

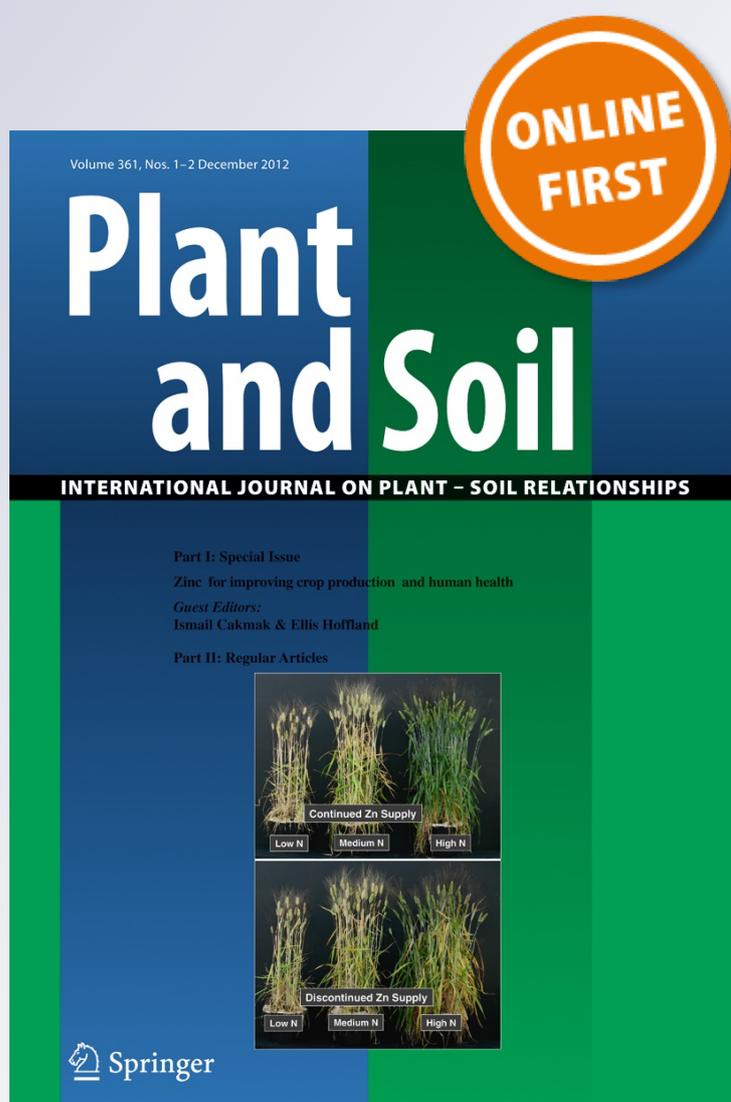
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Mixtures with grass litter may hasten shrub litter decomposition after shrub encroachment into mountain grasslands

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Abstract

Aims Shrub encroachment in mesic grasslands alters the identity and quality of litters entering the system. As litter from shrubs and grasses can differ in their quality, this can lead to differences in litter decomposition by the direct effect of quality, but also to litter interaction during decomposition. The objective of this study was to examine the occurrence of non-additive effects of litter mixtures on the decomposition rates of legume

shrub litter (poor in P) or conifer shrub litter (poor in N) and grass litter.

Methods In addition to single litter type litterbags for the three species, we mixed litters of each pair of possible combinations to determine the influence of each species on mass loss. Litterbags were placed in the field and collected after 1, 6, 8, 12 and 24 months. In each collection, litter of each species remaining in mixed bags was separated, dry weighed and analyzed for C, N and P.

Results With respect to shrub litter decomposing alone, mass loss of shrub litter when mixed with grass showed a 9–10 % increase in decomposition rate for conifer and a 3 % increase for legume litter. These litter mixture effects varied with time and they were detected after a decomposition period of 1 year in legume litter and of 2 years in conifer litter.

Conclusions Grass litter hastened conifer and legume litter decomposition in leaf litter mixtures, at least during the first stages of the process. The potential consequences of this result to alter litter accumulation patterns and thus carbon sequestration rates after shrub encroachment into grasslands will depend on whether the observed trends are maintained in the advanced decomposition stages.

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Introduction

Woody plant proliferation into grasslands is a world-wide phenomenon (e.g., Archer et al. 1995; Fang et al. 2001), affecting not only arid and semiarid (Van Auken 2009; Maestre et al. 2009), but also mesic grasslands (Briggs et al. 2005). Despite the global importance of shrub proliferation and the several studies dealing with its biogeochemical impact, the response of soil C stocks to woody encroachment remains unclear (Jackson et al. 2002; Asner et al. 2004). Understanding the processes affecting changes in C stocks after shrub encroachment should greatly help to improve the assessment and prediction of regional and global C cycling in a context of climate or land use changes.

