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Leaf litter mixtures and neighbour effects: Low-nitrogen and high-lignin species increase decomposition rate of high-nitrogen and low-lignin neighbours

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ABSTRACT

In natural ecosystems plant litter is typically a mixture of more than one species and the rate of decomposition can be faster (synergistic) or slower (antagonistic) than the average of its component species (non-additive effects). We analysed the decomposition rates of two-species mixtures to determine if there were consistent non-additive effects of litter mixing on decomposition and how do they compare with the effects of species identity on mixture decomposition. Then we tested if non-additive effects were consistently associated with the presence of particular species in the mixture, to the combination of Fast- or Slow-decomposing species, or to initial litter quality of mixtures. We found: (a) that species identity was the primary determinant of the decomposition rate of mixtures, and (b) we detected significant, but weak, non-additive effects which were consistently synergistic in the most chemically heterogeneous mixtures. However, slower decomposing species appeared to increase the decomposition rate of faster decomposing species (30 times out of 41 after 2 months of incubation, and 17 times out of 24 after 9 months of incubation). During the initial stages of decomposition, low-lignin mixtures showed mostly synergistic effects, whereas high-lignin mixtures showed antagonistic effects. At more advanced stages of decomposition, mixtures containing species with highest difference in initial N content had more synergistic effects, whereas those with similar initial N content showed both synergistic and antagonistic effects. Our results confirm previous findings about the importance of chemical heterogeneity of mixtures as a driver of decomposition rates of litter mixtures. We propose that mechanisms related to carbon priming may be related to synergistic effects in most heterogeneous mixtures, while nitrogen interaction with carbon may be resulting in antagonistic effects in homogeneous and Slow-decomposing species mixtures.

1. Introduction

Leaf litter quality is known to be a major driver of species decomposition rates across biomes (Swift et al., 1979; Cadisch and Giller, 1997; Cornwell et al., 2008). During early stages of decomposition, nutrients such as nitrogen and phosphorus, and water-soluble compounds have the largest effects, whereas at later stages, lignin is the primary determinant of decomposition dynamics (Berg and Staaf, 1980; Berg, 2000; Rahman et al., 2013). As a consequence of their structural and chemical attributes, each species, when incubated in isolation, has a characteristic decomposition rate ("decomposability", Pérez Harguindeguy et al., 2013). However, in nature litter typically falls and decomposes in mixtures and physicochemical interactions between decomposing leaves can increase or decrease decomposition up to 30% of their expected mass loss (Hättenschwiler and Gasser, 2005; Hoorens et al., 2010a; Tardif et al., 2013). Previous research has shown that mixtures may decompose faster (synergistic effect Seastedt, 1984; Taylor et al., 1989a; Wardle et al., 1997; Salamanca et al., 1998) or slower (antagonistic effect – Zech and Kogel-Knabner, 1994; Dijkstra et al., 2009) than expected relative to the decomposition rates of their component species in isolation, i.e., non-additive effects. Non-additive effects are mainly driven by the mixture components, such as the presence of Fast- or Slow-decomposing species, the magnitude of the difference in decomposability between the mixture components, the physical characteristics of litter that increase its water retention capacity, or the presence of recalcitrant compounds (Gartner and Cardon, 2004; Hättenschwiler et al., 2005). Among the

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mechanisms proposed to explain synergistic effects on decomposition in heterogeneous mixtures nutrient transfer from litter of high quality to litter of lower quality has been frequently invoked (McTiernan et al., 1997; Kuziakov et al., 2000) but not always confirmed (Staaf, 1980; Klemmedson, 1992; Hoorens et al., 2003). Antagonistic effects have been mainly related to the presence of recalcitrant compounds such as lignin and phenols which may form resistant complexes with proteins (Hättenschwiler and Vitousek, 2000), inhibiting microbial growth and activities (Schimel et al., 1998). In any case, only a small number of those studies separated the components of the mixture after incubation and were able to test if nutrient transfer had indeed occurred or which element (carbon, nitrogen or other) has changed as result of litter mixing (Staaf, 1980; Hoorens et al., 2010b).

Here we present the results of an analysis of decomposition in two-species mixtures incubated in litter-bags in a common garden experiment. By keeping the number of species constant we avoided richness effects (Pérez Harguindeguy et al., 2008). Initially, we determined if there were consistent synergistic or antagonistic effects on the decomposition of litter mixtures (i.e., non-additive effects, Hättenschwiler et al., 2005) and how important they were compared with the effects of species identity. We then tested whether the observed effects were related to specific combinations of Slow- and Fast-decomposing species or to the decomposability of the other component in the mixtures. We also tested whether non-additive effects were related to initial litter quality or decomposability, or to initial heterogeneity in litter quality of each mixture specifically in their content of carbon, nitrogen, and lignin.

