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REGULAR ARTICLE

Effects of exotic and native tree leaf litter on soil properties of two contrasting sites in the Iberian Peninsula

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Abstract

Aims We assessed the effects of native and exotic tree leaf litter on soil properties in two contrasting scenarios. The native *Quercus robur* and *Pinus pinaster* tree species coexist with the aliens *Eucalyptus globulus* and *Acacia dealbata* in acid soils of NW Spain. The native trees *Fraxinus angustifolia* and *Ulmus minor* coexist with the aliens *Ailanthus altissima*, *Robinia pseudoacacia* and *Ulmus pumila* in eutrophic basic riparian soils in Central Spain.

Methods Four plastic trays per species were filled with homogenized top-soil of the site and covered with leaf litter. Before and after 9 months of incubation, litter mass, soil pH, organic matter, mineral and total N were measured. Available mineral N (NO_3^--N and NH_4^+-N) was assessed every 2 months.

Results Soil biological activity was higher in the basic than in the acid soil. Litter of the exotic trees tended to decompose less than litter of native species, probably due to the presence of secondary metabolites in the former. Soil pH, mineral and total N responded differently to different litter types, irrespective of their exotic or native origin (acid soil), or was similar across litter treatments (basic riparian soil). The similar response of the basic soil to the addition of different litter types may be due to the low contrast of litter quality between the species. *E. globulus* litter inhibitied soil microbial activity much more than the rest of the studied litter types, leading to a drastic impoverishment of N in soils.

Conclusion Litter of exotic N-fixing trees (*A. dealbata* and *R. pseudoacacia*) did not increase soil N pools because of the inhibition of microbial activity by secondary compounds. Therefore, secondary metabolites of the litter played a major role explaining exotic litter impact on soil properties.

Keywords Invasive species · Litter decomposition · N cycle · Soil pH · Soil organic matter · Soil N content

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Introduction

Changes in species composition or abundance driven by exotic plant introductions may alter many ecosystem functions, such as disturbance regime (Grigulis et al. 2005; Levine et al. 2003; Mack and D’Antonio 1999), water cycle (Ehrenfeld 2003; Le Maitre et al. 1996; Tennesen 2008; Ohte et al. 2003) or nutrient cycles (Vitousek and Walker 1989; Ehrenfeld 2003; Gomez-Aparicio and Canham 2008; Vanderhoeven et al. 2005). Nutrient availability, particularly nitrogen, limits primary production in many terrestrial ecosystems (Schlesinger 1991; Bowman et al. 1993; Soudzilovskaia and Onipchenko 2005) and affects the diversity of plant communities (Stevens et al. 2010; Gilliam and Dick 2010; Bai et al. 2007). Therefore, any alteration of the N cycle because of exotic plant invasion may also alter the ecosystem services provided by plant communities.

The introduction of exotic plants may alter the nutrient cycle of the system either directly, by modifying the quality and quantity of litter entering the soils beneath, or indirectly, by altering the physical-chemical site properties below their canopy (Tateno et al. 2007; Yelenik et al. 2007; Vitousek and Walker 1989; Follstad Shah et al. 2010). Both site and litter properties affect the structure and activity of macro-detritivores and microbial decomposers (Hawkes et al. 2005; Mack et al. 2001; Mayer et al. 2005), controlling litter decomposition and organic matter mineralization rates (Cornelissen and Thompson 1997; Gallardo and Merino 1993, 1992; Xiong et al. 2008), and finally affecting nutrient availability for plants.

Previous information suggests that exotic invasive plants tend to speed up litter decomposition and increase nutrient pools readily available for plant uptake (reviewed in Ehrenfeld 2003; Liao et al. 2008). This trend accords with the opportunistic resource-acquisition strategy frequently reported among exotic invasive plants (Grotkopp and Rejmánek 2007; Pattison et al. 1998; Baruch and Bilbao 1999), which involves leaf traits typically associated with high decomposition rate, i.e. low leaf mass per area, high N content, low investment in secondary metabolites, etc. (Lake and Leishman 2004; Cornelissen and Thompson 1997). However, it is not clear the extent to which this trend derives from a case-selection bias (i.e. many studies report the effects of N-fixing species invading N-poor soils (Vitousek and Walker 1989; Yelenik et al. 2007; Stock et al. 1995; Ley and D'Antonio 1998)) and/or publication bias over-representing the most significant results. The few studies performed on the Mediterranean Basin showed that exotic invasive plants have little effect on N-related soil properties (Vilà et al. 2006; Castro-Díez et al. 2009), or they even tend to slow down litter decomposition (Godoy et al. 2010). These contrasting results suggest that the effects of exotic invasive plants on the N cycle largely depend on the interaction between the functional properties of the exotic plant (N-fixing ability, lignin content, growth rate, etc.) and the properties of the invaded ecosystems (soil properties, climate, etc.). Most studies comparing exotic and native litter decomposition and mineralization have been performed in the field using reciprocal incubation designs (Castro-Díez et al. 2009; Ehrenfeld et al. 2001; Ashton et al. 2005; Rothstein et al. 2004). However, soil conditions are difficult to replicate due to the inherent high variability of soil properties at small scales (Castro-Díez et al. 2009; Gallardo and Parama 2007), blurring the effects of the exotic plants on N cycling processes.