Litter decomposition controls nutrient and C cycling in most ecosystems (Berg and McClaugherty 2003). After shrub encroachment into mesic grasslands, the identity and quality of litters entering the system are altered. As litter from shrubs and grasses can differ in their quality, this can lead to differences in litter decomposition by the direct effect of quality, but also litter interaction during decomposition. In the Pyrenees, shrub expansion into mesic grasslands has occurred mainly by two woody plant species: a legume shrub (broom, *Cytisus balansae* ssp. *europaeus* (G. López & Jarvis) Muñoz Garmendia) and a conifer shrub (juniper, *Juniperus communis* L. ssp. *alpina* (Neir.) Celak). The encroachment of these shrub species resulted in increased soil organic carbon in the upper soil mineral profile compared to the grassland soil (Montané et al. 2007). As the aboveground litter decomposition of either shrub was slower than that of grasses, litter accumulated under shrubs, and the transfer of litter derived C may be responsible for the increase of organic C found in the upper mineral soil layer under shrubs (Montané et al. 2010). However, shortly after shrub proliferation into mesic grasslands, shrub litter does not decompose alone but mixed with grass litter. In this scenario, the effects of litter mixtures on litter decay may be relevant to C stocks and their modeling after shrub encroachment.

When the effects of mixed-species litters are additive (e.g., decay dynamics are the sum of the individual species decay rates) decay rates of single litters can be used to predict decay rates of mixed litter (Gartner and Cardon 2004). Alternatively, litter decay in mixtures could be dependent on the litter species, giving rise to non-additive effects on litter decomposition

(Gartner and Cardon 2004). These authors found that only 30 % of the studied litter mixtures decomposed additively, while synergistic and antagonistic non-additive effects were observed in 50 % and 20 % of all litter mixtures, respectively. When considering different decomposition phases, both negative and positive effects may occur (De Marco et al. 2011). Such changes between negative and positive effects for a given litter mixture with time, which might be related with changes in lignin degradation with time (De Marco et al. 2011), are probably due to the complex nature of the decomposition process in which several litter constituents are simultaneously being broken down at different rates being further complicated by the various interactions occurring between the litter components within the mixtures (Wardle et al. 1997).

Non-additive effects in litter mixtures may be caused by chemical interactions between the component litters or by changes in the micro-environment in which the litter is decomposed (Lecerf et al. 2011; Hättenschwiler et al. 2005). In general, the chemical characteristics of the litter, mediated by decomposer activity, constrains decomposition dynamics of single species (Manzoni et al. 2010), whereas chemical heterogeneity of the litter mixtures appears to better predict the decomposition of mixtures (Ball et al. 2008; Meier and Bowman 2010; Vivanco and Austin 2011; Bonanomi et al. 2010). In mixtures, the relatively high amount of nutrients in one litter could stimulate the decomposition of the other litter, most likely through nutrient transport from one litter type to another either by diffusion in a water film and/or actively transported through the hyphae of fungi connecting the different litter types (Hättenschwiler et al. 2005; Quedsted et al. 2003). Thus, nutrient release from nutrient rich litter would promote the decomposition of other species, leading to a positive non-additive effect (Madritch and Cardinale 2007). Conversely, nutrient poor litter would induce negative non-additive effects through the enhancement of microbial nutrient immobilization. In addition, antagonistic non-additive effects may occur as a consequence of the release of recalcitrant compounds during the decay of one litter acting as inhibitors of the decomposition of the mixtures (Hättenschwiler et al. 2005).

Research on single-species litter dynamics has revealed a strong correlation between the rates of decay and the chemical composition of litter, and especially initial N and P concentrations and the ratios C:N, Lignin:N and N:P (Manzoni et al. 2010; Moore et al.

2011; Wardle et al. 2009; Prescott 2005). In the Pyrenees, litters of grasses, legume and conifer shrubs show marked differences in their chemical composition (Montané et al. 2010), and these differences may drive complex interactions in decomposition dynamics of mixed litter. Thus, given that shrub litter has a high concentration of recalcitrant compounds (e.g., lignin, lipids, suberin) and a low concentration of either N or P relative to grass litter (Montané et al. 2010), shortly after shrub proliferation, when shrub and grass litter coexist, both shrub litters may decompose at higher rate than when decomposing alone. In this study, we examined the occurrence of non-additive effects of litter mixtures on the decomposition of litters from a legume shrub, a conifer shrub and grasses. Given that conifer litter decomposition seems to be N-limited and legume litter decomposition P-limited (Montané et al. 2010), we hypothesized that: i) litter with high concentrations of both N and P (i.e., grass) would accelerate the decomposition of litter that is either N- or P-limited (i.e., conifer and legume shrub, respectively) in mixtures, and that in turn ii) the litter with highest N concentration (i.e., legume shrub) would accelerate the decomposition of the rest of litter types in mixtures (i.e., grass and conifer). Conversely, iii) negative non-additive effects may be expected on litter with high proportion of labile compounds (i.e., grass) when decomposing with litter with high concentration of recalcitrant compounds (i.e., conifer and legume shrub). Because mixtures could show non-additive effects but species could be interacting with opposite effects (e.g., grass having a positive effect on conifer decomposition, but conifer having a negative effect on grass decomposition) the different litter types in the mixture were separated. Separating the different components allowed us to test if the decomposition of litter mixtures deviated from theoretically expected patterns due to the litter mixtures interactions. Moreover, as interactions among different litter types during decomposition are not static (Gartner and Cardon 2004), we expected changes through time of non-additive effects for a particular litter mixture.