We predict that as the heterogeneity of the mixture increases (for example in Slow–Fast mixtures) decomposition rate of the mixture will be greater than expected (synergistic effects). Specifically, we predict that nutrient-rich Fast-decomposing species will transfer nutrients to Slow-decomposing species and thereby increase the decomposition rate of the Slow-decomposing species. Within less heterogeneous mixtures, we expect to find antagonistic effects in Slow–Slow mixtures associated with the presence of recalcitrant compounds, such as lignin. Finally, we predict that if synergistic interactions do occur, after decomposition nitrogen (or carbon) will be lower than expected in at least one of the mixture components. If antagonistic interactions occur, nitrogen (or carbon) will be higher than expected in at least one of the mixture components. In turn, if antagonistic interactions are caused by the formation of nitrogen–lignin resistant complex (Berg, 1986; Camiré et al., 1991), the higher nitrogen in mixtures showing antagonistic interactions should be associated with high initial lignin content in one of the components of that mixture.

2. Materials and methods

2.1. Species selection

We collected leaf material from eight dominant species in the Sierras Chicas, Córdoba Mountains, central Argentina, from May to August 2007, depending on the peak litter fall of each species. The vegetation in the area is characteristic of Chaco montane woodlands (Luti et al., 1979). The climate is temperate/warm temperate, with rainfall concentrated in the warm season (October–April). Annual precipitation is about 850 mm and mean annual temperature is 15 °C (De Fina, 1992). The selected species have leaves that can be clearly classified as either of Fast- or Slow-decomposing species, based on the results of previous studies on decomposition dynamics (Vaieretti et al., 2005). The Fast-decomposing species selected were: *Acalypha communis* Mull. Arg., *Ambrosia tenuifolia* Spreng., *Celtis ehrenbergiana* (Klotzsch) Liebm., and *Zanthoxylum coco* Gillies ex Hook. f & Arn., and the Slow-decomposing species were: *Acanthostyles buniifolius* (Hook. & Arn.) R.M. King & H. Rob., *Lithraea molleoides* (Vell). Engl., *Schizachyrium condensatum* (Kunth) Ness, and *Jarava ichu* Ruiz & Pav. var. *ichu* (Table 1).

2.2. Litter preparation

We collected fresh litter of at least 10 individuals of each of the eight species and determined decomposition rate using the widely used nylon bag technique (Bocok and Gilbert, 1957; Schlesinger, 2000; Vaieretti et al., 2005; Pérez Harguindeguy et al., 2007). We prepared litter-bags following the methodology of Cornelissen (1996) and Pérez Harguindeguy et al. (2013); all litter was air-dried (±0.1 g), weighed, and sealed in 0.3 mm mesh nylon bags. This mesh size excludes most macrofauna which have been shown to contribute little to the decomposition process (Vaieretti et al., 2010) but permits entry of mesofauna, bacteria, protozoa and fungi, the major decomposers in our system. To estimate litter water content, we oven-dried subsamples of air-dried litter at 60 °C for 48 h. Difference in leaf litter mass as a correction factor of the initial dry mass of each sample.

2.3. Decomposition treatments

We made all possible 2-species combinations of the Slow–Slow and Fast–Fast mixtures. Of the 16 possible combinations of Fast–Slow mixtures we randomly selected eight in order to improve the balance in the number of Slow–Slow and Fast–Fast combinations. Overall, we made 20 species combinations: six Slow–Slow combinations, six Fast–Fast combinations, and eight Slow–Fast combinations. We incubated all mixtures, together with individual species samples, on a decomposition bed (1.0 ± 0.1 g for single species; and 0.5 g ± 0.1 of each component species in mixtures). We performed 10 replicates (per period of incubation) of the individual species samples and of each of the two-species combination.

To maintain almost natural and homogeneous conditions during the decomposition process, we incubated all samples simultaneously in a 4 m × 3 m purpose-built decomposition bed (common garden experiment) placed within the area where the litter had been collected. Before placing the litterbags, we cleaned the area by removing the most conspicuous plants, stems and litter. Then, we randomly placed litterbags on the decomposition bed and covered them with natural litter previously removed from the area. We protected the bed from damage by birds and small mammals by placing 3 cm-mesh galvanised metal net over the top of it. Samples remained in the decomposition bed for either 2 or 9 months (total period: January–September 2008, i.e., summer–winter–spring), under the natural temperature and rainfall conditions of the area.

