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Soil characteristics determine soil carbon and nitrogen availability during leaf litter decomposition regardless of litter quality

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ABSTRACT

Climate and litter quality have been identified as major drivers of litter decomposition, but our knowledge of how soil characteristics (e.g. microbial community and chemical properties) determine carbon (C) and nitrogen (N) availability derived from the decomposition of litter of different qualities is still scarce. We conducted a microcosm experiment to evaluate how soils with contrasting microbial communities and soil properties (denoted Soils A and B hereafter, where Soil B has higher bacterial and fungal abundance, fungal:bacterial ratio, and organic C than Soil A) determine the availability of soil C (carbohydrates, proteins, amino acids and phenols) and N (dissolved organic and inorganic N, microbial biomass N and available N) during the decomposition of litter of contrasting quality (C:N ratios ranging from 20 to 102). We also evaluated the relative importance of soil characteristics and litter quality as drivers of C and N inputs to the soil during this process. Overall, higher soil C and N availability after litter decomposition was found in Soil B than in Soil A. Soil characteristics had a higher positive effect on soil C and N contents than litter quality during litter decomposition. We also found that changes in N availability and organic matter quality registered after litter decomposition, linked to different soil characteristics, were able to promote dissimilarities in the potential mineralization rates. In conclusion, our study provides evidence that soil characteristics (e.g. microbial communities and chemical properties) can be more important than litter quality in determining soil C and equally important for N availability during the decomposition of leaf litter.

1. Introduction

Litter decomposition is one of the main processes controlling the availability of carbon (C) and nitrogen (N) in terrestrial ecosystems (Schlesinger, 1996). Identifying the main factors controlling this process has been a major topic in ecological research over the last decades (Meentemeyer, 1978; Cleveland et al., 2014). Climate and litter chemistry have been traditionally considered as the main drivers of litter decomposition (Meentemeyer, 1978; Hättenschwiler et al., 2005; Cornwell et al., 2008; García-Palacios et al., 2013a), with soil playing a minor role in this process (Schimel, 1995; Reed and Martiny, 2007; Green et al., 2008). However, a growing number of studies are showing that soil with different characteristics (e.g. different microbial communities) have different functionalities ("functional breadth" *sensu* Keiser et al., 2013), and thus differentially affect ecosystem processes such as litter decomposition (Strickland et al., 2009; Wallenstein et al., 2010; Keiser et al., 2013; Cleveland et al., 2014). For example, it is well known that litter decomposes faster with its local soil microbial community than when it is exposed to a non-native soil (Ayres et al., 2009; Strickland et al., 2009; Wallenstein et al., 2010; Keiser et al., 2011, 2013). In addition to their direct effects on litter decomposition, soil microbial communities largely determine the availability of C and N in soils through a number of mechanisms. For instance, fungal-dominated microbial communities use N more

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efficiently, and thus accelerate mineralization, than bacterial-dominated ones, enhancing N availability in the soil (Paul and Clark, 1996; Austin et al., 2004). In addition, complex processes, such as the depolymerization of large organic molecules into dissolved organic N (DON), require the cooperation of diverse groups of microorganisms (Schimel et al., 2005; Delgado-Baquerizo et al., 2013a). This is not the case for processes such as nitrification, which is carried out by specific groups of microorganisms (Schimel et al., 2005; Delgado-Baquerizo et al., 2013a).

