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17 **Effects of litter mixing on litter decomposition and soil properties along**  
18 **simulated invasion gradients of non-native trees**

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24 **Running title:** Litter mixing effects along invasion gradients

25

26 **Key-words:** invasive trees, litter decomposition, litter properties, non-additive effects, per-  
27 capita impact, soil properties

28

29 **Abstract**

30 **Aims:** Plant invasions generally lead to mixtures between native and non-native litter. We assess  
31 the interactions between leaf litters from two invasive non-native trees (*Robinia pseudoacacia* or  
32 *Ailanthus altissima*) and the native *Populus alba* on litter decomposition, nutrient release and  
33 soil properties along two gradients of invasion. **Methods:** Microcosms with field-collected soil  
34 covered by varying proportions of native and non-native litter simulated the two invasion  
35 gradients. We assessed the proportion of remaining litter mass and nutrient mass (N, P, C), and  
36 soil C, N-NO<sub>3</sub><sup>-</sup>, total N, and pH, five times throughout a period of 11 months. Observed values  
37 were compared to the expected values on the assumption of no interactions. **Results:** Litter  
38 mass and C mass decayed slower in *Robinia* and faster in *Ailanthus* than in *Populus*. The three  
39 species immobilized N and P. Soil properties did not differ across pure litters. Both litter mixture  
40 gradients showed additive or antagonistic interactions on litter decomposition, whereas N and  
41 P mass were equal or higher than expected. The proportion of non-native litter in the mixture  
42 had non-linear effects on most variables, suggesting that the impact of these non-native trees  
43 on litter decay levels off or even declines as they become more abundant. **Conclusion:** The  
44 impacts of *Ailanthus* and *Robinia* litter on soil processes should not be derived from single  
45 species experiments, both due to non-additive effects and to non-linear responses to litter  
46 abundance.

47

48 **Introduction**

49 Litter decomposition is a key process for nutrient cycling in forest ecosystems, and thus for  
50 primary production (Berg and McClaugherty 2014; Hickman et al. 2013; Vogt 1991). The litter  
51 decomposition process is controlled by climatic and edaphic factors, by the nature of the soil

52 community, and by the quality of the litter (Aerts 1997; Berg and McClaugherty 2014; Gallardo  
53 and Merino 1993; Jo et al. 2016). Thus, invasion of ecosystems by non-native plants with litter  
54 properties different from those prevailing in the native community may alter the nutrient cycle  
55 and soil properties (Castro-Díez et al. 2014a; Ehrenfeld and Scott 2001; Incerti et al. 2018;  
56 Kourtev et al. 2002). Impacts may be dramatic when the invaders represent novel functional key  
57 traits and/or when they are very abundant (Chapin et al. 1996; D'Antonio and Corbin 2003;  
58 Vitousek and Walker 1989).

59 One of the most widely described impacts of invasive plants is that of the litter on soil properties  
60 and processes (Castro-Díez et al. 2014a; Ehrenfeld and Scott 2001; Hulme et al. 2013; Liao et al.  
61 2008; Pyšek et al. 2012). Overall, invasive plants tend to be more productive than natives (Pyšek  
62 and Richardson 2007; van Kleunen et al. 2010) and to produce litters with properties associated  
63 with a fast decomposition (e.g. high nutrient content, high specific leaf area (SLA), low lignin and  
64 phenolic content) (Castro-Díez et al. 2014a; Ehrenfeld 2003; Pyšek et al. 2012). However,  
65 exceptions to this trend can be often found (Godoy et al. 2010; Marchante et al. 2019; McArthur  
66 et al. 1994; Nilsson et al. 1999), and recent studies suggest that this general trend may be the  
67 result of a biased selection of the non-native species with the largest impacts on ecosystem  
68 processes (Incerti et al. 2018; Jo et al. 2016).

69 The pattern of non-native tree invasion over space and time generally leads to litter mixtures  
70 between native and non-native species (Chen et al. 2013; Gartner and Cardon 2004; Hickman et  
71 al. 2013). Yet, most studies aiming to assess the impact of this litter on soil processes rely on  
72 comparisons between situations with pure native litter and pure non-native litter (Castro-Díez  
73 et al. 2009; Godoy et al. 2010; Incerti et al. 2018). Thus, these studies implicitly assume that  
74 native and non-native litters do not interact with each other, so that the dynamics of the litter  
75 mixtures might be derived from the dynamics of isolated litters knowing their proportions in the  
76 mixture. This situation is referred to as “additive effects” between litters, i.e. the whole is the  
77 sum of the components. Yet, the growing number of studies addressing the dynamics of litter  
78 mixtures highlights that non-additive effects are more frequent than additive effects and that  
79 positive effects or synergies (i.e. faster decomposition –or lower litter mass remaining– than  
80 predicted from component species decaying alone) are more frequent than negative or  
81 antagonistic effects (Gartner and Cardon 2004; Hattenschwiler et al. 2005; Lecerf et al. 2011).  
82 Mechanisms behind interactions between litters in mixtures include the transfer of nutrients or  
83 other compounds across litters, increased habitat complexity in litter mixtures, enhancing  
84 consumer diversity (Chen et al. 2013; Gartner and Cardon 2004; Lecerf et al. 2011; Zhang et al.  
85 2014), or feedbacks between edaphic organisms and the environment (Elgersma and Ehrenfeld  
86 2011).

87 Another widespread assumption is that invaders impacts are proportional to the invaders  
88 density, i.e. that the per-capita impact is constant (Parker et al. 1999). Yet, the few studies that  
89 specifically address the impact-density relation showed a variety of responses, including  
90 threshold effects (i.e. no impact below certain threshold density) (Elgersma and Ehrenfeld 2011;  
91 Maron and Marler 2008; Thiele et al. 2010). Given that most studies supporting impact  
92 assessment are focused on high-density invasions (Elgersma and Ehrenfeld 2011; Strayer et al.  
93 2006; Yokomizo et al. 2009), the assumption of per-capita constant impact, if false, may involve  
94 high management costs (Yokomizo et al. 2009).

96 In spite of recent advances and reviews, the dynamics of litter mixtures have revealed to be  
97 complex and difficult to predict. For instance, non-additive effects of litter mixtures have been  
98 suggested to be greater when component litters differ greatly in functional traits (Wardle et al.  
99 1997), but empirical evidences provide mixed support to this hypothesis (Chen et al. 2013;  
100 Hoorens et al. 2003; Lecerf et al. 2011; Quested et al. 2002). Length of the decay period may  
101 also affect the sign of the interactions (Chen et al. 2013; Gartner and Cardon 2004; Lecerf et al.  
102 2011). Finally, the proportion of different litters in the mixture may also interfere with the  
103 strength of the interactive effect (Gartner and Cardon 2004; Scowcroft 1997; Zhang et al. 2014).  
104 In this line, similar proportions of litters with different traits in mixtures have been suggested to  
105 promote non-additive effects on litter decomposition (Chen et al. 2013). However, few studies  
106 evaluate the effects of mixtures with different proportions of litters (Gartner and Cardon 2004,  
107 but see Elgersma and Ehrenfeld 2011; Scowcroft 1997). Thus, there is an urgent need to fill these  
108 gaps with empirical knowledge to prevent managers to adopt scarcely tested assumption (as the  
109 constant per-capita impact) that may lead to implementing ineffective management actions  
110 (Sofaer et al. 2018; Yokomizo et al. 2009).

111 In Europe, including Spain, riparian forests, along with coastal ecosystems, are the most invaded  
112 natural terrestrial habitats (Chytrý et al. 2008; Vilà et al. 2001). Riparian forests are particularly  
113 prone to invasion because they match many of the conditions hypothesized to increase invasion  
114 success (Castro-Díez and Alonso 2017; Catford et al. 2011; Catford et al. 2009; Hood and Naiman  
115 2000; Tabacchi et al. 2005; Tickner et al. 2001): 1) they receive intermittent pulses of resources  
116 thanks to periodic floods; 2) floods reduce the biotic resistance of the native community and  
117 create gaps that can be readily invaded by any pioneer species; 3) abiotic conditions are mild,  
118 thanks to the climatic buffering exerted by the tree canopy and the presence of a permanent  
119 water table; 4) rivers and floodplains are corridors for species propagules—either native or non-  
120 native—; 5) land-uses nearby rivers (agriculture, urbanization) increase propagule pressure of  
121 non-native plant species; 6) altered hydrologic regimes change the abiotic context where native  
122 species were selected and create opportunities for newly-arrived species.

123 In the inner Iberian Peninsula, riparian forests have been invaded by different non-native tree  
124 species (Cabra-Rivas and Castro-Díez 2016; Castro-Díez et al. 2009; Medina-Villar et al. 2015b).  
125 Two of the most common are *Ailanthus altissima* (Mill.) Swingle and *Robinia pseudoacacia* L.  
126 These species now coexist with dominant native trees, such as *Populus alba* L., *Populus nigra* L.,  
127 *Fraxinus angustifolia* Vahl or *Ulmus minor* Mill., leading to litter mixtures between native and  
128 invasive trees.