After incubation we retrieved 10 replicates from each species and mixture and stored them at −14 °C until processing in the laboratory. Samples were defrosted and carefully cleaned by manually removing adhering soil and extraneous material. Samples were then dried for 48 h at 60 °C and weighed. Decomposition rate was defined as the percentage of litter mass loss (%LML) during the incubation period.

We were able to separate, and independently weight, only the component species of Slow–Slow and Fast–Slow mixtures. Fast–Fast mixtures were too decomposed and we were unable to identify and separate their component species; hence, they were not included in the analysis of neighbour effect (Hoorens et al., 2010b).

2.4. Litter chemical quality

We determined the initial litter quality of each species and then calculated initial litter quality of mixtures (as the average quality of

Table 1
Litter chemical quality and decomposition (standard errors between brackets) of species used in litter mixture treatments. Significant differences between species groups (Fast- and Slow-decomposing species) are indicated with an asterisk (Tukey test, $p \leq 0.05$).

	C (%)		N (%)		Lignin (%)	Cellulose (%)	Hemicellulose (%)	Decomposition (%LML)	
	Initial	Final	Initial	Final				Two months	Nine months
Fast-decomposing species									
Acalypha communis (Ac)	45.8	32	1.8	2.4	4.3	20.4	3.1	52.5 (2.8)	64.1 (2.4)
Ambrosia tenuifolia (At)	45.2	34.4	2.4	2.7	10.4	22.3	2.8	58.0 (1.3)	64.6 (0.9)
Cetis ehrenbergiana (Ce)	39.9	24.3	2.1	2	5.5	13.6	2.9	54.1 (0.9)	62.4 (1.9)
Zanthoxylum coco (Zc)	46.7	32.2	2.1	3.1	7.6	14.1	2.3	74.6 (1.3)	81.7 (1.3)
Fast-decomposing species average	44.4 (1.5)	30.7 (2.2)	2.1 (0.1)*	2.6 (0.2)	6.9 (1.3)	17.6 (2.2)	2.8 (0.2)	59.8 (1.6)*	68.2 (1.6)*
Slow-decomposing species									
Acanthostyles buniifolium (Ab)	47.5	48	1.5	2.1	20.9	17.7	0.3	31.0 (1.6)	35.3 (1.9)
Lithraea molleoides (Lm)	51.3	47.4	1.2	2.5	12.7	10.8	1	30.1 (1.6)	31.7 (0.8)
Schizachyrium condensatum (Sc)	48.6	38	0.9	1.4	14.3	37	16.3	19.2 (3.3)	30.1 (3.1)
Jarava ichu (Ji)	52.3	41.9	0.6	1.4	10.3	44.5	23.7	24.6 (2.3)	29.9 (2.3)
Slow-decomposing species average	49.9 (1.1)*	43.8 (2.4)*	1 (0.2)	1.9 (0.3)	14.5 (2.3)*	27.5 (7.9)	10.3 (5.8)	26.3 (2.2)	31.8 (2.0)

its component species) on additional samples of the same leaf litter used for the decomposition experiment. We determined Carbon (C) and Nitrogen (N) content using an Alpkem RFA 300 autoanalyzer (Alpkem, Wilsonville, OR, USA). We also measured lignin, cellulose and hemicellulose contents following Goering and Van Soest (1970). From these data, we calculated the lignin/nitrogen ratio, the holocellulose/hemicellulose ratio (HLQ = (cellulose + hemicellulose)/lignin + cellulose + hemicellulose)), and the lignocellulose index (LCI = lignin/(lignin + cellulose + hemicellulose)), which are typical indicators of litter quality related to decomposability (Berg et al., 1984; McClaugherty and Berg, 1987; Cortez et al., 1996; Cadisch and Giller, 1997). We also calculated total fibre content in litter (LCH = lignin + cellulose + hemicellulose), which is an accurate predictor of decomposition dynamics on this flora (Vaieretti et al., 2005).

After sample incubation, we measured total remaining N and C content in all species decomposing in isolation and in all mixtures. For these determinations we used a Flash EA 1112 NC Soil analyser (Thermo Electron Corporation). Unfortunately, because the remaining material was so scarce, we were not able to perform a separate chemical analysis of the component species in each two-species mixture.

