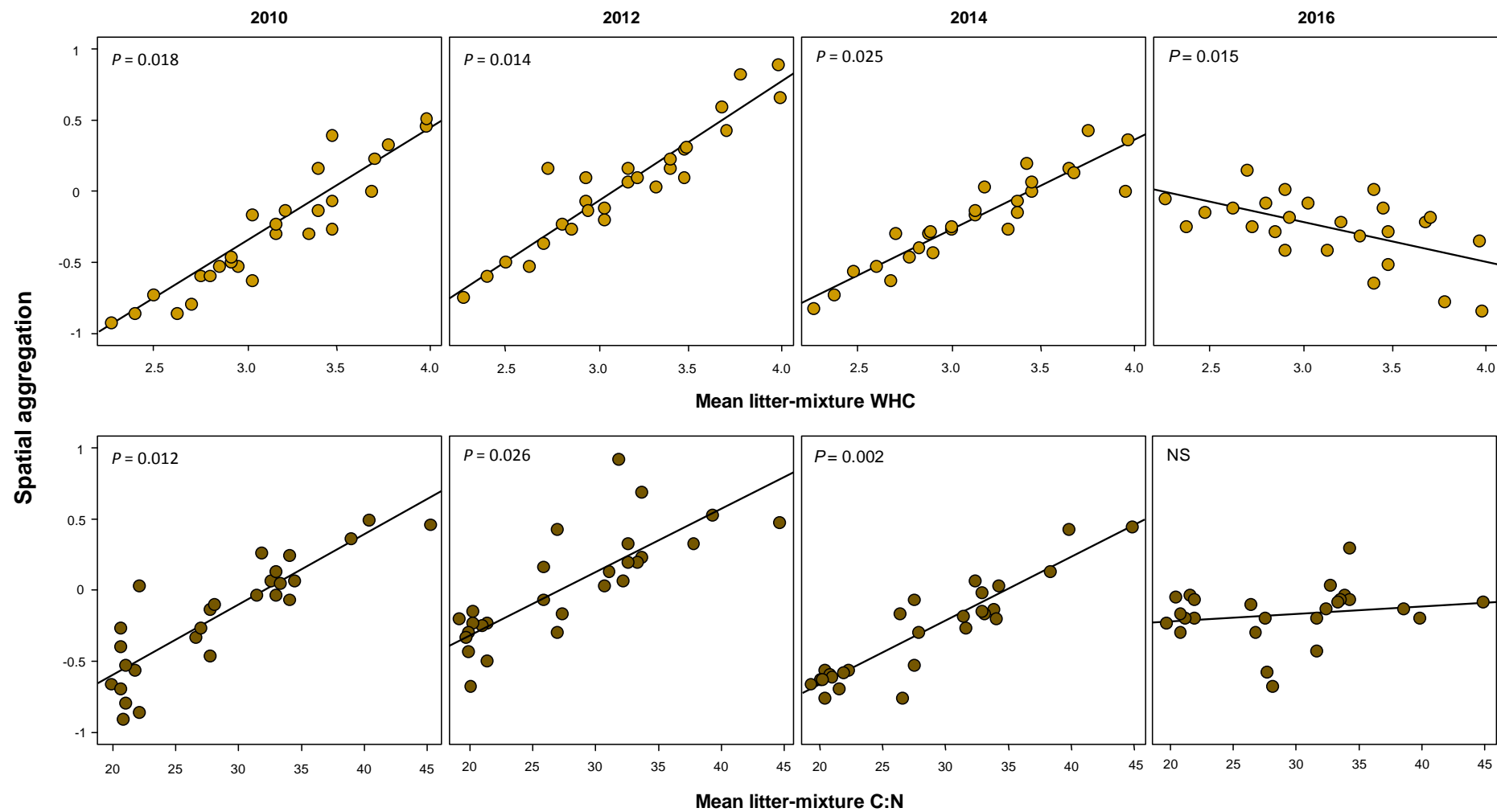


Figure 2.

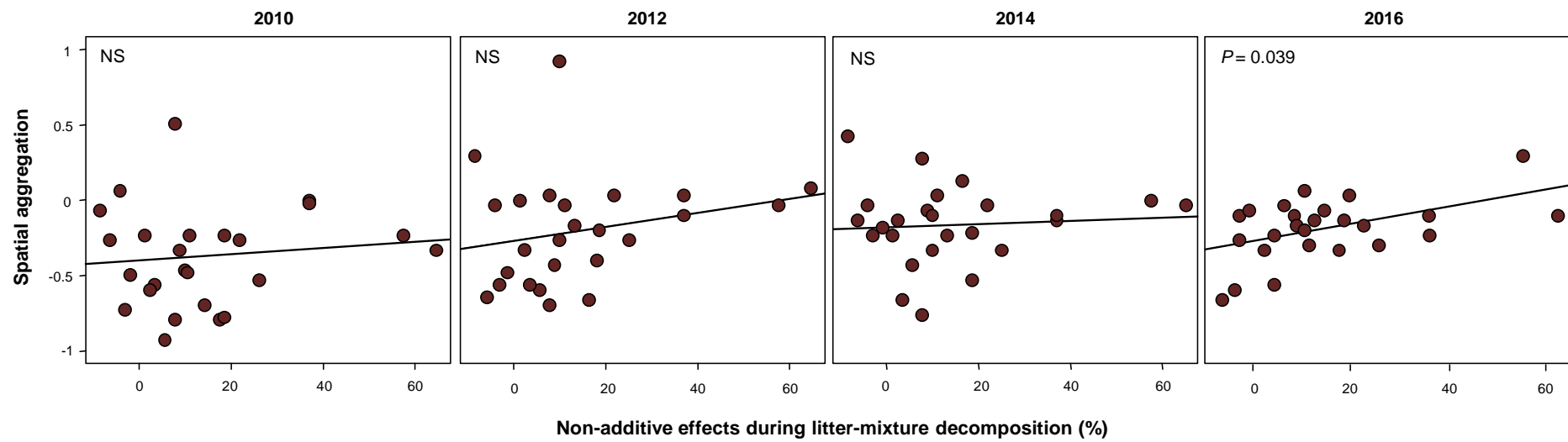


Figure 3.