Materials and methods

Study site

Research was conducted in Collada de Montalto, Campirme (42° 37' 47" N, 1° 11' 15" E; 2100 ma.s.l.),

a mesic mountain site in the Pyrenees in the Alt Pirineu Natural Park. Mean annual temperature and annual precipitation were estimated as 4.7 °C and 1052 mm (Ninyerola et al. 2000). The area is usually snow-covered from December to April and, during the study period, the mean temperature in this non-growing period is -3 °C, whereas mean temperature in the growing season is 6.1 °C. The site is relatively flat (slope < 10 %). Soils are classified as humic dystrodepts (Soil Survey Staff 1999) originating from slate. The soil profile is about 60–80 cm deep and the texture in the upper horizons is loamy (25.6 % clay, 35.4 % fine silt, 6.6 % coarse silt, 32.4 % sand). In the first 15 cm of soil, pH (H₂O, 1:2.5 w:v) is 4.26 and bulk density is about 1.14 g cm⁻³.

In the Pyrenees, subalpine vegetation is commonly constituted by a mosaic of grasslands and shrublands. Mesic grasslands are dominated by either *Festuca nigrescens* (Lam) or *Festuca eskia* (Ramond ex DC) patches. Other species such as *Nardus stricta* L., *Trifolium alpinum* L., *Ranunculus pyrenaicus* L. and *Erythronium dens-canis* L. are less dominant but frequent in the grasslands. Woody plant encroachment has been widespread in these grasslands, mainly driven by a legume shrub, *Cytisus balansae* ssp. *europaeus*, and a conifer shrub, *Juniperus communis* ssp. *alpina*. Shrub encroachment in the area may be directly linked with reduction in grazing rates. Over the last century, the site has been grazed by cattle, horse and sheep. Although Pyrenean shepherds have traditionally used fire as a management tool to transform encroached land into grassland, the site has not been burned for at least 30 years (Forest Office Pallars Sobirà, pers. comm. 2007). Historical aerial photographs confirm that shrubland surface has increased at the site and that shrub encroachment has been taking place since the mid 20th century.

Shrub species characteristics

Both *J. communis* and *C. balansae* are two shade-intolerant species that spread in sites with low overstorey cover (Gracia et al. 2007). The shrub *J. communis* usually grows as isolated individuals while the shrub *C. balansae* is a clonal species that grows in large clusters. Both shrub species have a prostrate form; the canopy of adult *J. communis* or *C. balansae* usually ranges between 0.2 and 1 m in height. The species *J. communis* is an evergreen conifer shrub and

C. balansae is a leafless legume shrub that drops senescent stems from the lower part of its canopy mostly at the end of winter. Leaf area index was about 4.84 ± 0.82 (mean \pm S.E.) for *J. communis* and 2.67 ± 0.10 for *C. balansae* (estimated as a cylinder surface of green stems) (Ninot, pers. comm. 2010).

Experimental design

Aboveground litter decomposition was measured in three 3×3 m plots, located in a mesic grassland. As the legume species is a leafless shrub, fine stems (diameter < 5 mm) were used in litterbags. Senesced needles were used as conifer litter. Branches were gently shaken to collect freshly senesced leaves or stems from shrubs in October 2006. Grass litter was obtained collecting standing dead mass of *F. nigrescens* at the end of summer. Litter from all plant types was air-dried and placed in litterbags. Single species litterbags and mixed litterbags, which comprised all possible pair combinations of litter, were placed in grassland plots in three replicate blocks at least 50 m apart. In all, 135 litterbags were incubated on 14 November 2006 (3 litter species \times 3 different mixtures \times 3 plots \times 5 collection dates). Three bags for each litter type or litter mixture (one bag per plot) were collected after 1 month (37 days), 6 months (178 days), 8 months (233 days), 12 months (363 days) and 24 months (701 days).

Litterbags construction and processing

Litterbags (10×10 cm) were made using a nylon 1 mm mesh on the top surface and a 0.1 mm mesh on the bottom to prevent loss of material. Single litter type litterbags were filled with either 10 g of shrub litter or 3 g of grass litter, with both litterbag types showing similar plant litter volume. Mixed litterbags were filled with 5 g of shrub litter for the conifer + legume combination and 4 g of shrub litter and 2 g of grass litter for the conifer + grass and legume + grass combinations. Subsamples of the initial litter were oven-dried (60 °C) to find the relationship between mass of air-dried and oven-dried litter. After field collection, the different litters in mixed litterbags were hand-sorted, oven-dried and weighed to determine mass loss. A subsample of each type of remaining aboveground litter was ground and C, N, P and corrected lignin were determined (see next section).