2.5. Data analyses

For both species in any mixtures we recorded species identity (species) and the identity of the other species (neighbour species). We then conducted ANOVAs using the linear modelling function (lm) in SPSS (SPSS for Windows, version 11.5.1) to compare the magnitudes of species and neighbour species effects on the decomposition of each mixture. We ran separate models for both incubation periods: 2 months and 9 months.

To evaluate the existence of consistent non-additive (synergistic or antagonistic) effects on decomposition we measured an observed mass loss (ObservedML) and calculated an expected decomposition (ExpectedML) for each species and mixture. Observed ML was the average percentage mass loss of 10 replicates after each incubation period (2 and 9 months); ExpectedML of mixtures was calculated using 10 pair-wise random averages of samples of the component species decomposing in isolation (Wardle et al., 1997; Pérez Harguindeguy et al., 2008). Thus, internal variation in decomposition within species was taken into account.

Based on the observed and expected decomposition values, we also calculated mixing effects (ME, or the interaction effect between litters), following Wardle et al. (2003), using the ratio:

$$\text{Mixing effects} = \frac{\text{ObservedML} - \text{ExpectedML}}{\text{ExpectedML}}$$

where positive values indicate synergistic effects and negative values indicate antagonistic interaction effects between species.

We assessed differences between observed and expected values for each two-species mixture using Student paired *t*-tests. Complementary, we grouped litter mixtures according to their composition: Slow–Slow mixtures, Fast–Fast mixtures and Slow–Fast mixtures, and compared differences between observed and expected values of those groups using Student paired *t*-tests.

To specifically evaluate the effect of each species (A) on the decomposition of its neighbour species (B), or neighbour effect (Hoorens et al., 2010b), we used the results of all litter-bags where we were able to separate and individually weight its component species. For each of those litter bag we calculated the effect of species A on B by subtracting the ExpectedML of species B, as incubated in isolation, from the ObservedML of the same species (B), in the presence of species A, as follows:

$$\text{Neighbour effect}_{\text{A on B}} = \text{ObservedMLB} - \text{ExpectedMLB}$$

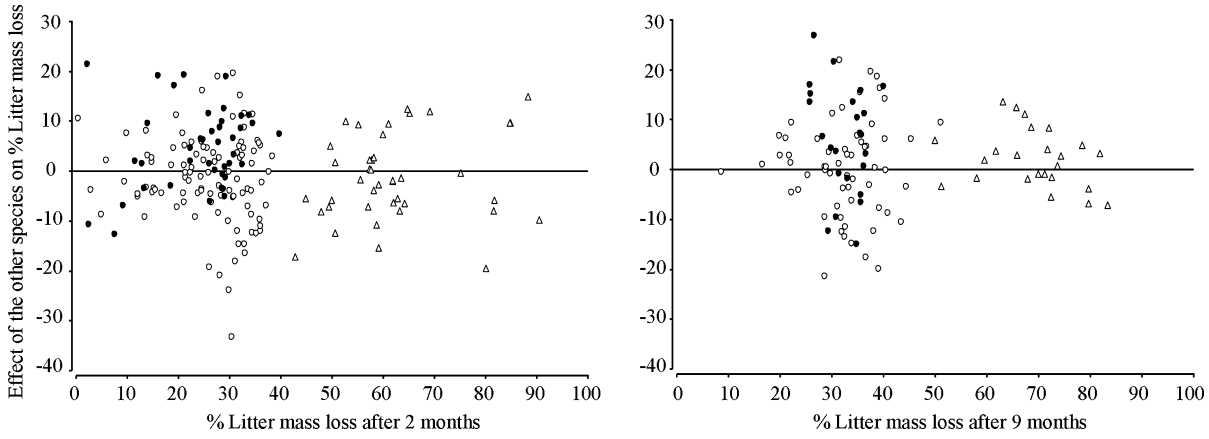

Fig. 1. Effect of "the other species" on % litter mass loss. Relationship between the % litter mass loss of the species after 2 and 9 months of incubation and the effect of these species on the % litter mass loss of the other species in the mixture ($r_{2m} = 0.004$, $p = 0.42$; $r_{9m} = 0.001$, $p = 0.80$). Positive values of the *Y* axis indicate synergistic effects on decomposition rates (accelerated loss rates) and negative values correspond to antagonistic effects on decomposition rates (depleted loss rates). Circles indicate the effects of Slow-decomposing species on the other Slow-decomposing species (white circles) and on Fast-decomposing species (black circles). Triangles indicate the effects of Fast-decomposing species on Slow-decomposing species. Each point in the graph is the average effect of one species (A) on other species (B), and then the average effect of the second species (B) on the first species (A), so each litter mixtures is twice in the graph.