129 In this study, we address the question of how the non-native litter of *A. altissima* and *R.*  
130 *pseudoacacia* alter nutrient cycling and soil properties. Previous field and lab experiments  
131 suggest that *A. altissima* litter decomposes faster than that of coexisting natives, while *R.*  
132 *pseudoacacia* litter does the opposite (Castro-Díez et al. 2009; Medina-Villar et al. 2015a). Under  
133 field conditions, *A. altissima* was found to decrease soil total N and *R. pseudoacacia* to increase  
134 soil mineral N (ammonium and nitrate) as compared to non-invaded forests (Medina-Villar et al.  
135 2016). By contrast, a microcosm experiment revealed no difference in the effects of native and  
136 non-native litter on several soil properties (Castro-Díez et al. 2012). However, to our knowledge

137 there is no information on how the litter of these two species interferes with the decomposition  
138 of native litter, or on the relationship between the abundance and the impact of the invaders.

139 Specifically, we address the following questions. 1) How do litter decomposition dynamics differ  
140 between the two invasive trees and a coexisting native tree? 2) Are there non-additive positive  
141 or negative effects of the non-native litter on litter decomposition and soil properties? 3) How  
142 do these effects vary through incubation time? 4) How do these effects vary along a simulated  
143 gradient of invasion (increasing proportion of non-native litter)?

144

## 145 **Materials and Methods**

### 146 Study species

147 *Ailanthus altissima* (Mill.) Swingle (Simaroubaceae) is native to South East Asia, while *Robinia*  
148 *pseudoacacia* L. (Fabaceae) is native to the Appalachian Mountains in Eastern North America.  
149 Both are fast-growing deciduous trees with compound leaves. They were introduced as  
150 ornamental plants in Europe, in the 18<sup>th</sup> and the 17<sup>th</sup> century, respectively (Sanz Elorza et al.  
151 2004). Now they are considered as global invaders, being among the 100 worst invasive species  
152 in Europe (Basnou 2009; Basnou and Vilà 2009). In Spain these species are widespread in  
153 floodplains, borders of crops and roads, and (sub)urban areas (Sanz Elorza et al. 2004). Their fast  
154 growth, high seed production and profuse resprout ability, along with the N-fixing capacity of *R.*  
155 *pseudoacacia*, and the efficient dispersal of *A. altissima* seeds by wind and water, have been  
156 listed among the causes explaining their invasive success (Cabra-Rivas and Castro-Díez 2016;  
157 Cierjacks et al. 2013; Kowarik and Saumel 2007). The native tree *Populus alba* L. (Salicaceae) has  
158 been selected as control species due to its high dominance in the riparian forest of middle-low  
159 altitudes and basic soils of inner Spain (Lara et al. 1996). It is a deciduous, simple leaves tree,  
160 which produces large amounts of tiny, wind-dispersed seeds (Prada and Arizpe 2008). For  
161 simplicity, we name the species by the genus name from now on.

### 162 Leaf litter sampling

163 The leaf litter sampling was performed in the riparian forest of the mid-low Henares River (or its  
164 subsidies) along a stretch of 15 km, between the municipalities of Cabanillas del Campo  
165 (40°38'04"N, 3°14'06"W) and Los Santos de la Humosa (40°30'58" N, 3° 17' 06"W), both in  
166 Guadalajara province (central Spain). In this stretch, four locations were selected (Cabanillas del  
167 Campo, Alovera, Chiloeches and Los Santos de la Humosa) and litter from each species was  
168 collected in one (*Populus*), two (*Ailanthus*) or the four locations (*Robinia*). The altitude ranges  
169 from 590 to 660 m.a.s.l. The climate is Mediterranean continental, with cold winters and arid  
170 summers. The mean annual temperature is 13.5°C and the mean annual precipitation is 358 mm  
171 (weather station of Alcalá de Henares-El Encín, 600 m.a.s.l., period 1970–2009). Soils are Calcic  
172 Cambisols + Calcaric Regosols or Calcaric Fluvisol + Calcic Cambisol + Gleyo-calcaric Fluvisols  
173 (FAO/IIASA/ISRIC/ISSCAS/JRC 2012).

174

175 Senescent leaves (those which detach with a gentle touch) were collected at the time of natural  
176 abscission (October-December 2015) from a minimum of 10 trees per species and location.  
177 Sampling trees were at least 15 m apart from each other to assure that they were different

178 genets. Once in the lab leaf litter was pooled by species, air-dried and stored in paper bags until  
179 being used. Given that leaflets and rachises of *Ailanthus* and *Robinia* tend to fall separately, we  
180 selected 10 full leaves (with rachis and all leaflets) to get the air-dried-weight proportion of  
181 rachis and lamina. This proportion was kept for subsequent chemical analysis and experimental  
182 setup.

183 From each pool of leaf litter, five pseudoreplicates of ca. 4 g of air-dried weight (keeping the  
184 rachis:lamina proportion in compound leaves) were selected; they were oven dried (60°C >48  
185 h) to obtain the dry weight, and subsequently grounded to a particle size of 1 mm in a Culatti  
186 mill for chemical analyses. The water content of these samples was used to correct the air-dried  
187 weight of the leaf litter in the experimental setup (see below).

#### 188 Soil sampling

189 In December 2015 we collected topsoil from 0 - 20 cm depth (after removing the litter layer) in  
190 the Henares River floodplain in the location of Los Santos de la Humosa, where vegetation was  
191 dominated by *Populus alba* and no non-native tree was present. Stones and large debris were  
192 removed. Soil was air-dried and passed through a 2 mm-sieve to remove large particles and  
193 homogenized. Five 350 g soil samples were separated, dried in the oven (60°C ≥ 72 h) and kept  
194 in the freezer at -20°C for subsequent chemical analyses.

#### 195 Soil moisture calibration

196 Prior to the beginning of the experiment we conducted a soil moisture – soil weight calibration  
197 in order to monitor soil moisture during the experiment. Six previously weighed 500 cm<sup>3</sup> (12 x 9  
198 x 5 cm) polypropylene containers with five drainage holes in the bottom were filled with 250 g  
199 of air-dried experimental soil. Containers were then kept in larger aluminum trays with 3 cm of  
200 water for several days until soils were fully saturated. Then containers were left to freely drain  
201 until they stopped dripping from the bottom. At that point, containers were weighed, left to air  
202 dry in the lab, and re-weighed one to four times per day, until the soil started to show cracks  
203 and to lose its structure. Finally, containers were oven-dried at 60°C for ≥ 72 h and weighed. Soil  
204 moisture was calculated for every weighing time as the proportion of soil water (full container  
205 weight minus empty container weight minus dry soil weight) with respect to the water content  
206 at soil field capacity (Castro-Díez et al. 2012). The relation between container weight and soil  
207 moisture was used to calculate the amount of water required to keep experimental containers  
208 at 70-90% of soil field capacity during the experiment.

209

#### 210 Experiment setup

211 The experiment was set up on 28<sup>th</sup> January 2016. We constructed microcosms by filling 162  
212 polypropylene containers like those used for soil moisture calibration with 250 g of air-dried  
213 homogenized soil (ca. 3 cm of soil depth). The soil of each container was directly covered with  
214 ca. 3.5 g of previously-weighed air-dried leaf litter, corresponding to one of the following  
215 treatments: 100% *Populus*, 100% *Ailanthus*, 100% *Robinia*, 75:25 *Ailanthus:Populus*, 50:50  
216 *Ailanthus: Populus*, 25:75 *Ailanthus: Populus*, 75:25 *Robinia:Populus*, 50:50 *Robinia: Populus*,  
217 and 25:75 *Robinia: Populus*. In this way, we simulated two invasion gradients of the riparian  
218 native forest (dominated by *Populus*) by each invasive tree. The quantity of leaf litter used was

219 equivalent to  $324 \text{ g m}^{-2}$ , which falls within the range of annual leaf litter production reported in  
220 the literature for these species in monospecific stands:  $305 \text{ g m}^{-2}$  for *Ailanthus* (González-Muñoz  
221 et al. 2013),  $310 \text{ g m}^{-2}$  for *Robinia* (Tateno et al. 2007) and  $412 \text{ g m}^{-2}$  for *Populus* (Medina-Villar  
222 et al. 2015b). We did not use litter bags, as usually done in this type of experiments, to  
223 maximize the contact between leaf litter and soil, as occurs in the field. However, this approach  
224 has the disadvantage of an increasing difficulty to separate the remaining litter from the soil –  
225 and thus an increasing variability – as decomposition proceeds. To compensate for this  
226 drawback, we doubled the number of replicates in the last harvest, as explained below.