Soil microbes interact with litter and soil chemistry to determine soil C and N availability. For example, a lower C:N ratio in the microbial substrate and/or a higher soil N availability have been related to a higher N mineralization and lower relative dominance of DON (Austin et al., 2004; Schimel and Bennett, 2004). The interplay between litter quality and microbial communities can have important implications on the total soil C and N storage in response to global change impacts (Wallenstein et al., 2010; Keiser et al., 2013). In this direction, litter quality is shifting in multiple biomes due to changes in plant community composition and/or phenotypic responses to global changes (Murphy et al., 2002; Kurokawa et al., 2010; Sardans et al., 2012). Similarly, climate change drivers, such as warming and drought, are shifting the soil microbial communities by promoting a higher fungal:bacterial ratio in many ecosystems worldwide (Zhang et al., 2005; Jung Kwon et al., 2013; Maestre et al., 2013), while increasing N deposition has been found to reduce the abundance of fungi relative to bacteria (Wallenstein et al., 2006; Strickland and Rousk, 2010). Improving our knowledge of how different soil characteristics (e.g. microbial and chemical properties) modulate the availability of soil C and N during litter decomposition in response to different litter qualities is of importance for understanding nutrient cycling in soils, and to predict how it will evolve with ongoing global environmental change (Cleveland et al., 2014). For example, a shift in the microbial community structure from fungal-dominated to bacterial-dominated communities may decrease N depolymerization and subsequent mineralization in soils with low quality litter inputs, which are expected to occur with global change (e.g. high litter C:N ratio as a consequence of increasing atmospheric CO_2, Sardans et al., 2012). Changes in the availability of nutrients linked to different microbial communities during litter decomposition may influence future decomposition and mineralization processes for a particular soil, as a consequence of the previous nutrient legacy (Keiser et al., 2011). Despite the importance of C and N cycles on ecosystem functioning and services (Schlesinger, 1996; Wardle, 2002; Robertson and Groffman, 2007), our understanding of how soil characteristics control soil C and N dynamics during leaf litter decomposition is still scarce (Bradford et al., 2013; Keiser et al., 2013).

García-Palacios et al. (2013b) used a litter decomposability assay to show that soils with higher levels of organic C, microbial abundance, microbial functional diversity and fungal:bacterial ratio accelerated litter decomposition in response to a wide variety of litter species. Here we used the experimental set up of García-Palacios et al. (2013b) to: i) evaluate changes in C and N availability in soils with contrasting characteristics (organic C, total N and microbial communities) during the decomposition of litters differing in its quality; ii) determine the relative importance of soil characteristics and litter quality as predictors of soil C and N availability; and iii) assess how changes in the nutrient availability derived from litter decomposition can distinctively affect potential net N mineralization and transformation rates in soils with contrasting soil characteristics. We hypothesized that soils with higher microbial functional diversity, bacterial and fungal abundance and fungal:bacterial ratio will increase C and N availabilities during litter decomposition (Austin et al., 2004). Additionally, we expected that changes in the N availability of the soil linked to different microbial communities would have a direct legacy effect on processes such as potential N mineralization rates (Schimel and Bennett, 2004).

2. Methods

2.1. Study site and soil sampling

Soils for this study were collected during Spring 2011 from two semiarid roadside grasslands from central Spain (Fig. S1). The first site was a 2- year-old roadside grassland, and represents an early-successional stage (39° 47′ N, 3° 12′ W, 731 m a.s.l.); the other was a >20-year-old roadside grassland representing a late-successional stage (40° 22′ N, 03° 53′ W; 615 m a.s.l.). For the top 10 cm, soil pH ranged between 7.2 and 8.3, organic C between 0.8% and 2.3%, total N between 0.08% and 0.24%, and total P between 0.03% and 0.07%, for the early- and late-successional grasslands, respectively (Table 1). We selected roadside grasslands for our study because they show rapid structural and compositional changes in their microbial communities, following a similar trajectory to that recorded during secondary succession in old fields (Harris, 2009; García-Palacios et al., 2011a,b), and thus represent a valid study system to investigate the functional role of soils with contrasting characteristics.

Thirty soil cores (0–10 cm depth) were randomly sampled at each grassland. Soil samples were bulked by site to get a representative microbial community, homogenized, and kept cold in the field until laboratory preparation. In the laboratory, the samples were sieved (2-mm mesh), and one fraction was immediately frozen at −80 °C for microbial analysis. The other fraction was kept at 4 °C for 1 day before conducting the decomposability assay. Before this assay, we characterized the microbial communities from both the early- and late-successional grasslands. The abundance of bacterial 16S and fungal 18S rRNA genes was obtained using qPCR as described by Evans and Wallenstein (2011). We calculated the fungal: bacterial ratio from these data. Additionally, the microbial functional diversity was measured by using Microresp (Campbell et al., 2003), as described in García-Palacios et al. (2011b). The

Table 1
Main characteristics of the studied soils. Data are means (SE), $n = 5$.