Then we did the same procedure but to calculate the effect of species B on species A (subtracting the ExpectedML of species A, as incubated in isolation, from the ObservedML of the same species (A), in the presence of species B). The neighbour effect values of A on B (neighbour effect$_{A\ on\ B}$) were then plotted against the ObservedML of species B, while the neighbour effect values of B on A (neighbour effect$_{B\ on\ A}$) were plotted against the ObservedML of species A (the same procedure for all mixtures). Note that litterbags of each mixture are shown twice in the graph because all species are present both as target species and as 'the other species' (Hoorens et al., 2010b). As proposed by Hoorens et al. (2010b), if a species has no effect on the other species in a mixture, then the difference between ObservedML and ExpectedML of each species would be zero. If a species tended to accelerate the decomposition of another species (synergistic effect), the difference would be greater than zero, whereas if a species tended to decelerate the decomposition of another species, then the difference would be less than zero (antagonistic effect). We used Student *t*-tests to evaluate if the neighbour effect of Slow-decomposing species on other Slow-decomposing species, Slow on Fast and Fast- on Slow were significantly different from zero.

We calculated the coefficient of variation (CV) of all the quality variables measured (N, lignin, lignin/nitrogen ratio, HLQ, LCI and LCH) for each mixture based on data for the initial litter quality of species. CV was also used to determine the initial chemical heterogeneity of the mixtures following Wardle et al. (1997).

To confirm that initial litter quality was a good predictor of species and mixtures decomposition we correlated decomposition with all litter quality variables using Spearman correlations. Then, to find a combination of initial litter quality variables that could explain mixing effects, we performed a backward regression procedure. We regressed the dependent variables (mixing effects) against initial chemical quality, and against initial chemical heterogeneity of mixtures. We checked normal distribution and dispersion plots of residuals to test independence of errors and homogeneity of variance (Sokal and Rohlf, 1995). All statistical analyses were carried out using SPSS (SPSS for Windows, version 11.5.1).

Finally, to test if the mechanisms proposed were reflected by the remaining nutrient content of the mixtures, we compared the observed and the expected C and N content of the mixtures using a Student paired *t*-test. We tested if synergistic effects were associated with lower N or C content in the mixture, and if antagonistic effects were associated with higher N or C content of the mixtures, by evaluating the final content of the two components in the mixtures.

3. Results

3.1. Species litter quality and decomposition

In general, and confirming our assumption, Fast-decomposing species had lower C and lignin content and higher N content than Slow-decomposing species (Table 1). The decomposition rate of individual species was fastest in species that had initially high N content (Table 1). Similarly, the decomposition rate of mixtures was fastest in mixtures that had initially high levels of N and low lignin:N ratios (Supplementary Table 1).

3.2. Species identity vs. neighbour identity

ANOVA showed that species identity had, by far, the strongest influence on mixture decomposition rate in both periods of incubation ($R^2 = 0.88$ at 2 months of incubation; $p = 0.0001$; $R^2 = 0.87$ at 9 months of incubation; $p = 0.0001$). The influence of neighbour identity was much weaker than species identity ($R^2 = 0.01$ at 2 months of incubation; $p = 0.005$; $R^2 = 0.02$ at 9 months of incubation; $p = 0.001$). In other words, the decomposability of the mixtures was mainly driven by the decomposability of its component species, with a small, but significant, influence of the neighbour (or other) species in the mixture.

The regression of 'neighbour effects' against litter mass loss of the neighbour was not significant (Fig. 1). Most species either increased or decreased decomposition of the neighbour species in the mixture at a rate irrespective of the other species identity. Only the Slow-decomposing species appeared to increase the decomposition of Fast-decomposing species (black circles in Fig. 1; 30 times out of 41 after 2 months of incubation, *t*-test, $p = 0.001$; and 17 times out of 24 after 9 months of incubation, *t*-test, $p = 0.01$). Fast-decomposing species did not increase decomposition rates of species that decompose slowly in isolation.