227 Eighteen containers (replicates) per treatment were prepared. Groups of four or six replicates  
228 were randomly placed in each of four drainage aluminum trays with ca. 2 cm of water in the  
229 bottom, to allow hydration of the containers' soil. The leaf litter was sprayed from above with  
230 deionized water. When both soil and leaf litter were hydrated, the trays with the containers  
231 were introduced in two thermostatic chambers (AQUA LYTIC<sup>®</sup>) at constant temperature of 20°C  
232 and in dark. Every week a subsample of 20 containers was weighed to assess soil moisture. The  
233 amount of deionized water required for attaining field capacity was added to the drainage tray  
234 and the leaf litter was sprayed also with deionized water. To avoid heterogeneity of conditions,  
235 trays were randomly rotated within and across chambers weekly. The average soil moisture  
236 during the experimental period was  $81.12\% \pm 1.58 \text{ SE}$  of soil field capacity.

237 After 62, 111, 161, 253 and 323 days of incubation we collected three randomly-selected  
238 containers per treatment (one per drainage tray), with the exception of the last date, when six  
239 containers were collected. This decision was made –at the expense of a sixth collection– to  
240 compensate for the increased variance of remaining litter mass detected in the later collections.  
241 In each container, we gently separated leaf litter from the soil, kept the litter in paper bags and  
242 dried it in the oven ( $60^\circ\text{C} \geq 48 \text{ h}$ ). Once dried, the sample was extended on a paper to additionally  
243 remove soil particles attached to the litter with a gentle brush before weighing the litter to  
244 obtain the proportion of litter mass remaining (LMR). Soils were also kept in paper bags, oven-  
245 dried ( $60^\circ\text{C} \geq 72 \text{ h}$ ) and kept in the freezer at  $-20^\circ\text{C}$  until analysis (Sun et al. 2015). By drying soil  
246 samples at a moderate temperature, we minimized the drying period, and then the chances of  
247 losing labile organic carbon (Sparling et al. 1985).

#### 248 Chemical analysis

249 **Leaf litter.** From the initial pool of leaf litter we selected 20 g (air-dried weight) per species,  
250 keeping the rachis:lamina ratio of full leaves of *Ailanthus* and *Robinia*. From each of the  
251 remaining collections, we kept the full amount of remaining litter. Each sample was ground in a  
252 Culatti mill to 1 mm- particle size. From the initial pool of leaf litter we collected four pseudo-  
253 replicates of 3.5 g for lignin analysis in Ainprot S.A. laboratory (Madrid, Spain), following the  
254 method of UNE-EN ISO 13906:2009. N, P and C concentrations of every litter sample were  
255 determined with an Automated Wet Chemistry Analyzer (Skalar San++ Breda, The Netherlands)  
256 after a digestion with  $\text{H}_2\text{SO}_4$  and  $\text{Cu-KSO}_4$ , in Nutrilab (Rey Juan Carlos I University, Móstoles,  
257 Madrid, Spain).

258 **Soils.** Soil pH, total N, total C, and  $\text{N-NO}_3^-$  (which represented 93.4% of the total inorganic N of  
259 this soil (Castro-Díez et al. 2012)) were measured at the beginning (day=0, 5 pseudo-replicates),  
260 at the middle (day =161, 3 replicates), and at the end of the incubation (day=323, 6 replicates).



261 Soils were defrosted at ambient temperature, homogenized and ground in a Culatti mill to a 2  
 262 mm soil particle. For soil pH assessment, 10 mL of deionized water were added to 5 g of each  
 263 soil sample, shaken and measured with a pH-meter (Allen et al. 1986). For total N and C, ca. 40  
 264 g per sample were digested with H<sub>2</sub>SO<sub>4</sub> and Cu-KSO<sub>4</sub>, and analyzed as before, with an  
 265 Automated Wet Chemistry Analyzer (Skalar San++ Breda, The Netherlands). Soil N-NO<sub>3</sub><sup>-</sup> was  
 266 extracted by adding 100mL of KCl 2M to 10 g of soil and shaking the mixture for 1 h. After 30  
 267 min, 20 mL of clear supernatant were kept in vials and frozen at -20°C before analysis at Nutrilab  
 268 with an Automated Wet Chemistry Analyzer (Skalar San ++, Breda, The Netherlands).

269

270 Statistical analyses

271 *Comparison of litter and soil properties across pure-litter treatments*

272 Initial chemical properties (N, P, C, lignin, C:N and lignin:N), the fraction of initial litter and  
 273 nutrient (N, P and C) mass that remained in the final collection, and soil properties in the final  
 274 collection, were compared across the three pure-litter treatments using one-way ANOVA or  
 275 Kruskal-Wallis when homoscedasticity was not met.

276 *Fitting litter mass loss models*

277 To fit the variation of LMR to time we used two models for each litter treatment: 1) the simple  
 278 exponential model which assumes that the substrate is completely decomposed at a constant  
 279 decomposition rate (*k*).

280 
$$LMR = e^{-kt}, \quad \text{eqn(1)}$$

281 *LMR* being the proportion of litter mass remaining, *k* the decomposition rate and *t* the time in  
 282 years (Berg and McLaugherty 2014). Although this is the most widely used model for litter  
 283 decomposition dynamics, it has been often found to poorly fit observations for late phases of  
 284 litter decomposition (Berg and McLaugherty 2014). Thus, we fit: 2) the asymptotic model, which  
 285 assumes that the decomposition proceeds progressively more slowly until approaching to zero,  
 286 and that a fraction of the litter mass is not decomposed (Berg and McLaugherty 2014).

287 
$$LMR = m + (1-m)e^{-k't/m}, \quad \text{eqn(2)}$$

288 *m* being the fraction of litter mass not decomposed at the end of the period (i.e. the model  
 289 asymptote), *k'* the initial decomposition rate, and the rest of parameters being the same as  
 290 before. The best model was considered the one with the lower residual standard error. Model  
 291 fit was performed with the nls() function of the 'stats' package in R.

292 *Expected values of litter mixtures*

293 For each litter mixture and collection day, we calculated the expected litter or nutrient (N, P and  
 294 C) mass remaining from the values obtained in the pure litter treatments as:

295 
$$MR_t^e(\text{mixture}) = x * MR_t^o(sp_1) + y * MR_t^o(sp_2), \quad \text{eqn(3)}$$

296  $MR_t^e$  being the expected mass remaining of the litter mixture at time  $t$ ,  $MR_t^o$  the observed mass  
 297 remaining of the species making up the mixture ( $sp_1$  and  $sp_2$ ) incubated in isolation, and  $x$  and  $y$   
 298 the proportions of  $sp_1$  and  $sp_2$  in the mixture.

### 299 *Comparison of observed and expected values*

300 We compared the observed and the expected values of litter (LMR) and nutrient (N, P, C) mass  
 301 remaining (NMR, PMR and CMR) for every litter mixture treatment and collection date, by  
 302 subtracting the latter from the former (observed - expected), and assessed whether the value  
 303 differs from zero using a Student's one-sample t-test.

### 304 *Effect of incubation time*

305 A two-way ANOVA was applied to assess the effect of incubation time, mixture treatment and  
 306 their interaction on the deviation of observed from expected values. To increase the statistical  
 307 power, values of the collections taken at days 62 and 111 (representing the initial phase) were  
 308 pooled and compared with collections from days 253 and 323 (representing the final phase).

### 309 *Variation of the effects of non-native litter with its abundance*

310 For all response variables that differ across pure litter treatments in the last collection, we fit  
 311 the response variable versus the proportion of non-native litter in the mixture using a linear and  
 312 a quadratic model. These two models were selected to represent different responses after a  
 313 visual inspection of the data. For each model, we assessed the residual deviance and the Akaike  
 314 information criterion (AIC). The quadratic model was considered to improve the linear model if  
 315 its AIC was at least two units lower than that of the linear model (Burnham and Anderson 2002).

316

## 317 **Results**

### 318 Comparison of pure-litter treatments

319 All litter chemical properties, except for carbon content, differed across species at the beginning  
 320 of the experiment. The two non-natives, *Ailanthus* and *Robinia*, showed higher N content, lower  
 321 C:N and lignin:N than the native *Populus*. The P content was the highest in *Ailanthus*, while lignin  
 322 was the highest in *Robinia*, followed by *Populus* and by *Ailanthus* (Table 1).

323 **Table 1.** Initial chemical properties (mean  $\pm$  SE) of the three studied species: *Ailanthus altissima*,  
 324 *Robinia pseudoacacia* and *Populus alba*. The last columns indicate the ANOVA results comparing  
 325 species. Different letters across columns indicate significant differences ( $P < 0.05$ ).