Litter chemical characteristics

Litter chemistry was determined at the start of the experiment, after 1 year, and after 2 years using the same methods. The initial chemical composition of aboveground plant material was characterised previously (Montané et al. 2010). Briefly, a subsample was ground in a ball mill (MM2000 Retsch®), and C and N were determined on a Carlo Erba NA2000 elemental analyzer. P was determined by ICP (Inductively Coupled Plasma, Varian, U.S.A.) after digesting the samples with nitric acid in a microwave oven. Corrected (true) lignin content was determined in the initial aboveground litter samples applying a biochemical fractionation (see a detailed description of the method and litter chemical characteristics in Montané et al. 2010).

Nutrient retention was calculated as the remaining total nutrient amount in the decomposed litter divided by the total nutrient amount in the initial litter mass, and expressed as a percentage.

Data analysis

Expected remaining mass was calculated as the sum of each monospecific litter's mass, weighted by its proportion in the mixture for each plot. Litter mass or nutrients dynamics during decomposition were analyzed using a General Linear Model (GLM)-Repeated Measures analysis with time as a within-subject factor and mixture as between-subject factor. In each litter type, the mixture factor had three levels: litter alone or litter mixed with each one of the two other litters. When the interaction between time and mixture was not significant, pairwise modified Bonferroni means comparisons were performed on the significant mixture effect from the repeated measures tests (Day and Quin 1989). All effects were considered statistically significant at $p < 0.05$. Statistical analyses were performed using SPSS v. 12. All the data were presented as mean \pm standard error ($n=3$).

Results

Litter chemical composition

Litter chemical characteristics were quite similar overall, but each plant species became separated from the other two by a single but very relevant property (Table 1). Thus, conifer showed the lowest N concentration,

Table 1 Chemical composition of initial aboveground litter from three plant types. cLignin = corrected (true) lignin. Data are means \pm S.E. ($n=3$). When significant ($p<0.05$), different lowercase letters indicate between-plant-type differences

	Conifer	Legume ¹	Grass
Concentration (mg g^{-1} dry matter)			
C	509.60 \pm 5.71 a	519.87 \pm 6.38 a	455.62 \pm 4.72 b
N	9.40 \pm 0.42 c	17.32 \pm 0.57 a	14.89 \pm 0.34 b
P	1.01 \pm 0.07 a	0.40 \pm 0.03 b	0.91 \pm 0.06 a
cLignin	242.54 \pm 6.78 b	255.21 \pm 2.41 a	112.1 \pm 3.51 c
Ratios			
C : N	54.2 \pm 5.6 a	30.1 \pm 2.3 c	30.6 \pm 2.8 b
C : P	509.6 \pm 9.2 b	1299.7 \pm 9.9 a	506.2 \pm 8.4 b
N : P	9.4 \pm 0.4 c	43.2 \pm 0.7 a	16.6 \pm 0.5 b
cLignin : N	25.8 \pm 0.9 a	14.7 \pm 0.4 b	7.5 \pm 0.7 c
cLignin : P	243.1 \pm 9.1 b	636.2 \pm 4.3 a	123.8 \pm 6.8 c

¹Legume aboveground litter was fine stems with a diameter <5 mm

whereas legume litter had the lowest P concentration, and grasses showed the lowest lignin concentration. Therefore, conifer showed the highest C:N and Lignin:N ratios, whereas legume litter showed the highest C:P, N:P and Lignin:P ratios. In contrast, grasses showed the lowest Lignin:N and Lignin:P ratio (Table 1). Finally, grass litter had a similar C:N ratio as legume litter and a similar C:P to conifer litter.

Litter mixing effects on individual litter decomposition

Grass litter alone decomposed faster than either shrub type litters alone, and the legume litter faster than conifer litter, with significant differences from the first month until the end of the experiment (Fig. 1). After

2 years, the percentage of the initial mass remaining in the mixtures was the highest in mixtures containing legume and conifer litter, and the lowest in mixtures containing legume and grass litter (Fig. 1). No significant differences were detected between the observed remaining mass of any mixture and the expected value calculated from the remaining mass of each litter in the mixture decomposing alone (Table 2). However, when considering the remaining mass of individual litter types decomposing alone or in mixture, the interactions between litter types became apparent (Fig. 2a). Shrub litter (conifer or legume) decomposed faster when mixed with grass litter than when decomposing alone or mixed with the other shrub type (Fig. 2a). In contrast, grass litter decomposition by itself did not differ significantly from grass decomposition when placed in a mixture with shrub litter. After 2 years, the conifer litter decomposed 9–10 % faster mixed with grass than when alone or mixed with legume litter. Similarly, legume litter decomposed 3 % faster when mixed with grass than when the legume litter decomposed alone (Fig. 2a). The temporal dynamics also differed; litter mixing effects in legume litter became apparent after 1 year and remained after 2 years, whereas litter mixing effects in conifer litter were only detected after 2 years.