In this study we aimed to isolate the effects of litter type on soil properties by means of a laboratory experiment, where different types of exotic and native litter were incubated in field-collected standard soils, under standard microclimatic conditions. We simulated two contrasting scenarios of invasion that occur in the Iberian Peninsula: 1) A temperate forest in NW Spain, developed on an acid soil and dominated by *Quercus robur* in the mature stage, and 2) a mixed riparian forest growing on eutrophic basic soils in central Spain, under continental Mediterranean climate. In the first scenario *Q. robur* has been largely displaced by forestry plantations of the native *Pinus pinaster* and the exotic *Eucalyptus globulus*. Both species are able to sustain stable populations in the region with no human assistance (Sanz Elorza et al. 2004). In addition, the N-fixing exotic tree *Acacia dealbata*, which was introduced for ornamental and agricultural purposes, has also naturalized in the region (Sanz Elorza et al. 2004). The four species can be found growing close to each other. The second scenario is dominated by native riparian trees, such as *Populus nigra, P. alba, Fraxinus angustifolia* and *Ulmus minor*. However, in disturbed places, near roads, crops or towns, these species coexist or have been displaced by exotic trees, such as *Ailanthus altissima*, *Robinia pseudoacacia* or *Ulmus pumila* (Sanz Elorza et al. 2004). All exotic trees of the study have been reported in Spain no longer than two centuries ago (Sanz Elorza et al. 2004), and the studied populations are not older than 30–50 years.

We address the following questions: Does the exotic litter tend to decompose faster and to increase soil N pools, as suggested by the literature, irrespective of the species and site? If not, is there any other consistent effect of all exotics in each site? Is there a correlation between litter properties and changes in soil and N cycle properties? Given the contrasting functional traits across exotic species we expect them to cause different effects on soil and N cycle properties (hypothesis 1). Given that the top soil largely derives from the decomposition of the incoming litter, and that native trees have been adding litter to the soil for a much longer time than exotic trees, we expect local soil properties to be closer to an equilibrium with the native litter, but not with the recently introduced exotic litter; therefore we expect exotic litter to alter the local soil properties more than native litter (hypothesis 2). Finally, litter decomposition is expected to be negatively related with leaf mass per area and positively with litter N content (hypothesis 3).

Materials and methods

Litter, leaf and soil collection

Between October and November 2007 we collected senescent or recently fallen leaf litter from 6–10 trees per species. *Pinus pinaster, Quercus robur* and *Eucalyptus globulus,* were collected in Poulo, Orense, NW Spain (latitude, 42° 12′ 05″N, longitude 8° 05′ 10″ W, altitude 489 m). In the case of *E. globulus*, we collected both true leaves of juveniles and phyllodes of adults. Although *Acacia dealbata* populations exist in the same site, leaves were not senescent at this time of the year, so its litter was collected in the colder place of Sierra de Gata, Cáceres, W-Spain (latitude 40° 14′ 33″, longitude 6°25′08″W, altitude 432 m). Although litter properties of *A. dealbata* may differ between populations, the magnitude of this variation is expected to be much lower than cross-species variation, which constitutes the focus of the study. Leaf litter of the native trees *Fraxinus angustifolia* and *Ulmus minor,* and the exotic trees *Ailanthus altissima, Robinia pseudoacacia* and *Ulmus pumila* was collected in Alcalá de Henares, Madrid, Central-Spain (latitude 40° 20–30′, longitude 3° 18–20′, altitude 600 m). Litter was taken to the lab, extended on paper sheets and left to dry at ambient temperature. All litter from the same species was pooled into a composite sample. Four replicates from this composite sample were oven-dried at 60°C for 48 h and grounded with a Culatti mill (<1 mm particle size) for analysis of N and ash content (see below). Additionally, five green leaves (both true leaves and phyllodes for *E. globulus*) or leaflets were collected from each of 7–9 trees per species to assess the leaf mass to leaf area ratio (see below). Soil was collected from the two main sites selected for the litter collection below the canopy of native forests (co-dominated by *Q. robur* and *P. pinaster* in Poulo and by *F. angustifolia* and *U. minor* in Alcalá de Henares), where no exotic trees were present. After removing the upper debris layer, the top soil was collected to a depth of 30 cm, dried at ambient temperature, pooled into a single composite sample, and passed through a 2 mm sieve to eliminate remaining debris and stones. A fraction of the pooled soil was kept for chemical analysis.

Experimental design

Thirty-six plastic trays (2 L, 6-cm depth, 22-cm long and 14-cm width), with holes drilled in the bottom to allow drainage, were filled with c.a. 900 g of air-dried soil (16 from Poulo and 20 from Alcalá) and covered with 10 g of air-dried litter of one species, matching the origin of litter and soil (four replicated trays per treatment). This amount of litter corresponds to the average litter layer thickness found below dense patches of the studied species during the peak of leaf abscission. Four additional samples of each soil type and litter type were kept to correct for the initial water content after >48 h at 60°C in the oven. The proportions of juvenile true leaves to adult phyllodes (*E. globulus*) and of leaflets to rachises (*A. altissima, R. pseudoacacia, F. angustifolia* and *A. dealbata*) was kept as found in the field, though *A. altissima* rachises had to be cut into smaller pieces to fit their size to the trays. On 2007 December 28th trays were introduced into a growth chamber (Walk-in Ibercex HR, A.S.L., S.A.) at dark and constant temperature (21°C) and air humidity (77%), until the end of the experiment on 2008 September 15th. During this time soil moisture was kept c.a. 70% of field capacity by weekly weighing every tray and adding the required amount of de-ionized water, after previous calibration. This moisture level was selected as the mean point between soil saturation and the point at which soil start to crack (see below). Litter moisture was kept high by weekly spraying the top of the trays with de-ionized water. After 9 months of incubation the litter was removed from the trays, gently brushed to remove the soil particles, and weighed after being oven-dried at 60°C for >48 h. A fraction of this litter (c.a. 1 g) was used to assess ash content after 2 h at

550°C in a muffle furnace. The percentage of mass loss by decomposition was calculated as the difference between the initial and final ash-free dry mass, divided by the initial mass.