	<i>Ailanthus</i>	<i>Robinia</i>	<i>Populus</i>	ANOVA <i>F</i>	ANOVA <i>P</i>
Litter N (mg/g)	8.79 $\pm$ 0.77 <sup>b</sup>	9.25 $\pm$ 1.04 <sup>b</sup>	4.66 $\pm$ 0.49 <sup>a</sup>	10.08	0.005
Litter P (mg/g)	0.97 $\pm$ 0.10 <sup>b</sup>	0.38 $\pm$ 0.06 <sup>a</sup>	0.40 $\pm$ 0.05 <sup>a</sup>	22.61	<0.001
Litter C (%)	29.15 $\pm$ 0.72 <sup>a</sup>	27.61 $\pm$ 0.55 <sup>a</sup>	28.25 $\pm$ 0.44 <sup>a</sup>	1.77	0.223
Litter C:N	33.17 $\pm$ 2.81 <sup>a</sup>	29.84 $\pm$ 4.26 <sup>a</sup>	60.67 $\pm$ 5.79 <sup>b</sup>	15.11	0.001
Litter lignin( %)	6.90 $\pm$ 0.26 <sup>a</sup>	11.03 $\pm$ 0.16 <sup>c</sup>	8.78 $\pm$ 0.12 <sup>b</sup>	116.93	<0.001
Lignin:N	8.11 $\pm$ 0.98 <sup>a</sup>	12.45 $\pm$ 1.61 <sup>a</sup>	19.56 $\pm$ 2.26 <sup>b</sup>	11.63	0.003

327 The decomposition rate obtained with the simple exponential model ( $k$ ) was the highest for  
 328 *Ailanthus*, followed by *Populus* and the lowest in *Robinia* (Table 2). Contrastingly, the asymptotic  
 329 model revealed that the initial decomposition rate ( $k'$ ) followed the opposite rank (*Populus*  
 330  $>$ *Robinia*  $>$ *Ailanthus*, Table 2, Online Resource Fig. S1). However, in contrast to *Ailanthus*, the  
 331 decomposition rate of *Populus* and *Robinia* litter slowed down quickly, leading to a residue ( $m$ )  
 332 of nearly two thirds of the initial mass (Table 2, Online Resource Fig. S1). According to their lower  
 333 residual standard error, the asymptotic model gave a better fit of litter decay dynamics for all  
 334 the treatments (Table 2).

335 **Table 2.** Summary of the models applied to fit the litter mass remaining to time (years). For the  
 336 simple exponential model we show the decomposition rate ( $k$ )  $\pm$  standard error (SE). For the  
 337 asymptotic model we show the initial decomposition rate ( $k'$ ) and the proportion of the initial  
 338 mass that is not decomposed ( $m$ ). For both models the residual standard error and degrees of  
 339 freedom ( $df$ ) are shown.

Treatment*	Simple exponential model			Asymptotic model			
	$k \pm SE$	Residual SE	$df$	$k' \pm SE$	$m \pm SE$	Residual SE	$df$
<i>Ailanthus</i>	1.38 $\pm$ 0.11	0.11	35	1.14 $\pm$ 0.44	0.34 $\pm$ 0.05	0.09	34
<i>Robinia</i>	0.40 $\pm$ 0.02	0.05	34	2.49 $\pm$ 0.48	0.73 $\pm$ 0.02	0.03	33
<i>Populus</i>	0.55 $\pm$ 0.05	0.08	34	4.01 $\pm$ 1.10	0.68 $\pm$ 0.02	0.05	33
A25P75	0.66 $\pm$ 0.05	0.09	35	3.73 $\pm$ 0.85	0.63 $\pm$ 0.02	0.05	34
A50P50	0.81 $\pm$ 0.06	0.08	35	1.11 $\pm$ 0.51	0.47 $\pm$ 0.07	0.08	34
A75P25	0.96 $\pm$ 0.06	0.07	35	1.66 $\pm$ 0.35	0.47 $\pm$ 0.03	0.05	34
R25P75	0.49 $\pm$ 0.04	0.07	34	5.16 $\pm$ 1.1	0.72 $\pm$ 0.01	0.03	33
R50P50	0.33 $\pm$ 0.03	0.06	35	2.62 $\pm$ 1.04	0.77 $\pm$ 0.03	0.05	34
R75P25	0.35 $\pm$ 0.03	0.07	35	4.64 $\pm$ 1.38	0.78 $\pm$ 0.01	0.04	34

340 \* Treatments: pure litter of *Ailanthus*, *Robinia* and *Populus*; mixtures between *Ailanthus* (A) or *Robinia*  
 341 (R) litter and *Populus* litter (P) at 25:75, 50:50 and 75:25%.

342 Nutrient dynamics during the decomposition of the litter differed across the three species. In  
 343 the first collection, the fraction of initial N remaining in the litter (NMR) increased above one in  
 344 the three species, indicating microbial N immobilization. This increase was particularly steep in  
 345 *Populus*, where the N litter content almost tripled. During the following collections, NMR  
 346 declined in *Ailanthus*, and remained similar in *Robinia* and *Populus* (Online Resource Fig. S2),  
 347 leading to a final net N content change of +155% in *Populus*, +54% in *Robinia* and -25% in  
 348 *Ailanthus* (Table 3). The P content of the litter (PMR) also increased in the first harvest in the  
 349 three species, again with a steeper increase in *Populus*. Later on, P slightly decreased in  
 350 *Ailanthus*, kept on increasing in *Robinia* and remained similar in *Populus* (Online Resource Fig.  
 351 S3), leading to a final net P content change of -4% in *Ailanthus*, +150% in *Robinia* and +187% in  
 352 *Populus* (Table 3). Finally, the C content of the litter, after an initial slight increase, tended to  
 353 decline through the remaining collections, leading to a net C loss in the three species, which was  
 354 the highest in *Ailanthus* (60%), followed by *Populus* (31%) and by *Robinia* (17%) (Online Resource  
 355 Fig. S4, Table 3).

356 **Table 3.** Proportion of litter mass (LMR), N (NMR), P (PMR) and C (CMR) remaining in the litter,  
 357 and soil properties (total carbon (C), total nitrogen (N), nitrate (N-NO<sub>3</sub><sup>-</sup>) and pH) in the last  
 358 collection (day=323). Values are means ± SE. The last two columns indicate the ANOVA results  
 359 comparing species, except for N-NO<sub>3</sub><sup>-</sup>, where the test was a Kruskal-Wallis. Different letters  
 360 across columns indicate significant differences ( $P < 0.05$ ).

	<i>Ailanthus</i>	<i>Robinia</i>	<i>Populus</i>	ANOVA <i>F</i>	ANOVA <i>P</i>
LMR	0.42±0.05 <sup>a</sup>	0.75±0.01 <sup>b</sup>	0.66±0.02 <sup>b</sup>	27.34	<0.001
NMR	0.75±0.10 <sup>a</sup>	1.54±0.06 <sup>b</sup>	2.55±0.19 <sup>c</sup>	53.19	<0.001
PMR	0.96±0.23 <sup>a</sup>	2.50±0.20 <sup>b</sup>	2.87±0.13 <sup>b</sup>	26.01	<0.001
CMR	0.40±0.05 <sup>a</sup>	0.83±0.03 <sup>c</sup>	0.69±0.02 <sup>b</sup>	35.09	<0.001
Soil C (%)	3.23±0.11 <sup>a</sup>	3.07±0.06 <sup>a</sup>	3.06±0.15 <sup>a</sup>	0.74	0.49
Soil N (mg/g)	2.83±0.11 <sup>a</sup>	2.72±0.08 <sup>a</sup>	2.88±0.10 <sup>a</sup>	0.77	0.48
Soil N-NO <sub>3</sub> <sup>-</sup> (µg/g)	1.12±0.10 <sup>a</sup>	0.78±0.18 <sup>a</sup>	2.03±0.58 <sup>a</sup>	3.62	0.16
Soil pH	7.88±0.02 <sup>a</sup>	7.81±0.03 <sup>a</sup>	0.79±0.05 <sup>a</sup>	1.67	0.22

361

362 Soil chemical properties and their dynamics were very similar across treatments (Online  
 363 Resource Fig. S5-S8). Soil N and soil N-NO<sub>3</sub><sup>-</sup> were similar at the beginning and at day 161, but in  
 364 the last collection (day =323) total N slightly increased, while N-NO<sub>3</sub><sup>-</sup> slightly decreased (Online  
 365 Resource Fig. S5-S6). Soil C and soil pH showed little variation through the study period (Online  
 366 Resource Fig. S7-S8). In the last collection, the ANOVA comparing soil properties across pure  
 367 litter treatments, revealed no significant difference for any of the soil properties (Table 3).

### 368 Are there interactive effects?

369 In all litter mixture treatments, LMR and CMR followed similar patterns, being equal or greater  
 370 than expected (Figs. 1-2). This indicates additive or antagonistic non-additive effects of mixtures  
 371 on mass and C decay. For *Ailanthus* mixtures, LMR was larger than expected in the 50:50 mixture  
 372 for collections taken at days 62, 11 and 253 (Fig. 1), while for *Robinia*, antagonistic effects were  
 373 only found in the last collection in the 25:75 and the 75:25 mixtures (Fig. 2).