Variable	Soil A	Soil B
pH	8.31 (0.29)	7.15 (0.02)
Total N[a]	0.08 (0.01)	0.24 (0.02)
Organic C[a]	0.77 (0.07)	2.30 (1.28)
Total P[b]	0.03 (0.01)	0.06 (0.02)
Microbial functional diversity[b]	1.88 (0.51)	2.63 (0.84)
Bacteria[c]	$1.36 \cdot 10^8$ ($8.52 \cdot 10^7$)	$3.56 \cdot 10^9$ ($1.31 \cdot 10^9$)
Fungi[c]	$9.24 \cdot 10^6$ ($3.71 \cdot 10^6$)	$9.27 \cdot 10^8$ ($4.40 \cdot 10^8$)
Fungal:bacterial ratio	0.07 (0.01)	0.26 (0.02)
Dissolved inorganic N (DIN)[d]	18.61 (1.36)	20.18 (1.38)
Dissolved organic N (DON)[d]	31.17 (7.59)	17.42 (1.85)
Available N[d]	49.78 (6.55)	37.60 (1.60)
Microbial biomass N[d]	22.72 (7.60)	23.16 (3.47)
Carbohydrates[e]	34.42 (2.04)	33.22 (2.69)
Amino acids[e]	6.46 (0.01)	3.36 (0.24)
Proteins[e]	20.76 (2.03)	19.84 (1.26)
Phenols[e]	8.22 (0.44)	7.15 (0.30)
DIN: DON	0.83 (0.28)	1.24 (0.21)
Pentoses: hexoses ratio	0.63 (0.15)	1.04 (0.08)
Carbohydrates: phenols ratio	4.42 (0.39)	4.59 (0.57)
Potential net mineralization[f]	2.39 (0.29)	1.94 (0.28)
Potential net N transformation[f]	1.02 (0.35)	1.57 (0.23)

[a] %.
[b] Decits.
[c] DNA copies g^{-1} soil.
[d] mg N kg^{-1} soil.
[e] mg C kg^{-1} soil.
[f] mg N kg^{-1} soil day^{-1}.

soil from the late-successional grassland (Soil B hereafter) showed higher levels of microbial functional diversity, but also more bacteria and fungi and a higher fungal:bacterial ratio than that from the early-successional grassland (Soil A hereafter; Table 1).

2.2. Litter decomposability assay

In this study, we used leaf litter from 16 different crops and wild crop relatives (Table S1). These species provide a wide range of litter qualities, as determined by contrasting litter C:N ratios (from 20.3 to 101.9; Table S1). We used the C:N ratio as our proxy of litter quality because it is a strong predictor of litter decomposition (Enríquez et al., 1993). This is especially true for our study, as showed in García-Palacios et al. (2013b). The focal plant species were grown during spring 2011 in a common garden at the plant growth facilities of Rey Juan Carlos University, located in Móstoles, central Spain (40°18′48″N, 38°52′57″W, 632 m a.s.l.; see García-Palacios et al., 2013a for details on this set up). At the end of the growing season, naturally senescent leaf litter were collected from the different plant species (from 5 individuals). The litter was air-dried for one month and then was analyzed for total C and N in an Elementar varioMAX N/CN (Hanau, Germany).

Sixty grams of each soil type (A and B) were introduced into 250 mL air-tight mason jars and moisture adjusted to 50% water holding capacity. Microcosms were constructed by carefully placing 0.75 g of air-dried litter (1 × 3 cm fragments) on top of the soil surface. We established five microcosms for each litter quality (16)× soil type (2) combination, rendering a total of 160 microcosms. Five controls (without litter) were also set for each soil type. Microcosms were placed in a plant growth chamber under 20 °C, 95% air humidity and darkness conditions for 9 weeks. The location of microcosms in the growth chamber was randomized weekly to avoid potential effects of subtle gradients in temperature and moisture present within the chamber. Litter decomposability was estimated along the 9 week incubation period by monitoring soil respiration rates with the method described in García-Palacios et al. (2013c). Soil respiration in each "litter" microcosms was corrected for soil contributions by subtracting the production rates measured in the "no-litter" soils, as explained in Strickland et al. (2009). The CO_2 concentration (%) was converted to CO_2 production rate (μg CO_2–C g^{-1} soil g^{-1} litter h^{-1}) as described in Campbell et al. (2003). The respiration rates are expressed per gram of litter to control for the fact that the amount of litter used in two species (*Lactuca serriola* L. and *Lactuca sativa* L.) was smaller (0.60 g) than in the rest. A Newton integration was applied to the CO_2 production rate to calculate the cumulative respiration rate at the end of the experiment (soil respiration was measured weekly) in each microcosm (García-Palacios et al., 2013b). Despite the litter:soil ratio used was smaller than the ratio used in similar litter decomposition incubations (e.g., Ayres et al., 2009), litter addition led to an increase in respiration ranging from 54.8 to 217%, and from 59.6 to 313%, in Soils A and B, respectively, as compared to microcosms without litter addition.