Soil moisture calibration

Before the beginning of the experiment, ten previously weighed trays were filled with 900 g of air-dry experimental soil (five with Poulo soil and five with Alcalá soil). Soils were saturated by submerging the bottom of the trays into water for several days and watering the top at intervals. Then trays were left to freely drain until they stopped dripping from the bottom. At that point trays were weighed, left to air-dry in the lab, and re-weighed 1–4 times per day until they reach c. a. 40% of the initial saturated weight. At this point the soil started to show cracks and to lose its structure. Finally, trays were oven-dried at 60°C for ≥3 day and weighed. Soil moisture was then calculated for every weighing time as the proportion of soil water mass (full tray weight minus empty tray weight minus dry soil weight) with respect to the saturated soil water content. The relation between tray weight and soil moisture (% of saturation water content) was used to calculate the amount of water required to keep trays at 70% during the experiment.

Leaf and litter properties

The four ground litter samples per species separated for analyses were divided into three aliquots. The first one was used to assess N concentration at Nutrilab (University of Rey Juan Carlos I, Móstoles, Madrid, Spain) with an Automated Wet Chemistry Analyzer (Skalar San ++, Breda, The Netherlands), after a digestion with H_2SO_4 and Cu-KSO_4, which converts all organic nitrogen into ammonium (NH_4^+-N). The second sample (c.a. 1 g) was used to assess water content, and the third one (1–1.5 g) to assess ash content after 2 h at 550°C in a muffle furnace. The two later values were used to express N content and litter mass per unit of ash-free dry mass. Values for two additional leaf litter properties (C/N and lignin content) were obtained from the literature. The leaf area of the fresh green leaves or leaflets (in the case of compound-leaf species) was assessed by means of a Delta-T Image Analysis System (Delta-T Devices LTD, Cambridge, England), and their dry mass was obtained after 2 days in the oven (60°C). The leaf mass per area (LMA) of each species was the average quotient between dry mass and leaf area of the eight sampled trees.

Soil properties

Soil pH, organic matter, mineral (NH_4^+-N, NO_3^--N and the sum of both) and total N content was measured at the beginning (4–7 replicates per soil type) and end (4 replicates per species, i.e. one per tray) of the experiment. For soil pH assessment, 40 mL of deionised water was added to 20 g of soil, shaken and measured with a pH-meter (Allen et al. 1986). Organic matter was assessed by the Walkley-Black acid digestion method (Porta Casanellas et al. 1982). Total N content was assessed as explained before for leaf N content. Mineral N was extracted by adding 100 ml KCl 2 N to 5 g of soil and shaking the mix for 2 h. The solution was passed through 0.45 µm Millipore filters and preserved at −20°C until analysis of NO_3^--N and NH_4^+-N at Nutrilab (University of Rey Juan Carlos I, Móstoles, Madrid, Spain) with an Automated Wet Chemistry Analyzer (Skalar San ++, Breda, The Netherlands). Available mineral N (NO_3^--N and NH_4^+-N) in soils was monitored through the experiment by means of ion exchange membranes. Two 2.5×2.5 cm anion and two 2.5×2.5 cation exchange resin membranes (types I-100 and I-200, Electropure excellion-inc., Laguna Hills, California) fixed to two plastic labels were introduced in each tray and extracted 15 days later (Abrams and Jarrell 1992; Subler et al. 1995). This process was repeated every 2 months. Resin membranes were previously conditioned in the lab by immersing them in deionized water at 82–90°C for 48 h. Ions were extracted from the resins by shaking them for 2 h in 20–25 ml of 2 N KCl solution. Ammonium and nitrate content in the solution were assessed as before.

Statistical analysis

Analyses were separately performed for each location. LMA of green leaves, litter N concentration and proportion of litter mass loss were compared across species by means of a one-way ANOVA followed by a post-hoc Tukey test. The relation between litter mass loss and litter properties was assessed by Pearson correlation coefficient. Resin-assessed ammonium and nitrate soil availability were compared across species through the time by means of a repeated-measures ANOVA, using time as intra-subject factor and species as between-subject factor. The two values obtained per tray were averaged prior to analysis. When data did not fulfil sphericity, the Huynh-Feldt correction was applied (Field 2005). Changes in soil properties (pH, organic matter, NO_3^--N, NH_4^+-N, total mineral N and total N content) were calculated by subtracting the average initial values from the final values in each treatment. The effects of litter origin (exotic or native) and litter species (nested in origin) on litter mass loss and on the change of soil properties between the beginning and the end of the experiment, were tested by means of a nested two-way ANOVA. Pair-wise comparisons of soil properties between trays covered by different litter types were performed by means of one-way ANOVA followed by a Tukey post-hoc test. In some cases data were Ln-transformed to achieve homogeneity of variances (checked by means of Levene test). All analyses were performed with SPSS 17.0.