374 In contrast to LMR and CMR, NMR and PMR in litter mixtures were equal or lower than expected  
 375 (except for PMR in the last collection of the 50:50 *Robinia:Populus* treatment), indicating  
 376 additive or synergistic non-additive effects. More synergies were observed in *Ailanthus* mixture  
 377 treatments – particularly in 25:75 and 50:50 mixtures (Fig. 1) – than in *Robinia* treatments (Fig.  
 378 2). Deviations from expected values were greater for PMR than for NMR, indicating stronger  
 379 interactions among litters for P decay (Figs. 1-2).

380

### 381 Do interactions vary through time?

382 The two-way ANOVA assessing the effects of collection date (day 62+111 versus day 253+323),  
 383 mixture treatment and their interaction on the deviation from expected values, revealed a  
 384 significant effect of collection date only for PMR in the *Ailanthus* mixtures and for LMR and CMR  
 385 in the *Robinia* mixtures. In the former, all deviations were negative, but larger in the early than  
 386 in the later phase (Fig. 1, Table 4). In the latter, the deviations from expected tended to turn

387 from negative or near zero in the early phase to positive values in the later phase (Fig. 2, Table  
388 4). The interaction between collection date and treatment was significant for *Ailanthus* CMR,  
389 where the deviation from expected was greater in the early phase for mixtures with low or  
390 medium proportion of *Ailanthus*, but the reverse occurred for the 75:25 *Ailanthus:Populus*  
391 treatment. In the case of *Robinia*, the interaction was significant for NMR, where the deviation  
392 from expected decreased from the early to the late phase in the 25:75 and the 75:25  
393 *Robinia:Populus* treatments, while the reverse occurred in the 50:50 treatment.

394 Table 4. Percentage of deviation of observed - expected values of litter (LMR), N (NMR), P (PMR) and C (CMR) mass remaining in each treatment (T) and  
 395 collection date (CD) (mean ± SE). The 2-way ANOVA columns indicate the significance of the two factors and their interaction for each of the two sets of  
 396 litter mixtures (*Ailanthus* + *Populus* on the left, and *Robinia* + *Populus* on the right).

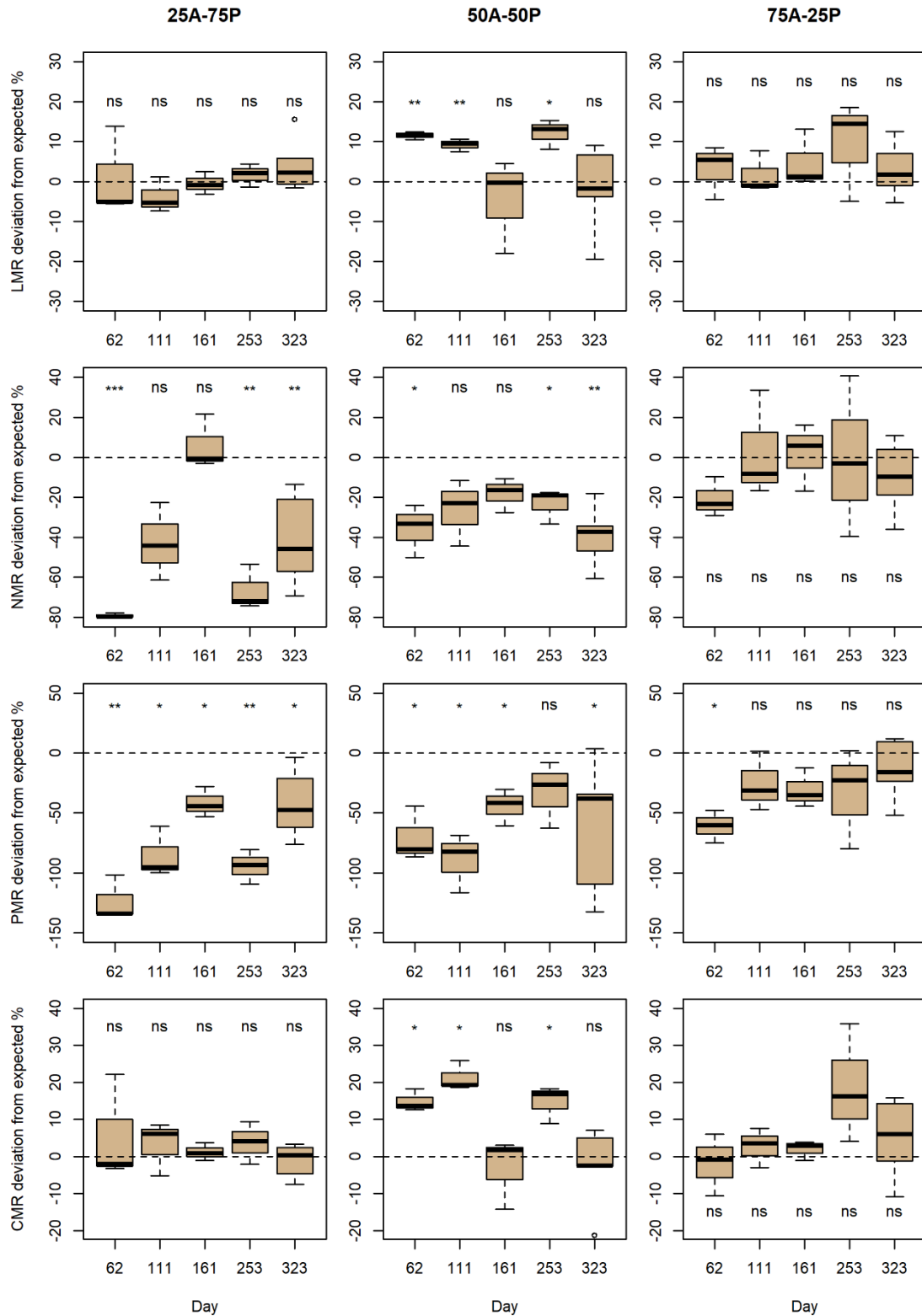
	Harvest	25A-75P	50A-50P	75A-25P	2-way ANOVA	25R-75P	50R-50P	75R-25P	2-way ANOVA
LMR(o-e)	Day 62+111	-1.4 ± 3.3	10.3 ± 0.7	2.4 ± 2.2	T: ns	-2.4 ± 1.7	5.6 ± 2.5	-0.5 ± 2.5	T: *
	Day 253+323	3.2 ± 1.8	2.8 ± 3.6	5.0 ± 2.9	CD: ns	2.2 ± 1.3	6.4 ± 2.5	6.2 ± 1.6	CD: *
					TxCD: ns				TxCD: ns
NMR(o-e)	Day 62+111	-61 ± 9.6	-31.0 ± 5.9	-8.9 ± 9.1	T:***	-26.9 ± 3.5	6.5 ± 8.6	-21.6 ± 3.3	T: ns
	Day 253+323	-50.2 ± 7.3	-33.8 ± 4.8	-6.8 ± 8.3	CD: ns	-12.3 ± 9.2	-15 ± 9.6	2.1 ± 6.2	CD: ns
					TxCD: ns				TxCD: *
PMR(o-e)	Day 62+111	-104.4 ± 11.3	-79.7 ± 9.7	-43.2 ± 10.8	T: **	-24.9 ± 12.8	42.1 ± 24.5	-15.2 ± 8.3	T: ***
	Day 253+323	-60 ± 11.3	-49.4 ± 15.0	-20.7 ± 9.9	CD: **	2.8 ± 17.6	53.2 ± 13.7	14.3 ± 9.2	CD: ns
					TxCD: ns				TxCD: ns
CMR(o-e)	Day 62+111	4.3 ± 4.2	18.0 ± 1.9	0.4 ± 2.8	T: ns	-0.2 ± 3.8	8.3 ± 4.3	-4.6 ± 5.2	T: ns
	Day 253+323	0.6 ± 1.7	3.0 ± 4.0	9.6 ± 4.4	CD: ns	2.9 ± 2.1	7.5 ± 3.2	9.7 ± 1.7	CD: *
					TxCD: **				TxCD: ns

397 Treatments: mixtures between *Ailanthus* (A) or *Robinia* (R) litter and *Populus* litter (P) at 25:75, 50:50 and 75:25%.