2.3. Soil C and N availability

Over 2.5 g of soil were taken from each microcosm before and after the litter decomposability assay. Soil samples (2.5 g of soil) were extracted with K_2SO_4 0.5 M in a ratio 1:5. Soil extracts were shaken in an orbital shaker at 200 rpm for 1 h at 20 °C and filtered to pass a 0.45-μm Millipore filter (Jones and Willett, 2006). The filtered extract was kept at 4 °C until colorimetric analyses, which were conducted within the 24 h following the extraction. Subsamples of each initial condition and incubated extracts were obtained to measure four C sources (Yu et al., 2012): proteins, amino acids, phenols, and carbohydrates (pentoses plus hexoses). These variables were assessed following Chantigny et al. (2006). In brief, amino acids, proteins and phenols were colorimetrically determined by using the Ninhydrin (read absorbance at 570 nm), Bradford (read absorbance at 620 nm) and Folin–Ciocalteu (read absorbance at 720 nm) reactions, respectively. Carbohydrates were determined by independently analyzing pentoses and hexoses in our extracts. Pentoses and hexoses were colorimetrically determined by using the orcinol (read absorbance at 655 nm) and anthrone (read absorbance at 620 nm) reactions, respectively. The concentration of carbohydrates was then calculated as the sum of pentoses and hexoses in our extracts. The DIN was colorimetrically estimated using the indophenol blue method (read absorbance at 655 nm), after incubating sub-samples of our extracts with Devarda alloy overnight (Sims et al., 1995). Similarly, available N was determined in the extracts by using the indophenol blue method (read absorbance at 655 nm), after the potassium persulphate digestion in an autoclave at 121 °C over 55 min (Sollins et al., 1999) and after incubating sub-samples of our digested extracts with Devarda alloy overnight (Sims et al., 1995). The concentration of dissolved organic N (DON) was determined as the difference between available N and DIN in our extracts (Delgado-Baquerizo et al., 2011). In addition, microbial biomass N (MB-N) was determined using the fumigation-extraction method (Brookes et al., 1985). We calculated the pentoses:hexoses, carbohydrates:phenols and the DIN:DON ratios from these variables. The pentoses:hexoses ratio provides information about the relative abundance of plant-derived carbohydrates in the soil organic matter (Chantigny et al., 2006). Additionally, the carbohydrates:phenols ratio gives an estimation of the quality of soil organic matter (Rovira and Vallejo, 2002).

Before and after the litter decomposability assay (once the litter was removed), we measured the potential net N transformation (production of available N) and mineralization rates of our soils. Air-dried soil samples were re-wetted to reach 80% of their water holding capacity and incubated in the laboratory for 14 days at 30 °C (Allen et al., 1986). Potential net N transformation (production of available N) and mineralization rates were estimated as the difference between initial and final available N (sum of DON and DIN) and DIN, respectively, by following Delgado-Baquerizo and Gallardo (2011).

2.4. Statistical analyses

Given the lack of normality in some of the studied C and N variables, the differences in the initial C and N concentrations between soil type (A vs. B) were tested by using one-way PERMANOVA (Anderson, 2001). PERMANOVA uses permutation tests to obtain *P* values, does not rely on the assumptions of traditional parametric ANOVA, and can handle experimental designs such as the one employed here (Anderson, 2001).

To estimate how litter quality and microbial community affected soil C and N variables throughout the duration of the decomposition assay, we calculated the absolute effect size (Ai) as Cl–Cwl, where Cl and Cwl are the values of a given C or N variable in the microcosms with litter and without litter (mean values) 9 weeks after the beginning of the incubation assay (for each microcosm). We used this approach to account for C and N releases from organic matter during the decomposition assay (Strickland et al., 2009).