Results

At the beginning of the experiment soils from Poulo exhibited lower pH, nitrate, mineral N and total N content, but higher ammonium content than Alcalá soils. Organic matter was similarly high in both sites (Table 1).

Native species from Poulo exhibited the highest (*P. pinaster*) and the lowest (*Q. robur*) LMA values, while exotic species (*E. globulus* and *A. dealbata*) showed intermediate and similar values. No significant difference of LMA was found between true leaves and phyllodes of *E. globulus*. Initial leaf litter N concentration was the lowest in *E. globulus* phyllodes and *P. pinaster* leaves, followed by *E. globulus* leaves and *Q. robur* leaves. The highest N concentration was found in *A. dealbata* leaf litter. Among Alcalá species, the two exotics, *A. altissima* and *R. pseudoacacia*, showed the lowest LMA values, followed by the native *F. angustifolia* and the two *Ulmus* species exhibited the highest values. Leaf litter N content was similar across species, being only differences between *U. minor* (the highest) and *U. pumila* (the lowest) (Table 2).

Table 1 Soil properties of the two study sites: Poulo- mesic *Quercus robur* forest in NW Spain, Alcalá - riparian forest in Central-Spain. Data represent the mean±standard deviation of seven (organic matter) or four (rest of the variables) pseudoreplicates collected from a composite soil sample of each site

	Poulo (Mesic forest)	Alcalá (Riparian forest)
Soil pH	4.41±0.12	8.03±0.04
Organic matter (%)	9.02±0.90	8.41±0.79
NO_3^--N (μg/g)	0.97±1.16	57.03±14.01
NH_4^--N (μg/g)	40.48±4.12	3.99±1.00
Total mineral N (μg/g)	41.45±4.30	61.02±15.04
Total N (mg/g)	2.91±0.17	4.61±0.24

The proportion of litter mass loss at the end of the incubation period was much lower in Poulo (0–15%) than in Alcalá species (25–48%, Fig. 1). Among Poulo species, the exotic litter tended to decomposed less than the native litter ($p=0.099$, Table 3), due to the negligible decomposition found in *E. globulus* (Fig. 1). The same trend was found among Alcalá species (Table 3), due to the low decomposition of the exotic litters of *R. pseudoacacia* and *U. pumila*. However, the litter of the remaining exotic, *A. altissima*, decomposed as fast as the litter of *U. minor*, which exhibited the highest decomposition (Fig. 1). Litter with higher N concentration tended to lose more mass than N-poor litter (Pearson correlation coefficients between litter N and proportion of litter mass loss for Poulo species, Alcalá species and all species were $r=0.74$ $p=0.26$, $r=0.74$ $p=0.16$ and for $r=0.70$ $p=0.049$, respectively); however, no significant correlation was found between litter mass loss and LMA ($r=-0.37$ $p=0.63$, $r=0.51$ $p=0.38$ and $r=-0.26$ $p=0.51$ for Poulo species, Alcalá species and all species, respectively).

Poulo soil properties showed contrasting responses to the addition of different litter types, while Alcalá soil showed virtually the same trends irrespective of the type of added litter. During the experiment, soil pH of Poulo tended to increase when covered with *P. pinaster* or *E. globulus* litter, but decreased below *Q. robur* litter, and remained similar below *A. dealbata* litter. However, the only significant pH difference was found between *E. globulus* and *Q. robur* treatments (Table 3, Fig. 2a). We found no support to hypothesis 2, which suggested that exotic litter changed soil properties more than native litter. The percentage of organic matter in Poulo soil increased below the native litter (*P. pinaster* and *Q. robur*), but decreased

Table 2 Mean leaf mass per area (LMA) of green leaves, and chemical properties of the litter (N and lignin content and C/N ratio) for each species and site. C/N and ligning were obtained from the literature for all species but *Acacia dealbata*. Origin: E-exotic, N-native to the Iberian Peninsula. Different letters within LMA or N columns means significant differences on the basis of an ANOVA test followed by a post-hoc Tukey test. (Significant values P≤0.05)

	Origin	LMA (mg cm^{-2})	N (mg/g)	C/N	Lignin (mg/g)
Species from Poulo (mesic forest)					
Acacia dealbata	E	10.01±0.73b	15.77±0.63d		
Eucalyptus globulus [a]	E	8.38±0.41b	5.41±0.18a	84.66^{c}	151.2^{c}
Eucalyptus globulus [b]	E	10.24±0.80b	7.49±0.18b		
Pinus pinaster	N	22.20±0.97c	4.35±0.06a	96.48^{c}	228.9^{c}
Quercus robur	N	5.09±0.15a	10.28±0.42c	37.70^{d}	233.5^{d}
Species from Alcalá (riparian forest)					
Ailanthus altissima	E	7.91±0.22a	12.65±1.03ab	47.24^{e}	52.3^{e}
Robinia pseudoacacia	E	7.28±0.51a	11.63±0.73ab	29.41^{e}	145.0^{e}
Ulmus pumila	E	10.70±0.71bc	10.47±0.96a	38.67^{c}	25.1^{c}
Fraxinus angustifolia	N	8.65±0.61ab	12.94±0.79ab	52.64^{e}	60.2^{e}
Ulmus minor	N	11.87±0.51c	15.69±1.97b	41.99^{e}	47.8^{e}