398 Significance of 2-way ANOVA: ns –  $P > 0.05$ ; \* –  $0.05 \geq P > 0.01$ ; \*\* –  $0.01 \geq P > 0.001$ ; \*\*\* –  $P \leq 0.001$

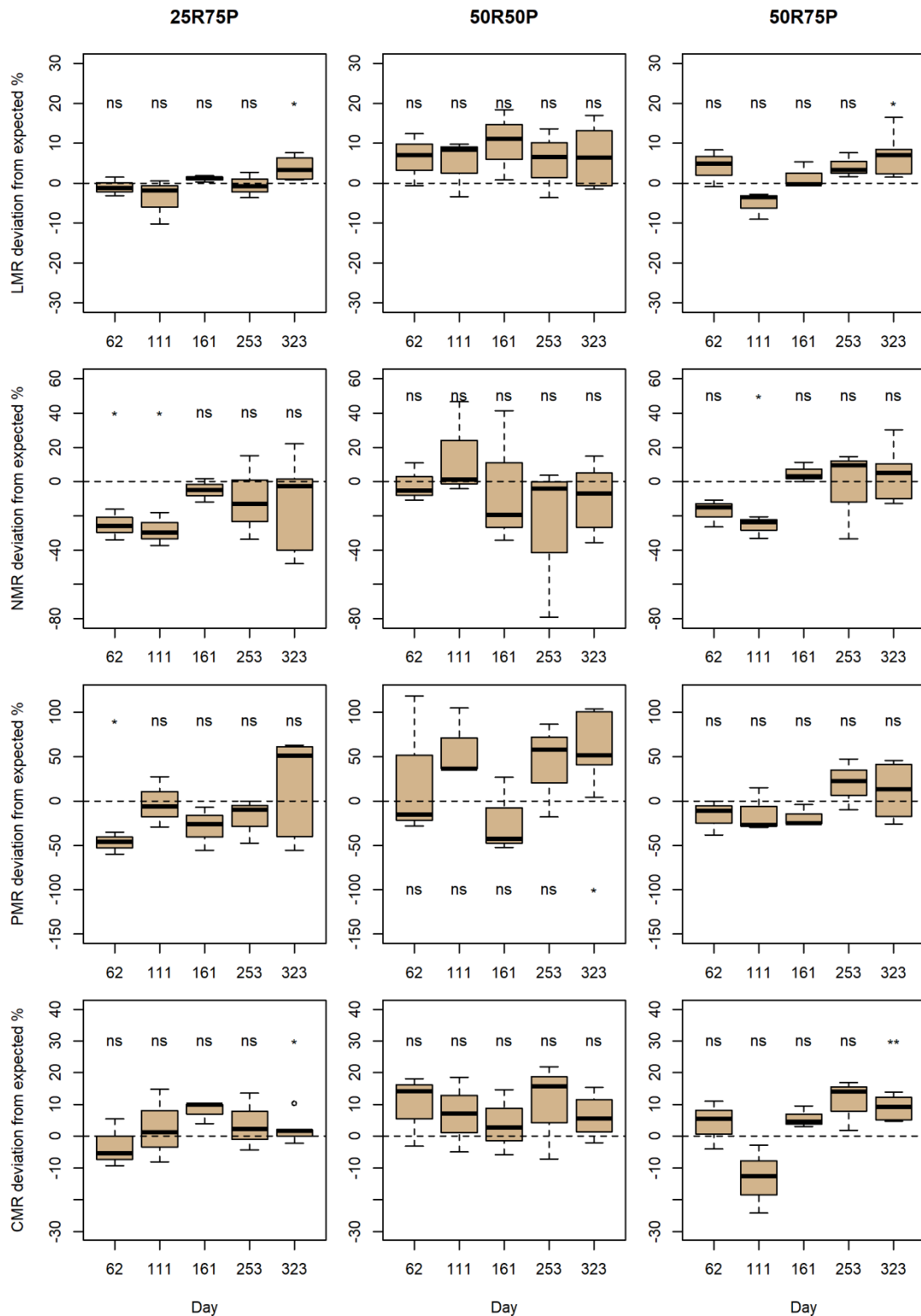
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400



401 **Figure 1.** Deviations of litter properties from the values expected under the assumption of  
 402 additive effects between *Ailanthus* and *Populus* litters in the mixtures. Litter properties were  
 403 proportions of: initial litter mass remaining (LMR), initial N mass remaining (NMR), initial P mass  
 404 remaining (PMR) and initial carbon mass remaining in the litter (CMR). Deviations are expressed  
 405 in percentage. The zero line (where observed an expected values are equal) are represented by  
 406 a dashed line. Values above zero suggest antagonistic effects, while values below zero suggest

407 synergistic effects. Asterisks indicate significant differences from zero (one-sample *t*-test,  
 408  $P < 0.05$ )



409 **Figure 2.** Deviations of litter properties from the values expected under the assumption of  
 410 additive effects between *Robinia* and *Populus* litters in the mixtures. Litter properties are  
 411 abbreviated as in Fig. 1. The zero line (where observed an expected values are equal) are  
 412 represented by a dashed line. Values above zero suggest antagonistic effects, while values below



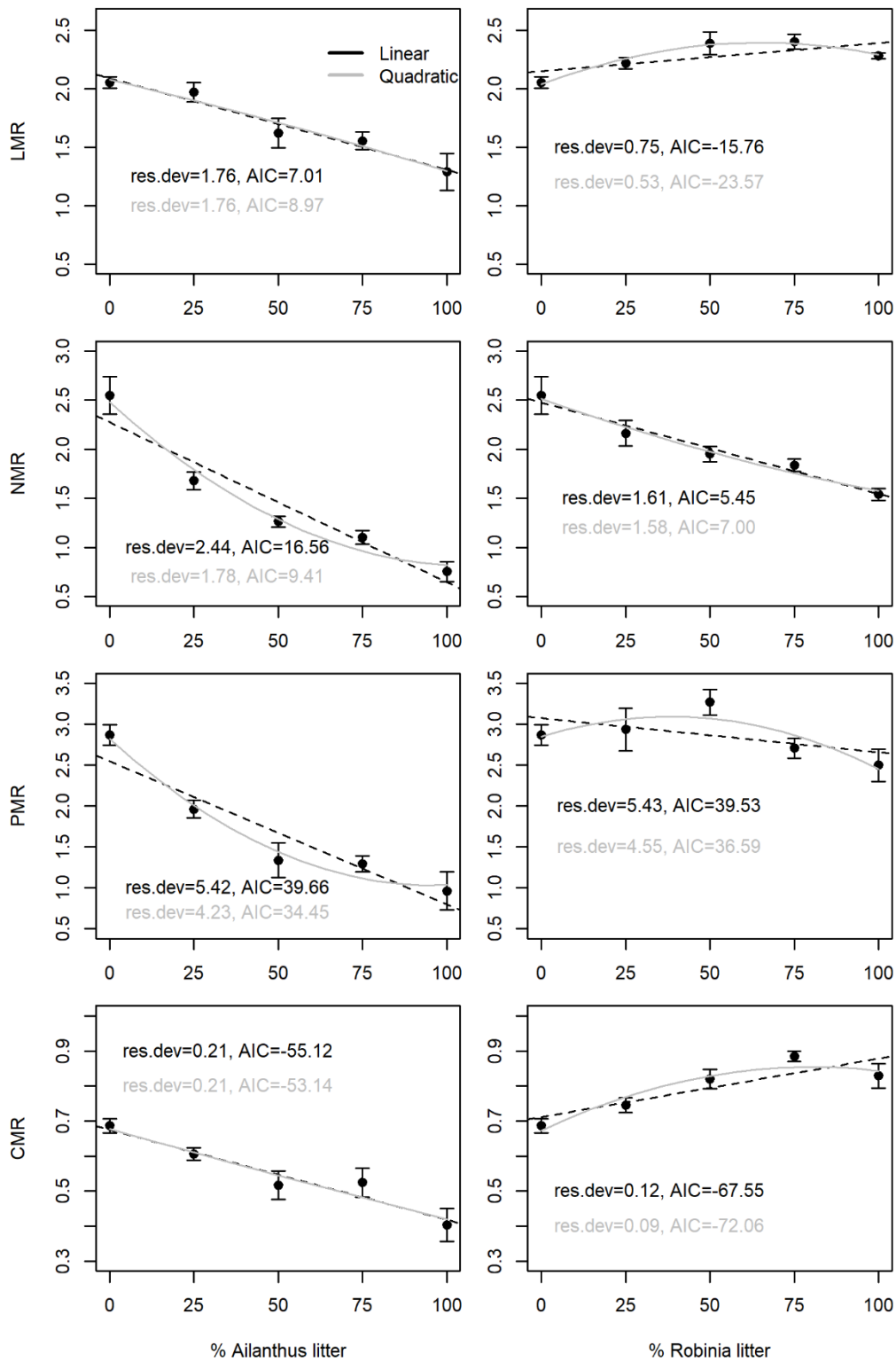
413 zero suggest synergistic effects. Asterisks indicate significant differences from zero (one-sample  
414 *t*-test,  $P < 0.05$ )

#### 415 Impact versus abundance of the non-native litter

416 In the *Ailanthus* mixtures, the two-way ANOVA showed that deviations from expected varied  
417 across treatments for NMR and PMR. In both cases, deviations were greater in the mixture  
418 where *Ailanthus* was at 25% (Table 4, Fig. 1). In the *Robinia* mixtures, treatment significantly  
419 affected the deviations from expected for LMR and PMR. In these cases, deviations were greater  
420 in the 50:50 mixtures (Table 4, Fig. 2).