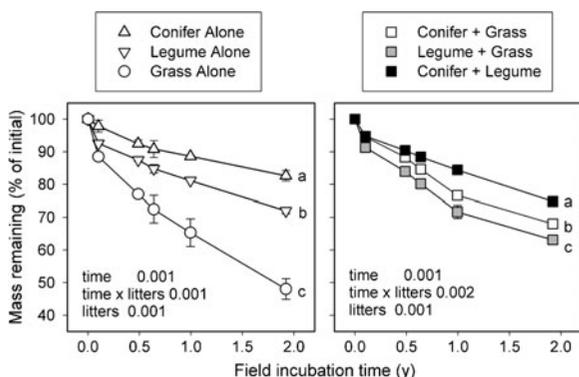


Fig. 1 Total mass remaining during the first 2 years of decomposition, expressed as a percentage of the original content for each treatment (litter alone and in mixtures). Lowercase letters denote significant differences between either single or mixed litterbags ($p<0.05$) in the repeated-measures ANOVA

N and P litter dynamics

The behaviour of nitrogen follows the pattern expected about transfer of nutrient between litters (Fig. 2). Thus conifer litter, with the lowest nitrogen concentration (Table 1), accumulated more nitrogen

Table 2 Observed and expected remaining mass (%) in each litter mixture after one and two decomposition years. Expected remaining mass was calculated as the sum of each monospecific

litter's mass, weighted by its proportion in the mixture for each plot. Mean \pm standard error ($n=3$). Differences were assessed by Student paired t -test between the observed and the expected values

	Litter Mixture								
	Conifer-Legume			Conifer-Grass			Legume-Grass		
	Observed	Expected	p -value	Observed	Expected	p -value	Observed	Expected	p -value
1 year	84.41 \pm 0.41	84.94 \pm 0.14	0.314	76.66 \pm 0.05	80.88 \pm 1.54	0.109	71.70 \pm 1.17	75.90 \pm 1.47	0.250
2 year	74.81 \pm 0.47	77.37 \pm 1.15	0.250	68.01 \pm 1.34	71.13 \pm 2.12	0.307	63.10 \pm 1.37	64.00 \pm 1.56	0.357

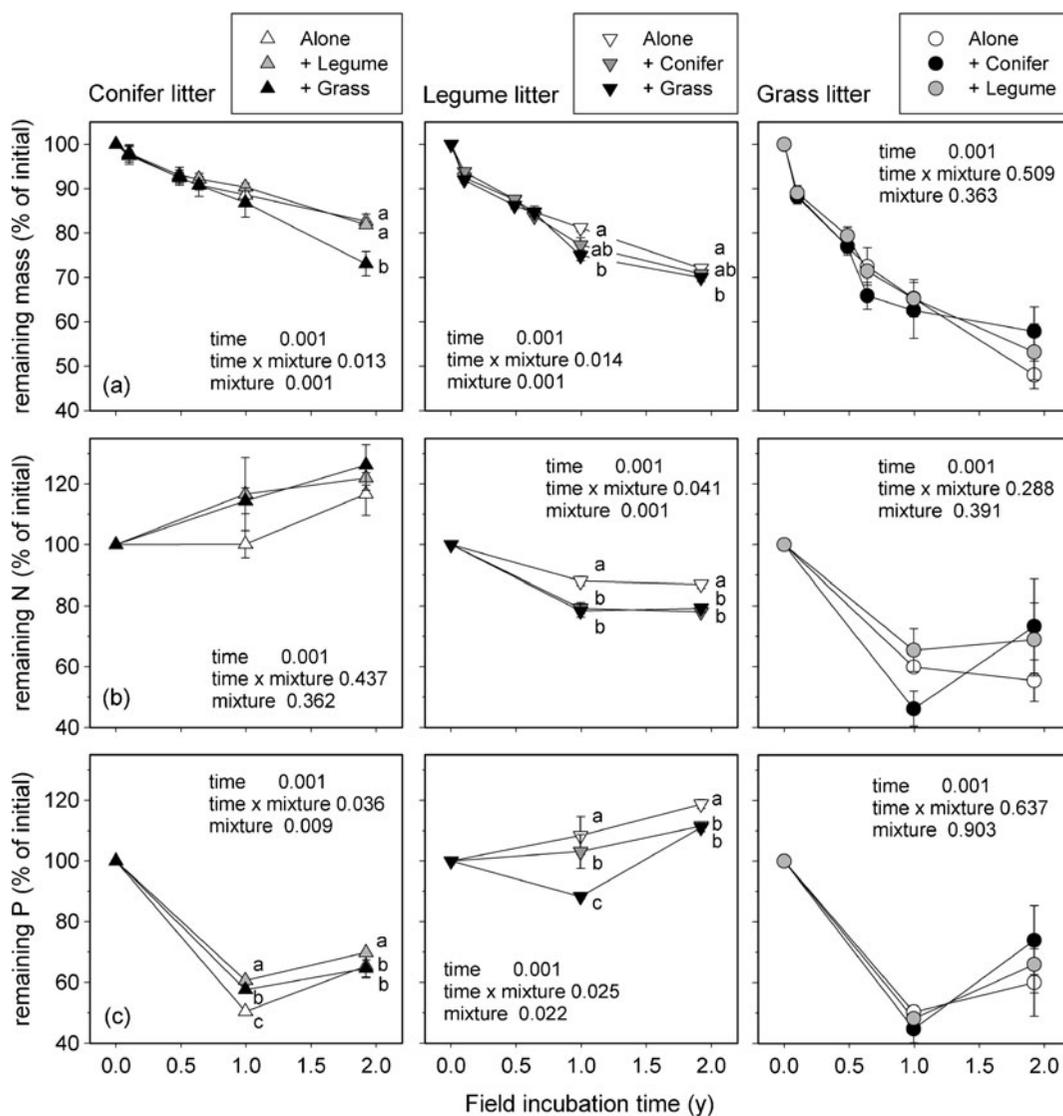


Fig. 2 Mass (a), N (b) and P (c), expressed as a percentage of the original mass content, for each litter type in all the treatments (conifer, legume and grass litters decomposing alone or in