3.3. Mixing effects and mixture composition

On average, Slow–Slow mixtures showed antagonistic effects after 2 months of decomposition (Fig. 2, and Supplementary Fig. 1

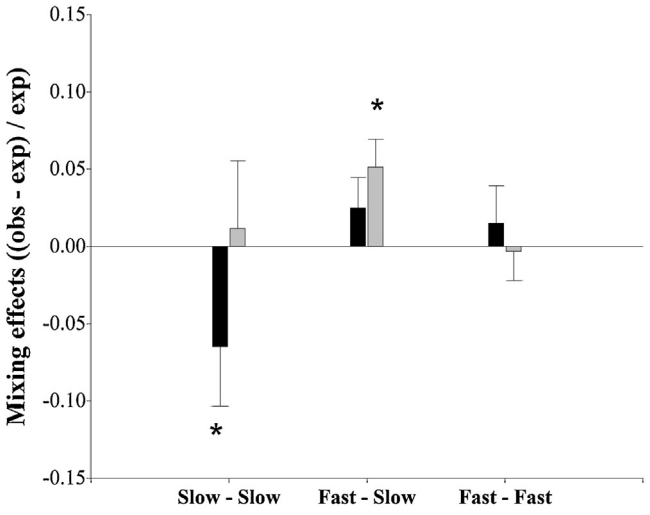

Fig. 2. Mixing effects of the same litter mixture composition after two and nine months of decomposition. Mixtures with two Slow-decomposing species (Slow–Slow), mixtures with two Fast-decomposing species (Fast–Fast) and mixtures with one Fast- and one Slow-decomposing species (Fast–Slow). Black columns indicate the mixtures after 2 months of incubation and grey columns, the mixtures after 9 months of incubation with the corresponding standard error bars. Mixing effects different from zero are indicated with an asterisk (*t*-test, $p \leq 0.05$).

for details on all mixtures) while Fast–Slow mixtures showed synergistic effects after 9 months of incubation (Fig. 2). Decomposition of Slow–Slow mixtures was 6% lower than expected after 2 months of incubation, whereas decomposition of Fast–Slow mixtures was 5% higher than expected after 9 months of incubation.

The individual analysis of decomposition on the component species of each mixture (component species separated and individually weighed) showed that none of the component species in Slow–Slow mixtures decomposed significantly more slowly than expected (species 1: %ObservedML = 29.8, %ExpectedML = 30.6; $p = 0.44$, species 2: %ObservedML = 21.7, %ExpectedML = 24.0; $p = 0.13$). This result, which cannot confirm the pattern shown by the average decomposition of Slow–Slow mixtures, suggests that when dealing with such small differences between observed and expected values (about 5%) it can be difficult to identify the source of variation in the component species. In Fast–Slow mixtures, Fast-decomposing species decomposed significantly faster than expected, and caused the synergistic pattern detected (%ObservedML = 69.1, %ExpectedML = 63.1; *t*-test, $p = 0.01$).

3.4. Mixing effects and heterogeneity and quality of litter mixtures

Of all the initial litter quality variables considered, only initial lignin content was significantly associated with mixing effects after 2 months of incubation (Fig. 3a). Although the variance explained was low (18%) at initial stages of decomposition litter mixtures containing species with low-initial lignin content had mostly synergistic effects (7 out of 11 mixtures), whereas high-lignin content mixtures showed mainly antagonistic effects (6 out of 9 mixtures). This pattern is consistent with the high lignin content of Slow–Slow mixtures, which were the ones showing significant antagonistic effects. None of the initial quality variables measured, or their combination (C, N, lignin, lignin:N, cellulose, hemicellulose, HLQ, LCI or LCH) accounted for any of the mixing effects after 9 months of incubation.

When we explored the relationships between mixing effects and the heterogeneity of the components of the mixture we found that after 9 months of incubation, mixtures containing species with different initial N content (i.e., higher CV N) had more synergistic effects. Mixtures with similar initial N content showed both synergistic and antagonistic responses (Fig. 3b). Accordingly, Fast–Slow mixtures generally showed the highest initial chemical heterogeneity and synergistic responses. In Fast–Slow mixtures we observed a significant synergistic effect only after 9 months of decomposition (Fig. 2).

3.5. Carbon and nitrogen content in decomposed litter mixtures

The analysis of final C and N content in mixtures showed that Slow–Slow mixtures, which have shown significant antagonistic effects, had higher final N content than expected (Table 2). This could indicate that N, being less consumed by microorganisms in Slow–Slow mixtures, is causing antagonistic effects in those mixtures. Despite the synergistic effects found in Fast–Slow mixtures after 9 months of incubation, final C and N content were not significantly lower than expected. We cannot discard that the magnitude of change in C and N content due to the interaction in these last mixtures could be too small to be detected.