421 In the *Ailanthus* gradient, the proportion of litter and nutrient mass remaining at the end of the  
422 experiment declined at increasing proportion of *Ailanthus* in the mixtures. In the case of LMR  
423 and CMR, this decrease was linear to the proportion of *Ailanthus* litter in the mixture (the AIC of  
424 the linear model was lower than that of the quadratic model). In the case of NMR and PMR, the  
425 quadratic model was the best (AIC at least two units lower than that of the linear model),  
426 showing a steeper decrease up to 50% *Ailanthus* litter proportion in the mixture, but a less steep  
427 response at higher proportions (Fig. 3).

428 In the *Robinia* gradient, LMR and CMR in the last collection increased with the proportion of  
429 *Robinia* litter in the mixtures, while NMR and PMR decreased (Fig. 3). In this case, only NMR  
430 showed a response linear to the proportion of *Robinia* litter. For the remaining variables, the  
431 quadratic model showed the best adjustment (AIC at least two units lower than the linear  
432 model), with the largest impact (i.e. the largest deviation from the value at 100% native litter)  
433 between 50 and 75% of *Robinia* litter in the mixture, and a lower impact with pure *Robinia* litter  
434 (Fig. 3).



435

436 **Figure 3.** Relation between the response variables (proportion of litter, N, P, and C mass  
 437 remaining (LMR, NMR, PMR and CMR) in the last collection) and the proportion of the non-  
 438 native litter in the mixture (*Ailanthus* left and *Robinia* right). Linear (black and dashed line) and  
 439 quadratic (grey and solid line) model fits, along with the residual deviation (res.dev) and Akaike  
 440 information criterion (AIC) are shown.

441

## 442 **Discussion**

443 Our experiment revealed that the pattern of litter decay varied across species and litter mixture  
444 treatments. However, we found little effect of litter types on soil properties at the end of the  
445 incubation period. The lack of edaphic macroinvertebrates in our soil sample probably delayed  
446 the incorporation of litter-derived material into the soil (Berg and McClaugherty 2014;  
447 Hattenschwiler et al. 2005). Also, the presence of high soil organic matter content, derived in  
448 this case from native litter, may buffer the effects of the new litter on soil properties in the short  
449 term. This result is in line with other studies reporting larger impacts of non-native plants on  
450 certain ecosystem processes or community structure than on soil properties (Berendse et al.  
451 1989; Castro-Díez et al. 2009; Castro-Díez et al. 2016; Mack et al. 2001). Thus, the time needed  
452 to translate altered litter properties into the soil may be much longer than the time needed for  
453 the expression of other impacts (Berendse et al. 1989; Castro-Díez et al. 2009; Marchante et al.  
454 2008; Muñoz Vallés et al. 2011).

### 455 How do litter decomposition dynamics differ across the three species?

456 We found different dynamics of litter and nutrient decay across the three tree species coexisting  
457 in riparian forests of central Spain: the native tree *Populus alba* and the two non-native invaders  
458 *Ailanthus altissima* and *Robinia pseudoacacia*. The initial litter properties of the invaders (higher  
459 N and lower C:N and lignin:N than the native) suggest a faster litter decay, according to previous  
460 studies on the litter properties controlling decomposition rates (Aerts 1997; Berg and  
461 McClaugherty 2014; Gallardo and Merino 1993). However, this prediction only holds for  
462 *Ailanthus*, which showed the smallest values of litter and nutrient mass remaining at the end of  
463 the experiment. By contrast, *Robinia* showed litter and nutrient decay patterns similar to those  
464 of *Populus*, in spite of its higher initial N content. Previous studies suggested that high initial N  
465 content in the litter may accelerate decomposition in the early phase, but retards it in the later  
466 phase (Berg 2000; Berg and Ekbohm 1991). This happens because low-molecular N reacts with  
467 the increasing fraction of lignin remains, turning them recalcitrant (Berg 2000; Gallardo and  
468 Merino 1993; Hattenschwiler and Vitousek 2000). This effect should be stronger in *Robinia* than  
469 in *Ailanthus* because of the higher lignin content of the former. In fact, other studies that found  
470 a relatively low decomposition rate of *Robinia* litter, attributed it to its high lignin content or  
471 lignin:nutrient ratio (Castro-Díez et al. 2009; Medina-Villar et al. 2015a). Our results are in line  
472 with recent studies suggesting that invasive species do not necessarily speed up nutrient cycles,  
473 as suggested by former reviews (Castro-Díez et al. 2014a; Ehrenfeld 2003; Pyšek et al. 2012),  
474 and this general trend may be the result of a publication bias towards the invasive species with  
475 the largest impacts (Incerti et al. 2018; Jo et al. 2016).

### 476 Are there interactions between non-native and native litter on the decomposition process?

477 We found different types of effects among the mixed litters across treatments, incubation times,  
478 and response variables. Specifically, the sign of the non-additive effects was different depending  
479 on the component of the litter considered: LMR and CMR tended to be equal or higher than  
480 expected (i.e. additive or antagonistic effects), while the NMR and PMR tended to be equal or  
481 lower than expected (i.e. additive or synergistic effects). Most studies assessing changes in N

482 and C dynamics caused by mixing different litters also report that the responses of both  
483 components were not correlated (Hattenschwiler et al. 2005; McTiernan et al. 1997; Quested et  
484 al. 2002). In our study, this apparent discrepancy can be explained by the fact that changes in  
485 NMR and PMR are due to N and P microbial immobilization, rather than to N and P decay. During  
486 the early fast decomposition period (up to day 62), N and P increased in the three litters, but  
487 more steeply in the species with faster initial decomposition (i.e. *Populus*, followed by *Robinia*,  
488 and *Ailanthus*). This increase suggests that litter mixtures are colonized by the fast-growth  
489 opportunistic microorganisms, which retain all N and P from the litter in their biomass, and take  
490 up additional N from the surroundings (Berg and McLaugherty 2014; Gallardo and Merino  
491 1992). After 62 days of incubation, N and P leveled off, or even decreased, coinciding with a  
492 deceleration of the decomposition rate (Online Resource Fig. S1, S2). Thus, lower NMR and PMR  
493 than expected in the mixtures would indicate antagonistic effects on microbial growth, rather  
494 than synergistic effects on N and P decay.

495 Previous reviews found that antagonistic interactions in litter mixtures were less frequent than  
496 synergistic interactions (Gartner and Cardon 2004; Lecerf et al. 2011). In our study, the  
497 antagonistic effects may be attributed to the relatively high lignin content of the litter of *Robinia*  
498 and *Populus*, as compared to *Ailanthus* or other coexisting riparian trees (Alonso et al. 2010;  
499 Castro-Díez et al. 2009; Medina-Villar et al. 2015a). As explained above, lignin forms complexes  
500 with proteins that are resistant to most decomposing organisms (Berg and McLaugherty 2014;  
501 Gallardo and Merino 1993; Hattenschwiler and Vitousek 2000). Thus, the transfer of lignin from  
502 *Populus* to *Ailanthus* or the accumulation of lignin from *Robinia* and *Populus* as decomposition  
503 proceeds may explain the antagonistic effects detected in some mixtures. The number and  
504 strength of the antagonistic effects were higher in *Ailanthus* mixtures, in line with previous  
505 suggestions of stronger non-additive effects among the species differing more in their litter  
506 properties (i.e. *Ailanthus* and *Populus*) (Quested et al. 2002; Wardle et al. 1997, but see Hoorens  
507 et al. 2003).

#### 508 Do interactions between litters vary throughout incubation time?

509 Previous studies detected changes in the sign of the interaction among litters throughout the  
510 process of litter decomposition and nutrient release (Chen et al. 2013; Gartner and Cardon 2004;  
511 McTiernan et al. 1997; Wardle et al. 1997). Litter decomposition has been often suggested to  
512 follow two phases (Berg and McLaugherty 2014; Gallardo and Merino 1993). The initial fast-  
513 decomposition phase occurs when most soluble and/or labile components are quickly leached  
514 or released from the litter, and it is probably driven by early-successional fast-growing bacteria  
515 (Berg and McLaugherty 2014; Elgersma and Ehrenfeld 2011). In the later phase, recalcitrant  
516 compounds, such as lignin, become dominant, explaining the slowing down of the  
517 decomposition (Berg and McLaugherty 2014; Gallardo and Merino 1993). The shift in the ratio  
518 of labile versus recalcitrant components in the decomposing substrata is probably accompanied  
519 by a shift in the community of decomposers (Berg and McLaugherty 2014; Elgersma and  
520 Ehrenfeld 2011; Gallardo and Merino 1993; Lecerf et al. 2011), which may explain changes in  
521 the strength or sign of the interactions through time.