As our data did not meet ANOVA assumptions (normality and homogeneity of variances), we analyzed the effects of soil type on the cumulative respiration and the Ai of each studied C and N variables by using one-way PERMANOVAs. Soil type was considered as a fixed factor, and litter quality was included as a covariable in these analyses.

We used structural equation modeling (Grace, 2006) to assess how direct and total effects of soil type and litter quality determined the Ai of our studied C and N variables. We included in our models the accumulated soil respiration measured during the decomposability assay as a proxy for microbial metabolism to check whether inputs of C and N from litter decomposition are linked to soil respiration (García-Palacios et al., 2013b). In our models, soil type is a categorical exogenous variable (Grace, 2006) with two levels: A (lowest values of levels of functional diversity, bacterial and fungal abundance and fungal:bacterial ratios) and B (highest values of functional diversity, bacterial and fungal abundance and fungal:bacterial ratios). Categorical exogenous variables are compatible with structural equation models because distributional assumptions do not apply to them (Grace, 2006). There is no single universally accepted test of overall goodness of fit for structural equation models, applicable in all situations regardless of sample size or data distribution. We used the Chi-square test (χ^2; the model has a good fit when $0 \leq \chi^2 \leq 2$ and $0.05 < P \leq 1.00$) and the root mean square error of approximation (RMSEA; the model has a good fit when $RMSEA\ 0 \leq RMSEA \leq 0.05$ and $0.10 < P \leq 1.00$; Schermelleh-Engel et al., 2003). Additionally, and because several variables were not normally distributed, we confirmed the fit of the model using the Bollen-Stine bootstrap test (the model has a good fit when 0.10 < bootstrap $P \leq 1.00$; Schermelleh-Engel et al., 2003). To aid with the interpretation of the results from our models, we also determined the standardized total effects (direct plus indirect effects from the structural equation modeling) of soil type, litter quality, and litter decomposition on the Ai of our studied C and N variables.

Finally, we used independent one-way PERMANOVAs to evaluate the effects of soil type on the potential net mineralization and N transformation rates before and after litter decomposition (with and without litter). Soil type (Soil A vs. B) was considered a fixed factor in these analyses, and litter quality was included as a covariable when we analyzed differences between soil soil types in the microcosms with litter.

PERMANOVA analyses were carried out using 9999 permutations (permutation of raw data) and the Euclidean distance with the PERMANOVA+ for PRIMER statistical package (PRIMER-E Ltd., Plymounth Marine Laboratory, UK). Structural equation modeling analyses were performed using AMOS 20 for windows (SPSS Inc., Chicago, IL, USA).

3. Results

Before the incubation, Soil A had a higher concentration of amino acids than Soil B ($P < 0.01$; Table S2). Non-significant differences between these soils were found in other C or N variables evaluated ($P > 0.05$; Table 1).

Nine weeks after the beginning of the experimental assay, Soil B showed a higher concentration of microbial biomass N, amino acids, proteins, phenols and pentoses:hexoses ratios than Soil A in the microcosms without litter ($P < 0.05$; Table S3). No differences were observed between soil types for the rest of C and N variables studied ($P > 0.05$; Table S4). Soil B had a higher Ai in cumulative soil respiration than Soil A (Fig. S2; Table S4; $P < 0.001$). In addition, Soil B showed higher values of Ai in DIN, available N, carbohydrates, proteins, phenols and in the DIN:DON, pentoses:hexoses and carbohydrates: phenols ratios than Soil A (Fig. S3). We did not find any differences between soil type for the Ai in DON (Fig. S3; Table S4). As a whole, soil type (i.e. higher microbes abundances, fungal: bacterial ratio, functional diversity, organic C and total N) had a