4. Discussion

Our research, focused on the analysis of two-species mixtures, shows new evidences of species interactions, both synergistic and antagonistic, during decomposition (Fig. 4). Consistent with results from most previous research, the effects of species interactions

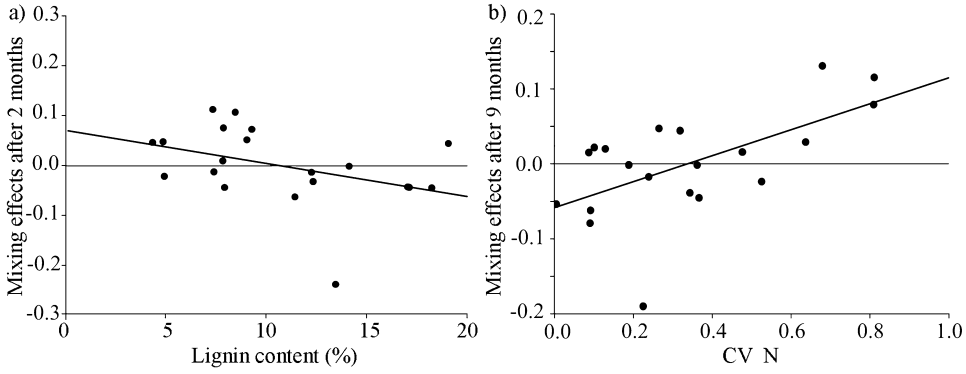

Fig. 3. Mixing effects in relation to initial quality and heterogeneity of mixtures. (a) Mixing effects ((obs – exp)/exp) after 2 months of incubation plotted against initial leaf litter lignin content in mixtures ($r = 0.18$, $p = 0.05$) and (b) mixing effects after 9 months of incubation plotted against initial coefficient of variation in N content (CV N = (N in species A – N in species B)/average (A–B)) between the two component species of each mixture ($r = 0.31$, $p = 0.006$).

Table 2
Final C and N content after two and nine months of incubation in mixtures with two Slow-decomposing species (Slow–Slow), mixtures with one Fast- and one Slow-decomposing species (Fast–Slow), and mixtures with two Fast-decomposing species (Fast–Fast). Standard errors are indicated between brackets. Significant different values of observed and expected differences are indicated with an asterisk (Student paired *t*-test, $p \leq 0.05$).

Mixture composition group	Carbon (%)				Nitrogen (%)			
	Two months		Nine months		Two months		Nine months	
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
Slow–Slow	40.9(2.3)	43.6 (0.5)	43.8 (1.2)	43.8 (1.1)	1.6(0.1)*	1.5 (0.1)*	1.9 (0.1)*	1.8 (0.1)*
Fast–Slow	36.5(1.6)	36.5 (0.4)	38.4 (1.3)	37.3 (1)	1.9(0.1)	1.8 (0.1)	2.3 (0.1)	2.2 (0.1)
Fast–Fast	30.4(0.8)*	29.4 (0.7)*	32.4 (1.3)*	30.7 (1)*	2.2(0.1)	2.2 (0.1)	2.6 (0.1)	2.5 (0.1)

within mixtures on decomposition were much weaker than the effect of species identity per se (Wardle et al., 2003; Hoorens et al., 2010b).

We detected synergistic effects in the most chemically heterogeneous mixtures (Pérez Harguindeguy et al., 2008; Hoorens et al., 2010a; Hättenschwiler and Jørgensen, 2010). Surprisingly, Slow-decomposing species increased the rate of decomposition of Fast-decomposing species, as reported by Hoorens et al. (2010b), but contrary to results reported from other studies (Staaf, 1980; Wardle et al., 2003; Schimel and Hättenschwiller, 2007).

C-priming could be a possible mechanism underlying the synergistic effect of Slow-decomposing species on Fast-decomposing ones (Fig. 4a). If decomposers are limited by the amount of C present in Fast-decomposing species, then when a higher C source, or a different C source is added (Slow-decomposing species), decomposition may be accelerated (Hobbie, 2000; Hoorens et al., 2010b; Berglund et al., 2013). The presence of different C substrates in the same mixture may have induced C priming through the production of a greater variety of enzymes, which, in turn, may increase the ability of microorganisms to decompose different types of substrates (Orwin et al., 2006; Chapman et al., 2013). If C were actually limiting decomposition of Fast-decomposing species, then it would be reasonable to find Slow-decomposing species to increase decomposition of Fast-decomposing species. Unfortunately, we were not able to fully confirm this mechanism because, despite detecting differences in species mass loss, we did not detect differences in either C or N content in mixtures after decomposition (Fig. 4a). In addition, we did not measure microbial identity or activities in mixtures.