[a] adult tree phyllodes, [b] juvenile tree leaves. [c] Data from Godoy et al. (2010). [d] Data from Hobbie et al. (2006). [e] Data from Alonso et al. (2010)

below the exotic litter of *E. globulus*. Below *A. dealbata* litter, soil organic matter slightly decreased, but its value neither differed from that of *E. globulus* nor the native litter treatments (Fig. 2b). On average, NO_3^--N increased more below the native than the exotic litter (Table 3). However, pair-wise comparisons revealed that this effect was driven by the negligible increase of nitrate below *E. globulus* litter, as the other exotic (*A. dealbata*) produced a similar effect as the native *P. pinaster*, and the highest increase of NO_3^--N was found below the native *Q. robur* litter (Fig. 2c). Accordingly, resin-assessed nitrate availability increased through time below all litter types, except *E. globulus* (Table 4, Fig. 3). NH_4^+-N increased signifincantly more below the exotic than the native litter (p=0.025, Table 3), although pair-wise differences across species were not significant (Fig. 2d). Resin-assessed ammonium availability in *P. pinaster*, *Q. robur* and *E. globulus* trays decreased the first 4 months, peaked in the sixth month and declined again in the last month of incubation. By contrast, below *A. dealbata* litter, ammonium constantly increased since the second month (Table 4, Fig. 3). The highest increase of total soil mineral N was found below the litter of the native *Q. robur* and the exotic *A. dealbata,* closely followed by *P. pinaster*. Below *E. globulus* litter we found the least increase of mineral N, which significantly differed from the values found in the *A. dealbata* and *Q. robur* treatments, but not from that of *P. pinaster* (Fig. 2e).

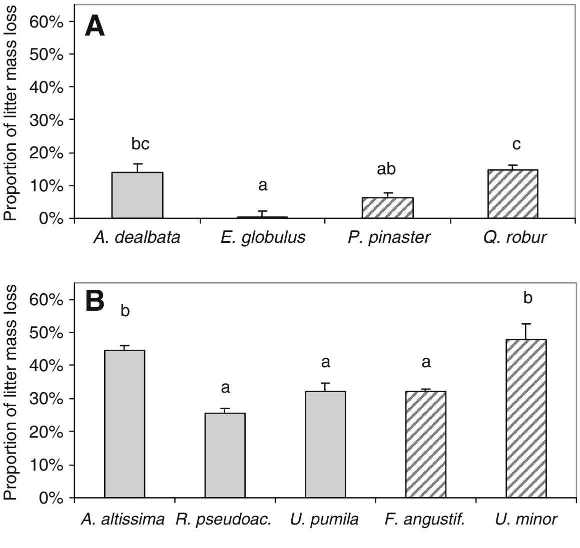

Fig. 1 Percentage of leaf litter mass loss after 9 months of incubation in a growth chamber. **a**) Species from Poulo (mesic forest of NW Spain) and **b**) species from Alcalá (riparian forest of central Spain). Grey columns represent exotic species and dashed columns native species. Different letters between columns in each graph means significant differences on the basis of an ANOVA test followed by a post-hoc Tukey test (Significant values P≤0.05)

Table 3 F-values for the ANOVA analyses assessing the effect of litter origin (native or exotic to the Iberian peninsula) and species litter (nested in origin) on the proportion of litter mass loss and on the change of soil properties between the beginning and the end of the experiment. ns – P>0.10; (+) 0.10≥p>0.05; * 0.05≥p>0.01; ** 0.01≥p>0.001; *** p≤0.001

	Poulo (Mesic forest)		Alcalá (Riparian forest)	
	Origin	Species	Origin	Species
% Litter mass loss	3.19(+)	17.62***	5.96*	14.07***
Soil pH	4.25 (+)	6.46*	0.01 ns	0.54 ns
Soil organic matter	15.71**	0.23 ns	5.44*	0.78 ns
NO_3^--N	45.82***	39.82***	2.90 ns	1.59 ns
NH_4^+-N	6.74*	0.19 ns	3.55(+)	2.69(+)
Total mineral N	2.35 ns	6.68*	2.41 ns	1.59 ns
Total N	2.64 ns	3.52(+)	0.81 ns	1.06 ns

Total soil N (including organic and inorganic N) increased in all cases, but slightly more below *P. pinaster* litter (the significance of the species factor in the ANOVA analysis was marginally significant (p=0.063) Table 3, Fig. 2f).

Among Alcalá species, litter origin only affected the organic matter content of the soil, decreasing more below the native than below the exotic litter (Table 3, Fig. 2h). Soil pH decreased, and NO_3^--N and mineral N increased below all litter types (Fig. 2g, i, k). Resin-assessed nitrate availability speeded up after 4–6 months of incubation (Table 4, Fig. 3). NH_4^+-N was marginally affected by origin and species (p=0.081 and p=0.086, respectively), decreasing slightly more below *F. angustifolia, U. minor* and *R. pseudoacacia* litter than below the rest of the species (Table 3, Fig. 2j). Resin-assessed ammonium availability decreased during the first 2 months, recovered until the sixth month and declined again in the last month of incubation (Fig. 3). Total N tended to increase only below *A. altissima*, and to remain constant below the rest of the species, but the species effect was not significant (Table 3, Fig. 2l).