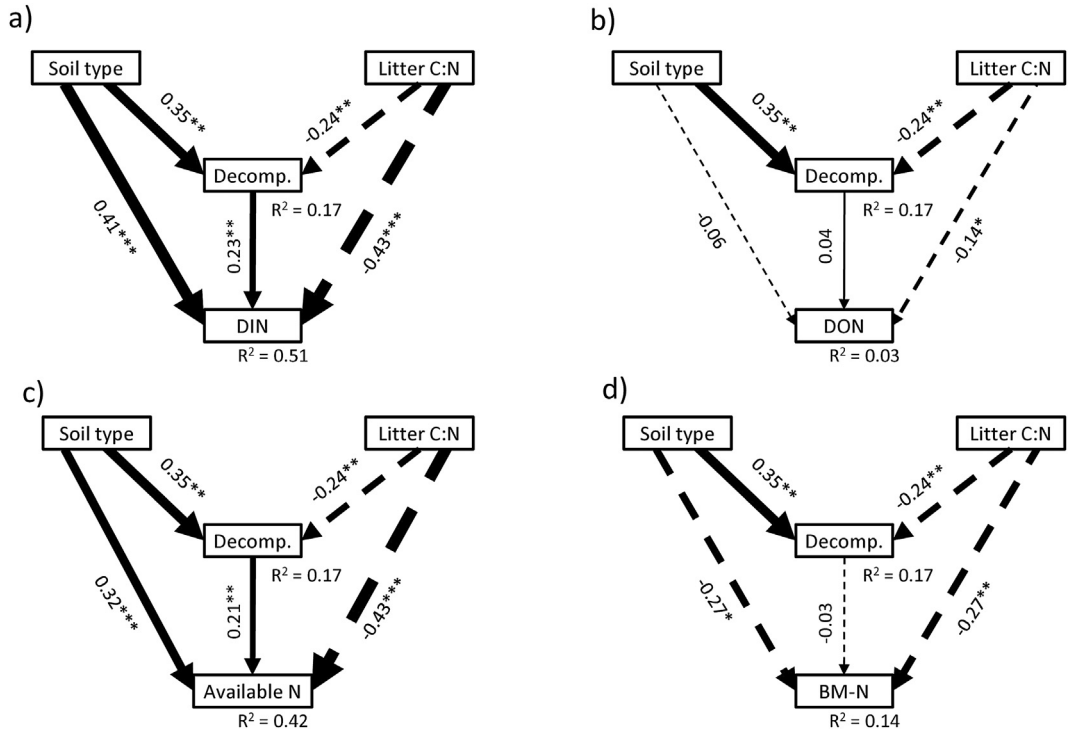

Fig. 1. Structural equation models based on the effects of soil type, litter quality (litter C:N ratio) and litter decomposition (Decomp.) on the Ai of the N variables (salt extractable) studied: DIN (a), DON (b), available N (c) and microbial biomass N (d; MB-N). Numbers adjacent to arrows are standardized path coefficients, analogous to partial regression weights and indicative of the effect size of the relationship. Continuous and dashed arrows indicate positive and negative relationships, respectively. Width of arrows is proportional to the strength of path coefficients. As in other linear models, R^2 indicates the proportion of variance explained and appears above every response variable in the model. Significance levels are as follows: ns = no significant, $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. The SEM models used (Figs. 1–3) satisfactorily fitted our data, as suggested by non-significant χ^2 values ($\chi^2 = 0.00$; $P = 1.00$; $df = 1$ in all cases), non-parametric Bootstrap $P = 1.00$ and by values of RMSEA = 0.00 ($P = 1.00$; Schermelleh-Engel et al., 2003).

positive direct effect on the Ai of DIN, available N, carbohydrates, proteins, phenols, DIN:DON, pentoses:hexoses and carbohydrates:phenols ratios ($P < 0.05$; Figs. 1–3, and a negative direct effect on that of microbial biomass N and amino acids ($P < 0.05$; Figs. 1–3). No significant direct effects of the soil type were observed on the Ai of DON ($P > 0.05$; Figs. 2 and 3; Fig. S3). Additionally, the litter C:N ratio showed a direct negative effect on the Ai of DIN, DON, available N, microbial biomass N, phenols and DIN:DON ratio ($P < 0.05$; Figs. 1–3; Fig. S3). Non significant direct effects from the litter C:N ratio were observed on the Ai of carbohydrates, amino acids, proteins, pentoses:hexoses and carbohydrates:phenols ratios ($P > 0.05$; Figs. 2 and 3; Fig. S3). In addition, litter decomposition only showed a significant positive direct effect on the Ai of DIN, available N and carbohydrates:phenols ratio, but a negative direct effect on the Ai of phenols ($P < 0.05$; Figs. 1 and 3; Fig. S3). We would like to highlight that all the C and N variables in this study indicate concentrations in the salt extracts, not from the whole soil.