mixtures). Lowercase letters denote significant differences between litters decomposing alone or in litter mixtures ($p < 0.05$) in the repeated-measures ANOVA

when decomposing in a mixture than when decomposing alone (Fig. 2). Conversely, legume litter, with the highest N concentration, lost more nitrogen when in mixed-litterbags than when decomposing alone. As for grass litter, no significant differences were detected between its behaviour in mixtures and when decomposing alone. Overall these results points to a transfer of N between litters, from the richest one (legume) to the poorest one (conifer). Grass, whose N content is intermediate between the other two, behaves neutrally as to N transfer.

For phosphorus the behaviour was less clear. Conifer litter was the richest in P, and in mixtures retained less P than when decomposing alone. But legume litter, the poorest in P, when decomposing in mixtures did not accumulate more P than when decomposing alone. Thus conifer apparently released more P in mixtures, but this excess of P released apparently is not captured by legume litter. Again, grass apparently behaves neutrally in this transfer: no significant differences in P accumulation were detected between grass decomposing alone and grass decomposing in mixtures. Losses of N in conifer litter and P in legume litter, and of both elements in grass occurred during the first year.

As a consequence of these N and P dynamics, N to P ratio in conifer litter increased during the first year, from an initial value of 10, and tended to stabilize around 15 to 20 in the second year; while in legume litter, with a higher starting point, the N to P ratio decreased during all the studied period (Fig. 3c). For grass litter, the N to P ratio slightly increased when mixed with legume litter (Fig. 3c).

Discussion

Decomposition of individual litters: limiting factors

The individual (non-mixed) litters decomposed in the order (1) grass, (2) legume, (3) conifer. This order follows that of the lignin : N ratio (Table 1), thus suggesting these two features (N content high enough, lignin content low enough) as the main drivers for decomposition. The low lignin content of grass is a clear explanation for the very fast decomposition of grass when incubated alone in the field (Fig. 1). The likely role of nitrogen as a limiting factor for the decomposing microflora is reflected in its transfer among litters in mixtures: legume, the N-richest litter, is source of N for the poorest one, conifer.

In comparison, phosphorus seems a less relevant factor. Thus, legume litter decomposed faster than conifer litter, in spite of its much lower P concentration and its very high C:P and lignin:P values. Conifer litter, the richest one in P, retains less P (i.e., releases more P) when mixed with litters poorer in P, such as legume or grass; but on the other hand, legume litter, the poorest one in P, does not accumulate more P when mixed with conifer. Overall, our data about P transfer between litters in mixtures are less consistent than those for N, thus suggesting that phosphorus is not a limiting factor for litter decomposition in our experiment.

Decomposition of litter mixtures

Despite the lack of differences between the observed and expected decomposition rates of the mixtures as a whole, after separating the individual litter types in the mixed litterbags positive non-additive effects were found when litter from either shrub decomposed in a mixture with grass litter. In contrast, no clear effect of either shrub litter over the decomposition of the other shrub or grass litter was detected. Opposite effects of the litters within the same mixture, synergistic and antagonistic interactions, may explain the lack of differences between the overall mass loss of the total mixture and the decomposition predicted based on single species dynamics.

In our studied mixtures, the overall mass loss agrees with the mass loss calculated from the weighed sum of the individual mass loss of their several individual components, as obtained from the experiments in which each litter type was incubated alone in the field. This points to a lack of non-additive, multiplicative effects of the mixtures on the overall litter decomposition, in line with the results of Hoorens et al. (2010a, b). However, this result could be more apparent than real, since we observed, for the individual litters in the mixtures, relevant deviations relative to the expected decomposition rates: particularly for conifer litter, whose mass loss in mixtures may be 9–10 % higher than when incubated individually, in contrast with the results of Hoorens et al. (2010b), who observed in litter mixtures small deviations in the expected mass losses, of only 1.5 %. Thus in our experiment, interactions between litters are more relevant than it could be suspected just from the weighed sum of decomposition rates. These interactions result in altered turnover rates of specific litter types and