Discussion

Temporal changes in soil properties were different between the two studied soils, which may be due to differences in their initial properties, differences between the litter added to each soil, or both. Growth and activity of decomposing soil microorganisms may have been more limited in Poulo than in Alcalá soil because of a lower litter quality among Poulo species (e.g. higher lignin content, see Table 2), because of the lower soil pH (Whalen and Sampedro 2009; Chamier 1987; Kittle et al. 1995; Motavalli et al. 1995) and/or because of the lower soil N content, which is a requirement for the growth of microbes (Whalen and Sampedro 2009; Yoshitake et al. 2007; Eiland et al. 2001). These arguments may explain the overall higher litter decomposition (Fig. 1) and the larger decline of soil organic matter (Fig. 2b, i) found in Alcalá treatments, as compared to Poulo treatments. Mineralization in Poulo soil was co-dominated by ammonification and nitrification processes, while in Alcalá it was dominated by nitrification processes (Fig. 2c, d, i, j). This suggests that there are differences in composition or activity rate of microbial communities between both sites. Nitrification in the Poulo soil may be inhibited by the low pH, while favoured in the Alcala soil by the lower C availability. This is suggested by the low organic matter to N ratio of the Alcalá soil, as compared to the Poulo soil, (Table 1) which may promote autotrophic nitrification (Aerts and Chapin 2000). In spite of the higher litter mass loss of Alcalá trays, total soil N tended to increase more in Poulo treatments (Fig. 2f, l), maybe because denitrification processes may compensate for N gains in the former.

The results of this study did not agree with the general trends of exotic invasive plants increasing soil N pools and speeding up the N cycle, shown by previous reviews (Ehrenfeld 2003; Liao et al. 2008). Conversely, the least decomposed litter in both soils belonged to exotic species, including the N-fixing *R. pseudoacacia* (Fig. 3). The higher decomposition of *Q. robur* and *A. dealbata* litter among Poulo species and of *U. minor* and *A. altissima* litter among Alcalá species may be attributed to their relatively low LMA and C/N, to a relatively high N content, or a combination of both (Cornelissen and Thompson

Fig. 2 Changes of soil properties between the beginning and the end of the experiment in trays filled with soil from Poulo (*left*, A–F) and Alcalá (*right*, G–L), and covered with leaf litter of different native (*dashed columns*) and exotic (*grey columns*) tree species. Different letters across columns mean significant differences after an ANOVA followed by a post-hoc Tukey test (Significant values P≤0.05)