522 In our study, interactive effects between *Robinia* and *Populus* on LMR turned from mostly  
523 additive in the early phase (days 62+111) to antagonistic – i.e. larger LMR than expected – in the

524 later phase (days 253+323). In this case, the shift may be attributed to an extraordinary  
525 accumulation of lignin from the two litters through time, as both possessed high initial lignin  
526 content. By contrast, the interactive effects between *Ailanthus* and *Populus* litter on PMR (less  
527 P immobilization than expected) were stronger in the early than in the later phase. In this case,  
528 the growth of early-successional microbes that colonizes *Ailanthus* litter (responsible for P  
529 immobilization) may be halted by the formation of recalcitrant bonds between the lignin from  
530 the lignin-rich litter of *Populus* and the proteins from the N-rich litter of *Ailanthus* (Berg and  
531 McClaugherty 2014; Gallardo and Merino 1993; Hattenschwiler and Vitousek 2000). Thus, shifts  
532 in the sign of interactions through time seem to be frequent, the direction of the shift depending  
533 on the released component and on the initial composition of the litters in the mixture. These  
534 complex interactions make it difficult to derive general conclusions by joining results from  
535 experiments performed with different litters and with different time schedules, as highlighted  
536 before (Hattenschwiler et al. 2005).

### 537 Are the impacts linear to the abundance of the non-native litter?

538 Most studies assessing the effects of plant invasions compare heavily invaded or monocultures  
539 with uninvaded situations (Strayer et al. 2006; Yokomizo et al. 2009). Although this is useful for  
540 identifying potential impacts, it does not inform on the impacts of invasions at intermediate  
541 abundances. This lack of information often leads researchers or managers to assume a linear  
542 relation between the impact and the abundance of the invader (Parker et al. 1999). However,  
543 we found in this study that the linear relation was the least frequent, only standing in three out  
544 of the eight case studies (two invaders x four response variables). For the remaining cases, the  
545 impact (assessed as deviation from the non-invaded situation) increased faster from zero to low  
546 or mid abundance, and slower –even leveling off or decreasing– from mid to high abundance.  
547 These non-linear responses may be attributed to the fact that the strength of the interactions  
548 across litters depended upon the proportion of the invader in the mixtures, being the highest  
549 when the invader was 25% (*Ailanthus* impact on NMR and PMR) or 50% (*Robinia* impact on LMR,  
550 PMR and CMR). This finding has important implications for the management of these invasive  
551 trees in riparian forests of central Spain, and suggests that the most effective reduction of their  
552 impacts on litter decomposition would occur at low (*Ailanthus*) or medium (*Robinia*)  
553 abundances. By contrast, at high abundance, the benefit of a small reduction of the invasive tree  
554 cover will be proportionally smaller (but more costly). Indeed, as previously reported, knowing  
555 the shape of the impact-abundance curve is crucial for defining an optimal strategy for the  
556 management of invasive plants (Sofaer et al. 2018; Yokomizo et al. 2009).

### 557 Limitations of the study and future perspectives

558 Using microcosm experiments, we have demonstrated that mixing the litter from native and  
559 invasive species of central Spain riparian forests may have additive or antagonistic effects on  
560 litter decomposition. We have also shown that in most cases the invader's litter impacts are not  
561 linear to abundance. Our approach has the advantage of allowing a tight control over  
562 environmental conditions, and the use of a standard soil allowed us addressing the potential  
563 effect of different litters on soil properties. However, the extent to which these results can be  
564 extrapolated to the field remains to be further explored, due to several limitation of our  
565 approach. First, our microcosms lack macroinvertebrates. These organisms play a major role on

566 the litter decomposition, fragmenting the litter and digesting the raw organic matter, facilitating  
567 further processing by smaller invertebrates and bacteria (Berg and McClaugherty 2014;  
568 Hattenschwiler et al. 2005). The structure and function of the macroinvertebrate community  
569 may be altered by the non-native litter (Gutiérrez-López et al. 2014; Medina-Villar et al. 2015a),  
570 which in turn may alter the effects that the non-native litter have on the decomposition  
571 processes (Hattenschwiler and Gasser 2005). Moreover, some studies revealed that the sign of  
572 interactions among litter species may shift depending on the presence or absence of certain  
573 macroinvertebrates (Hattenschwiler and Gasser 2005; Hattenschwiler et al. 2005). Second,  
574 although here we did not find differential effects of the litter mixtures on soil properties,  
575 differences may probably arise in the long term, and such difference might interfere with the  
576 litter decomposition process. Third, although our experiment covered an incubation period  
577 longer than most decomposition studies, the final proportion of litter mass remaining was still  
578 quite high (42-75%), so we cannot provide information about litter interactions in later phases  
579 of decomposition. Fourth, we have only observed above-ground effects of the non-native leaf  
580 litter, but several studies suggest that the belowground plant inputs (root litter and exudates)  
581 have stronger effects on soil processes than above-ground plant inputs (Brant et al. 2006; Keith  
582 et al. 2009; Pollierer et al. 2007). Besides, other components of the litter, differing from leaves  
583 in physical-chemical properties (inflorescences, fruits, or bark) may represent important inputs  
584 of organic matter to the soil in certain periods of the year (Castro-Díez et al. 2014b; Medina-  
585 Villar et al. 2015b). Finally, microclimatic conditions in the field vary throughout the seasons,  
586 and these variations may interfere with the interactions of the litter mixtures (Aerts 1997; Berg  
587 and McClaugherty 2014; Gallardo and Merino 1993). Thus, future research should include  
588 macroinvertebrates and other litter components in microcosms and/or combine microcosms  
589 with field experiments.

## 590 Conclusions

591 *Ailanthus altissima* can accelerate the litter decomposition in forests dominated by *Populus alba*  
592 due to the production of high-N, low-lignin, fast-decomposing litter. However, the antagonistic  
593 effects observed in some litter mixtures might buffer this acceleration. By contrast, *Robinia*  
594 *pseudoacacia* would delay litter decomposition in *Populus* forests, probably due to the high lignin  
595 content of its litter. In this case, the delay may be even exacerbated by the antagonistic effects  
596 on decomposition observed in litter mixtures, mainly when both litters have similar proportions  
597 in the mixture. Effects of non-native litters on soil properties were negligible in the time-scale of  
598 this experiment (ca. 1 year). The final effects of the non-native litters on different variables  
599 characterizing the decomposition process were non-linear in most cases, with greater changes  
600 when the abundance increased from zero to 25 or 50% of the mixture. Impacts leveled off or  
601 even decreased at greater abundances. This suggests that control efforts to minimize impacts  
602 on litter decay should focus on the earlier or mid stages of the invasions, where the reduction  
603 of the non-native tree abundance would have greater benefits. Future studies should include  
604 macroinvertebrates in microcosm designs and/or should combine microcosms with field  
605 experiments.

606

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613

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848

## 849 **Supplementary Material**

850 This article contains the following Supplementary Material:

851 **Figure S1.** Litter mass remaining of pure litter of *Ailanthus altissima* (100A), *Robinia*  
852 *pseudoacacia* (100R), *Populus alba* (100P), and different litter mixtures along the incubation

853 period (*Ailanthus:Populus* litter mixtures at 25:75, 50:50, 75:25 proportions are named 25A-  
854 75P, 50A-50P and 75A-25P. *Robinia:Populus* litter mixtures at the same proportions as above  
855 are named 25R-75P, 50R-50P, 75R-25P, respectively). The red line represents the exponential  
856 model fit, while the blue line represents the asymptotic model.

857 **Figure S2.** Proportion of initial nitrogen (N) mass remaining in the pure litter of *Ailanthus*  
858 *altissima* (100A), *Robinia pseudoacacia* (100R), *Populus alba* (100P), and different litter  
859 mixtures (abbreviations as in Fig. S1) during the incubation period.

860 **Figure S3.** Proportion of initial phosphorous (P) mass remaining in the pure litter of *Ailanthus*  
861 *altissima* (100A), *Robinia pseudoacacia* (100R), *Populus alba* (100P), and different litter  
862 mixtures (abbreviations as in Fig. S1) during the incubation period.

863 **Figure S4.** Proportion of initial carbon (C) mass remaining in the pure litter of *Ailanthus*  
864 *altissima* (100A), *Robinia pseudoacacia* (100R), *Populus alba* (100P), and different litter  
865 mixtures (abbreviations as in Fig. S1) during the incubation period.

866 **Figure S5.** Soil N content in different pure litter and litter mixture treatments (abbreviations as  
867 in Fig. S1) during the incubation period.

868 **Figure S6.** Soil nitrate content (N-NO<sub>3</sub><sup>-</sup>) in different pure litter and litter mixture treatments  
869 (abbreviations as in Fig. S1) during the incubation period.

870 **Figure S7.** Soil carbon content (C) in different pure litter and litter mixture treatments  
871 (abbreviations as in Fig. S1) during the incubation period.

872 **Figure S8.** Soil pH in in different pure litter and litter mixture treatments (abbreviations as in  
873 Fig. S1) during the incubation period.

874