Our SEM results show that soil type was more important than the litter quality and decomposition in determining the Ai of DIN, microbial biomass N, carbohydrates, amino acids, proteins, phenols, and DIN:DON, pentoses:hexoses and carbohydrates:phenols ratios (Fig. 4). However, litter quality had a higher importance modulating the Ai of DON and available N (Fig. 4). Similarly, and following our PERMANOVA results, soil type had a higher pseudo-F value, therefore suggesting a higher importance than litter quality for DIN, microbial biomass N, carbohydrates, amino acids, carbohydrate: available N, amino acids, proteins, and phenols (Table S4).

Finally, differences between the two soil communities were not observed in the potential net mineralization and N transformation (production of available N) rates in the microcosms incubated without litter after the decomposability assay (Fig. 5; $P > 0.05$; Table S4). However, Soil B showed higher potential net mineralization and N transformation rates than Soil A in the microcosms previously incubated with litter after the decomposability assay (Fig. 5; $P < 0.05$; Table S4).

4. Discussion

Our analyses provide strong evidence that soil characteristics (e.g. microbial communities) impact C and N availability more than litter quality during litter decomposition. In this respect, we found that microbial communities from soils with a higher level of microbial abundance, fungal: bacterial ratio and microbial functional diversity promoted an overall higher soil C and N availability than those present in soils with lower levels in these microbial variables. We highlight the lack of significant differences between the two soils used in the initial values of the studied C and N variables, as well as in other factors such as pH (Table 1). In addition, the differences observed in the total N and organic C in the initial conditions of these soils (Table 1) did not lead to significant differences in the potential net mineralization and N transformation rates before the decomposability assay, suggesting that a large amount of the organic matter originally present in our soils was not available for the soil microbial communities. Even so, and to account for any possible C and N release from organic matter during the decomposition assay, the available C and N variables in this study were corrected by subtracting the C and N levels measured from the corresponding "no-litter" soils (Strickland et al., 2009). As a whole, our results suggest that soil characteristics such as microbial abundance and functional diversity are the main driver of soil C and N dynamics during leaf litter decomposition.

The positive total effect of soil type on the availability of C and N (Fig. 2) may be the consequence of particular soil characteristics such as the fungal: bacterial ratio, microbial abundance and