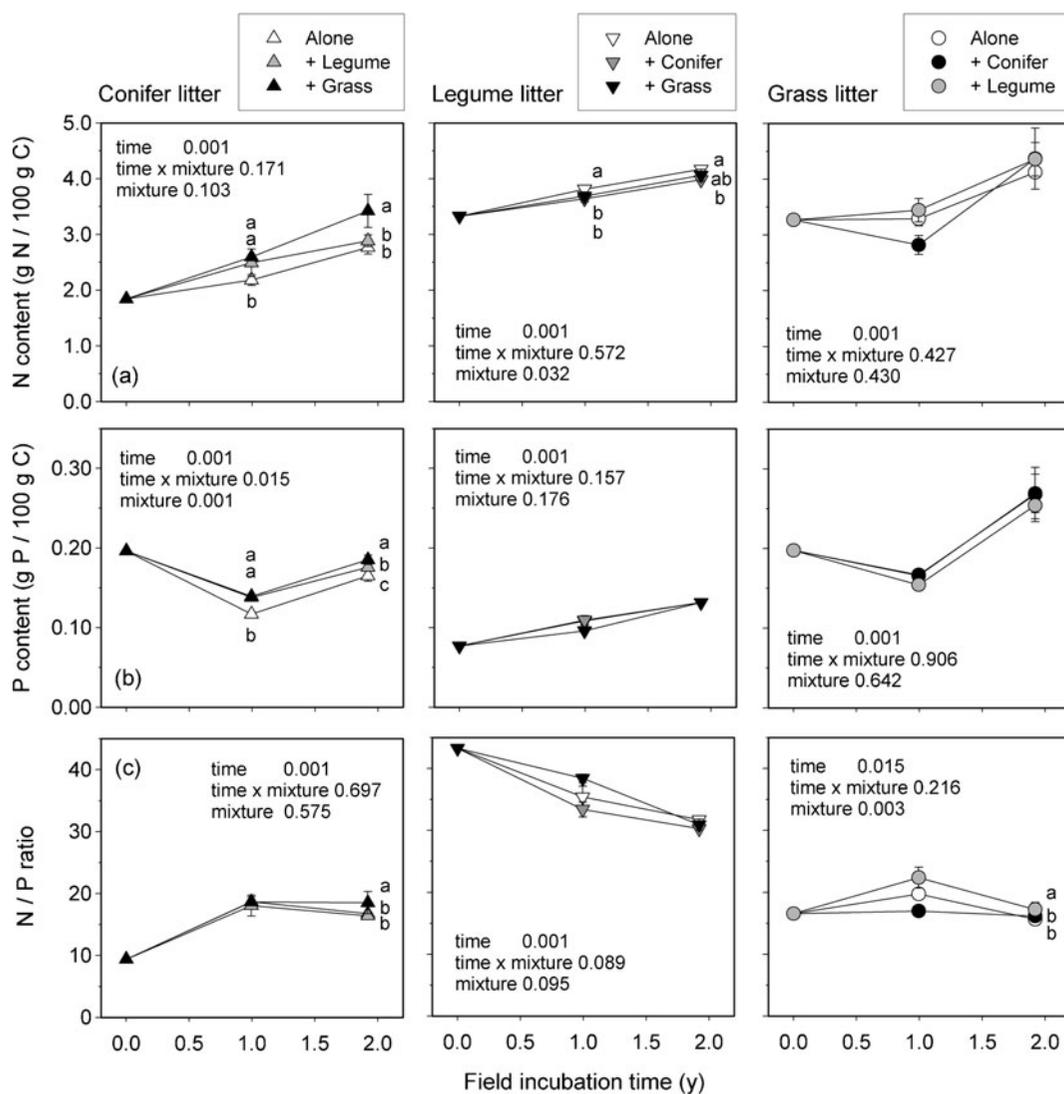


Fig. 3 N (a), P (b), and N to P ratio of the remaining C mass for each litter type in all of the treatments (conifer, legume and grass litters decomposing alone or in litter mixtures). Lowercase

letters denote significant differences between litters decomposing alone or in litter mixtures ($p < 0.05$) in the repeated-measures ANOVA

might have important implications for nutrient dynamics and soil organic carbon formation (Hättenschwiler 2005).

According to Seastedt (1984), when mixed together during decomposition, nutrient rich litter may induce a priming effect on the nutrient poor litter, facilitating faster decomposition of this litter without necessarily retarding the decomposition of the high quality litter. Thus, the positive effect of grass litter on shrub litter decomposition in mixtures may be explained as a microorganism benefit from the higher N or P concentrations of grass leaf litter. Based on the low N concentration and high C:N ratio of conifer litter (C:N=54), decomposing conifer litter

retained N. In the case of mixtures, this N may have been transferred from the other litters, which experienced net N losses, at least during the first year. Our results are consistent with previously reported patterns of N transfer between leaf litter mixtures of differing N status (Schimel and Hättenschwiler 2007). Moreover, the N loss from legume litter was lower when decomposing alone. In contrast, legume litter, with a low P content and high C:P ratio (C:P=1300), retained P during the 2 years of decomposition. As both grass and conifer litters lost P during the first year, the decomposition of legume litter may be enhanced when mixed with these litters. Our

results may agree with reported changes in patterns of P transfer between litters with different chemical composition (Staaf 1980).