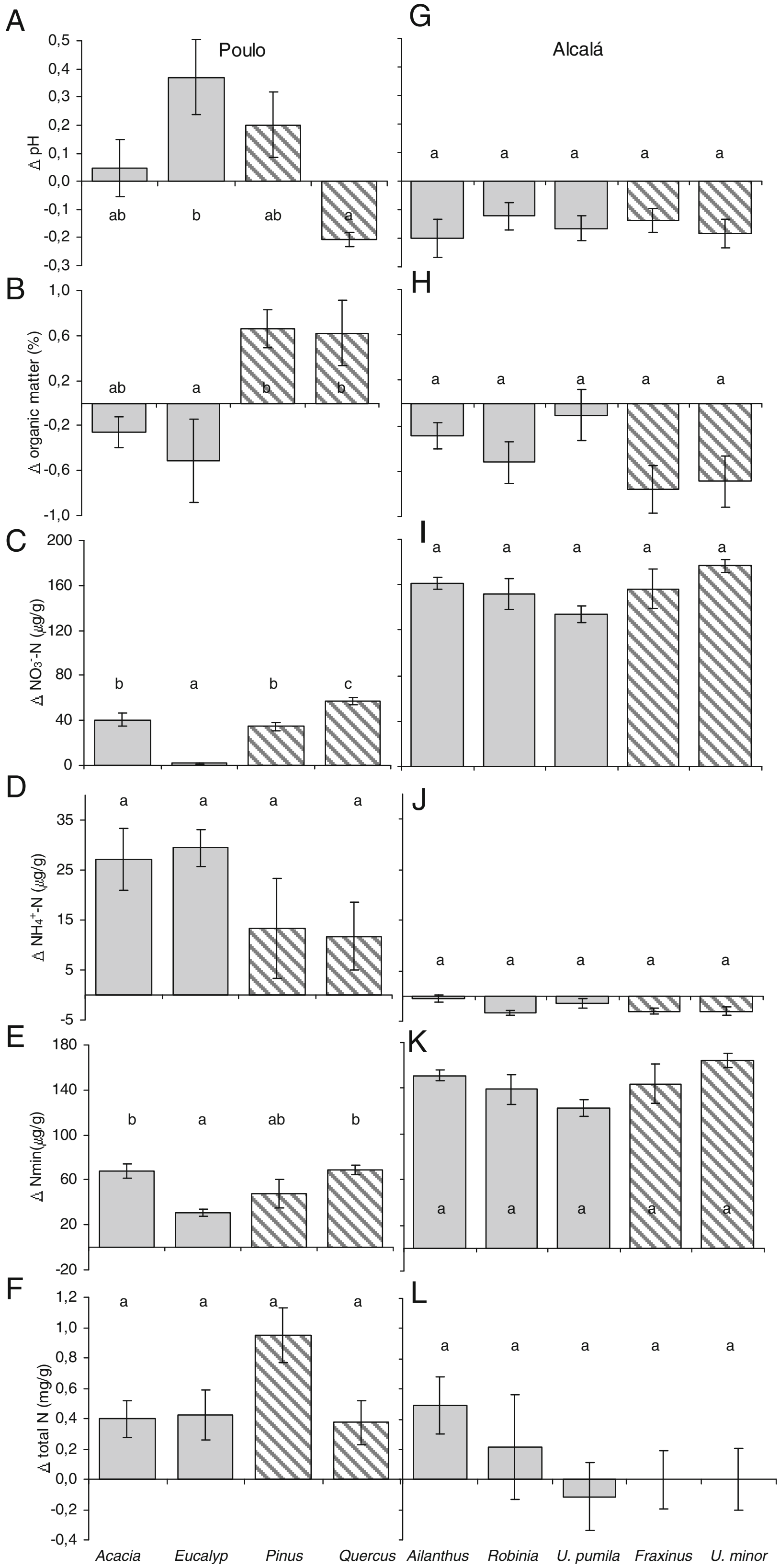

Table 4 Summary results of the repeated-measures ANOVA, assessing the effects of time (within-subject factor) and litter species (between-subject factor) on the nitrate and ammonium soil availability, assessed by means of ion exchange resins. F-values and their significance are provided

	NO_3^--N		NH_4^+-N	
	F	Sig.	F	Sig.
POULO (mesic forest)				
Within-subject effects				
month	93.964	<0.001	16.803	<0.001
month*species	6.492	<0.001	4.490	<0.001
Between-subject effects				
Species	46.355	<0.001	26.608	<0.001
ALCALÁ (riparian forest)				
Within-subject effects				
month	24.014	<0.001	80.699	<0.001
month*species	3.096	0.002	2.900	0.002
Between-subject effects				
Species	2.840	0.068	0.783	0.556

1997; Gallardo and Merino 1993). However, contrary to hypothesis 3, these litter properties failed to explain the low decomposition of some exotic litters. For instance, *R. pseudoacacia* showed the lowest LMA and C/N among Alcalá species. Instead, the presence of unassessed recalcitrant compounds, such as lignin in *R. pseudoacacia* litter (Castro-Díez et al. 2009; Alonso et al. 2010), or soluble polyphenols, as reported in *E. globulus* litter (Corbeels et al. 2003), may explain this result.

Organic matter content in the acid soil of Poulo increased below the native litter (*P. pinaster* and *Q. robur*) and decreased below the exotic litter (*E. globulus, A. dealbata,* Fig. 2b). Therefore, the release of organic matter from the native litter seemed to over-compensate the soil losses by mineralization. By contrast, organic matter declined below *A. dealbata* litter, in spite of the relatively high litter decomposition, suggesting a higher soil microbial activity. The same occurred in the basic Alcalá soil, whose organic matter declined in all cases. This decline was lower

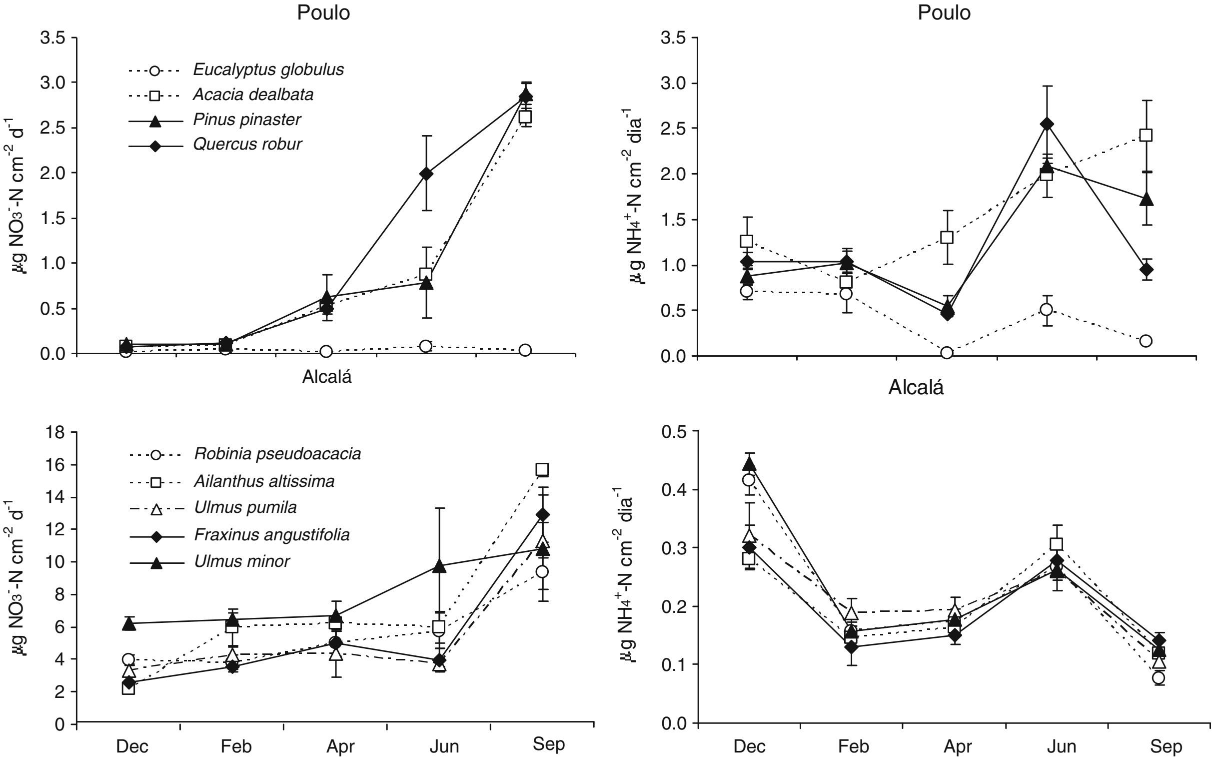

Fig. 3 Nitrate (NO_3^--N) and ammonium (NH_4^+-N) availability over time assessed by means of adsorption resin membranes in soils of Poulo (*above*) and Alcalá (*below*) covered by different species litter (exotic litter represented by open symbols and native litter by filled symbols). Note the different scales between graphs