In Slow–Slow mixtures, decomposers could be limited by the quality of the C source (e.g. proportion of lignin), by the amount of N or by their interaction (Fog, 1988; Taylor et al., 1989b; Hobbie, 2000; Hoorens et al., 2010b). After 2 months of decomposition, we found that final N content was higher than expected in Slow–Slow mixtures (Table 2; Fig. 4b). This higher N content found in mixtures with initial high lignin content suggests that antagonistic effects could be related to the formation of recalcitrant lignin–N complexes in those mixtures (Fig. 4b; Berg, 1986; Fog, 1988; Camiré et al., 1991).

In most studies the effect of litter mixing on overall decomposition rates is small compared to the effect of species identity (Pérez Harguindeguy et al., 2008; Hoorens et al., 2010b). Thus, in most cases the dominance of Slow- or Fast-decomposing species in an ecosystem would be expected to drive its decomposition, nutrient cycling and C stocks. However, identifying and understanding the mechanisms underlying litter interactions in mixtures is still necessary to predict when and why interactions can be significant and relevant from the ecosystem perspective and in the context of ecosystem management. Our study provides some evidences that can shed light on some of those mechanisms, but additional research on microbial identity, abundance and activities related to the mechanisms proposed is critical. In particular there is a need to determine if (and how) litter interactions are effectively translated in changes in ecosystem functioning at the landscape level, and how the decomposition environment (e.g. soil characteristics, soil fauna, temperature and precipitation) affects those effects (McLaren and Turkington, 2011; Tardif et al., 2013).

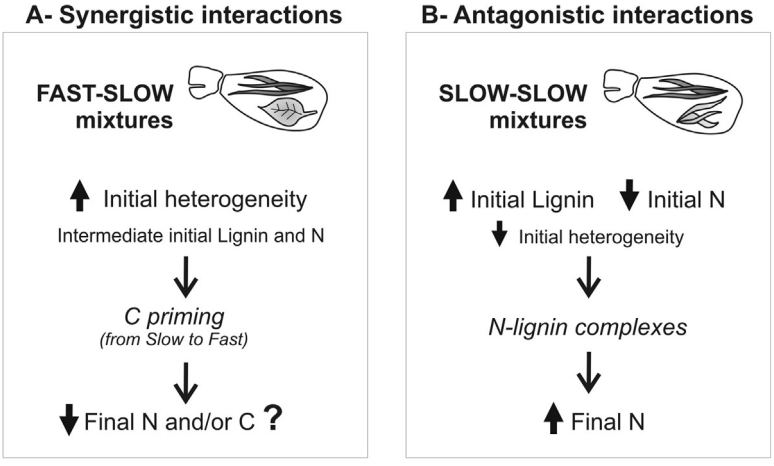

Fig. 4. Types of non-additive patterns found in mixtures. (A) Synergistic interactions were found in mixtures of Fast–Slow decomposition mixtures. These mixtures have an initially high chemical heterogeneity (or greater difference between the chemistry of its component species) and intermediate initial lignin and N content. High heterogeneity may favour C priming (or labile C transfer) from Slow- to Fast-decomposition species. This mechanism was not confirmed because final content of N (or C) was not lower than expected. (B) Antagonistic interactions were found in Slow–Slow decomposition mixtures (which have low heterogeneity, or a smaller difference between the chemistry of its component species). These mixtures have an initially high lignin content and low N content. This composition may lead to the formation of lignin–N complexes, slowing decomposition and, as a result, final N content would be higher than expected in decomposed litter, as found in Slow–Slow mixtures.

5. Conclusions

In the studied species, mixture decomposition was mainly driven by the component species identity (and decomposability). In addition, litter mixing affected decomposition rates weakly but significantly. In particular, at initial stages of decomposition, low-lignin appears to result in synergistic mixing effects, whereas high lignin tends to result in antagonistic mixing effects. At more advance stages of decomposition, mixtures containing species with different initial N content had more synergistic effects, whereas those with similar initial N content showed both synergistic and antagonistic effects. Based on the individual mass loss of the components, as well as on the final chemistry of the decomposed mixtures. We propose that several mechanisms related to carbon priming and nitrogen interaction with carbon (probably carbon from lignin) may be operating simultaneously.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2014.05.004.

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