One of the mechanisms that may explain our results is nutrient transfer among litter types (Hättenschwiler et al. 2005). The behaviour of the three litter types in our experiment stresses the existence of critical C:N and C:P ratios which determine whether or not N and P will be mineralized from the litter and, in a litter mixture, transferred from one litter to another (Klemmedson 1992). This nutrient transfer may be either active (e.g., by fungi) or passive (e.g., by leaching) (Gessner et al. 2010). The relevance of each type of nutrient transfer may depend on the mobility of different litter constituents and for example, P is much more easily leached from litter than is N (Gessner 1991). These different behaviors may explain the differences observed in the retention patterns of N and P in the mixtures. Thus, N concentration in the conifer litter increased when it was mixed with grass (and with legume in the first year). In contrast, although the legume litter retained P, the remaining P was lower when mixed with both conifer or grass litters despite the fact that decomposition was enhanced (at least in the mixture with grass). Also, because conifer and legume litter showed low and high N:P ratios respectively, fungi and bacteria may have different relative importance in litter-associated microbial communities (Gusewell and Gessner 2009), and litter nutrient dynamics. Moreover, such nutrient transfer may occur not only between the litter within the bag but also between the litter inside the bag and the underlying soil (Chigineva et al. 2011).

Besides nutrient transfer among litter types, additional mechanisms may contribute to explain differences in litter mixtures decomposition. For instance, heterogeneity in lignin concentration in mixtures between shrubs and grasses may enhance decomposition of shrub litters, as reported previously for litter mixtures with heterogeneity in non-labile compounds (Perez Harguindeguy et al. 2008). Moreover, our mixtures are comprised of different litter sizes, shapes and surface structures that confer a distinct geometric organization and water-holding capacity (Wardle et al. 2003). Such differences influence microclimatic conditions and microhabitat structure for soil biota and, therefore, have indirect consequences for decomposition (Hättenschwiler et al. 2005; Chapman and Newman 2010).

Our results also suggested that the non-additive effects of litter mixture in decomposition varied with

time, as previously reported by others studies (Prescott et al. 2000; Liu et al. 2007). For instance, non-additive effects in conifer litter decomposition were only found after 2 years, whereas non-additive effects in legume litter appeared in the first year. While a comprehensive explanation is somewhat constrained by our study design, we may speculate that the biotic degradation of organic compounds could be responsible for the time dependence of the non-additive effects of litter mixtures (Blair et al. 1990). The different chemical components of litter show decomposition rates that change with time, following a two-phase model (Berg and Staaf 1980). In the early phase mainly soluble material (with fast turnover time), cellulose and hemicellulose are degraded, whereas in a later phase increased lignin concentration retards the decomposition rate (Berg and Staaf 1980). During these two phases the decomposition rate is regulated by different factors, with nutrient content of litter mainly controlling the early stage, and lignin content becoming more important as decomposition progresses (Berg and McLaugherty 2003). It should be emphasized that our experiment is of a limited duration (701 days) and does not allow to predict the effect of litter mixing in the more advanced stage of decomposition (i.e., humus-near stage).

Implications for C dynamics after shrub encroachment into grasslands

Mixtures of grass and shrub litters may play an important role in C sequestration rates after shrub encroachment into grasslands. Our previous work (Montané et al. 2010) shows that although leaf litter quantity is similar between grass and both shrubs, the lower litter quality (e.g., higher recalcitrance) of shrub litter may cause increases in soil organic carbon under shrubs. Besides differences in litter quality, litter interactions may also play an important role in C dynamics after shrub encroachment into grasslands. According to our results, the litter accumulation pattern may be altered by the effect of litter mixing. Grass litter, with high N and P concentrations, may prime decomposition of shrub litter with low N or P content, as both shrub litters decomposed faster when mixed with grass litter. However, as leaf area index is higher in conifer than in legume patches, grass density below conifer shrubs is generally lower than the one under legume shrubs. This may affect C sequestration, basically in two ways: (i) several years after conifer encroachment, the absence of grasses under

conifer shrubs does not allow litter mixing between conifer and grasses, consequently reducing litter decomposition. In contrast, grasses remain abundant under legume shrubs even several years after legume encroachment. In this case, the presence of grass litter may prime legume litter decomposition. (ii) Lower grass cover under conifer than under legume shrubs may allow contact and mixing between soil and litter, as pointed out by Throop and Archer (2007), and may therefore accelerate above-ground litter incorporation in the mineral soil under conifer shrubs. Finally we must mention that our experiment involved only the first 2 years of decomposition, during which the studied litters loss less than 60 % of their weight. Thus, the validity of the above statement on the long term will depend on whether the observed trends are maintained in the advanced stages of decomposition, which are out of the scope of our paper.

Conclusion

This study showed the occurrence of non-additive mixture effects in decomposing litters of both shrubs species (conifer and legume shrubs) when mixed with grasses. Therefore, litter mixtures of different plant functional types may alter litter accumulation pattern and, thus, C sequestration rates after shrub encroachment into mesic grasslands. Furthermore, the cycling of nutrients, such as N and P, may be altered significantly due to shrub and grass litter mixtures in mesic grasslands after shrub encroachment.

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