below the exotic litter (Table 3), suggesting that compounds released by this litter may partially inhibit soil mineralization activity. In the case of *R. pseudoacacia* the inhibitory compounds may be polyphenols, which are known to form complexes with proteins that are highly resistant to decomposition (Taylor et al. 1989; Hattenschwiler and Vitousek 2000). In the case of *A. altissima*, it is known to release allelopathic compounds, such as ailanthone, that are toxic to many plant species (Heisey and Heisey 2003; Kowarik and Saumel 2007), although their effects on soil microbes are not clear (Heisey and Heisey 2003; Castro-Díez et al. 2009).

In Alcalá soil no differences were found in pH, mineral N or total N across litter type treatments (Table 3). This can be attributed to the fact that the variation range of litter properties was lower across Alcalá species than across Poulo species (Table 2). In the case of Poulo, litter impacts on soil properties depended more on the species which produced the litter than on the origin of the species (native or exotic). Soil pH changes may reflect a shift in the balance between incomes (through litter decomposition) and losses (through mineralization) of organic acids (Finzi et al. 1998). In the case of the *E. globulus* treatment, where soil organic matter declined and litter mass remained unchanged at the end of the incubation (Figs. 2a, b and 1a), this balance was negative, leading to a pH increase. In the case of *Q. robur* treatment, the fast litter decomposition may lead to a positive balance and to a pH decrease. The relatively high increase of ammonium and nitrate at the end of the incubation period below *A. dealbata* litter (Fig. 1c, d, e) indicates a notable N mineralization activity, which accords with the decline of soil organic matter. By contrast, the small increase of mineral N in soils beneath *E. globulus* litter (Fig. 1f), fails to explain the relatively large decline of soil organic matter and suggests that the litter of this species inhibits more the mineralization of organic N than the mineralization of organic C, as suggested by previous studies (Mendham et al. 2004; Rovira and Vallejo 1997). Accordingly, the increase of mineral N, particularly nitrate, below *E. globulus* litter, was the lowest among all the studied litter types.

The relatively low nitrate availability shown by adsorption resins in all treatments during the first months of incubation (Fig. 3) can be attributed to N-poor litter organic compounds (simple sugars, starch, etc.) decomposing earlier than N-rich compounds (Whalen and Sampedro 2009). The subsequent nitrate increase suggests that the peak of protein breakdown occurred after 4–6 months of incubation. Ammoniun availability showed declines and increases during the incubation period, consistent across most treatments, indicating that the balance between ammonification activity and microbial uptake and/or conversion to nitrate was reversed several times during the 9-months incubation period.

The above results mostly support hypothesis 1, suggesting that the effects of plant litter on soil properties were species-specific, rather than origin-specific (i.e. native or exotic). Although some soil properties were significantly affected by the origin of species, the found trends differed between the two scenarios (i.e. soil organic matter and ammonium), or were clearly driven by one species (i.e. soil pH and nitrate trends found below exotic litter in Poulo were driven by *E. globulus*).

We found no support to hypothesis 2, suggesting that the addition of native litter would alter soil properties less than the addition of exotic litter, in either scenario, as native plant litter was expected to be in equilibrium with the local soil. However, the properties of other plant parts shed by the same species, such as fine roots or stems, may largely differ from those of the leaf litter (Hobbie et al. 2010). In the case of Alcalá floodplain soil, deposition of sediments from upstream represents an additional and relevant source of nutrients and organic matter to the soils (Noe and Hupp 2005; Olde Venterink et al. 2006). Therefore, soil properties are only partially determined by the input of native leaf litter. Our experiment also lacks detritivorous meso- and macrofauna, which fragment and make organic compounds available for microbial attack (Whalen and Sampedro 2009; Gallardo and Merino 1993). Under field conditions these fauna might be negatively affected by the addition of exotic litter, amplifying the effect of this litter on soil processes and properties. Further studies are needed to explore these possibilities.

We conclude that the litter produced by the main exotic trees living in two scenarios of the Iberian Peninsula did not decompose faster than that of the native trees. Moreover, litter of three out of the five exotic trees decomposed less rapidly than the coexisting native litter, contrary to the general trends reported in the literature. However, this trend was not consistently translated into changes of soil pH, total and mineral N pools, which varied irrespective of the added litter (Alcalá soil), or responded to each litter type, irrespective of their origin (Poulo soil). *E. globulus* litter caused the most drastic impact on the soil, strongly inhibiting nitrification and reducing soil N content. Litter of the N-fixing exotic trees did not increase soil N pools more than the rest of the species, probably because the transfer of litter N to the soil was slowed down by the presence of recalcitrant secondary compounds in the litter.

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