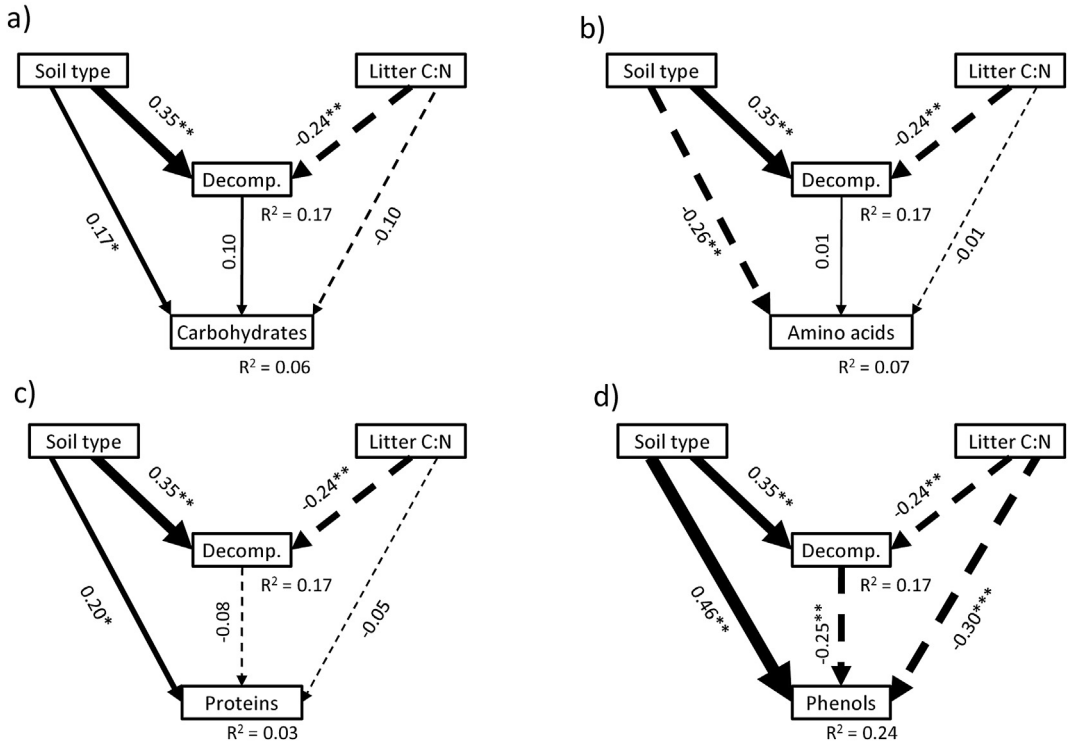

Fig. 2. Structural equation models based on the effects of soil type, litter quality (litter C:N ratio) and litter decomposition (Decomp.) on the Ai of the C variables (salt extractable) studied: carbohydrates (a), amino acids (b), proteins (c) and phenols (d). Numbers adjacent to arrows are standardized path coefficients, analogous to partial regression weights and indicative of the effect size of the relationship. Continuous and dashed arrows indicate positive and negative relationships, respectively. Width of arrows is proportional to the strength of path coefficients. Rest of legend as in Fig. 1.

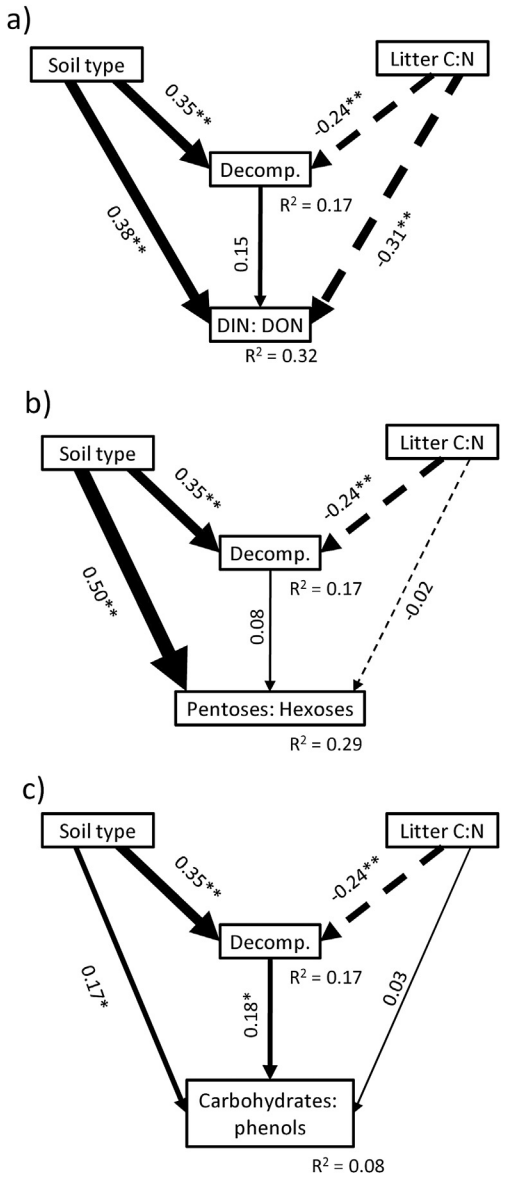

Fig. 3. Structural equation models based on the effects of soil type, litter quality (litter C:N ratio) and litter decomposition (Decomp.) on the Ai of the C and N ratios (salt extractable) studied: DIN:DON (a), pentoses:hexoses (b), and carbohydrates:phenols (c) ratios. Numbers adjacent to arrows are standardized path coefficients, analogous to partial regression weights and indicative of the effect size of the relationship. Continuous and dashed arrows indicate positive and negative relationships, respectively. Width of arrows is proportional to the strength of path coefficients. Rest of legend as in Fig. 1.

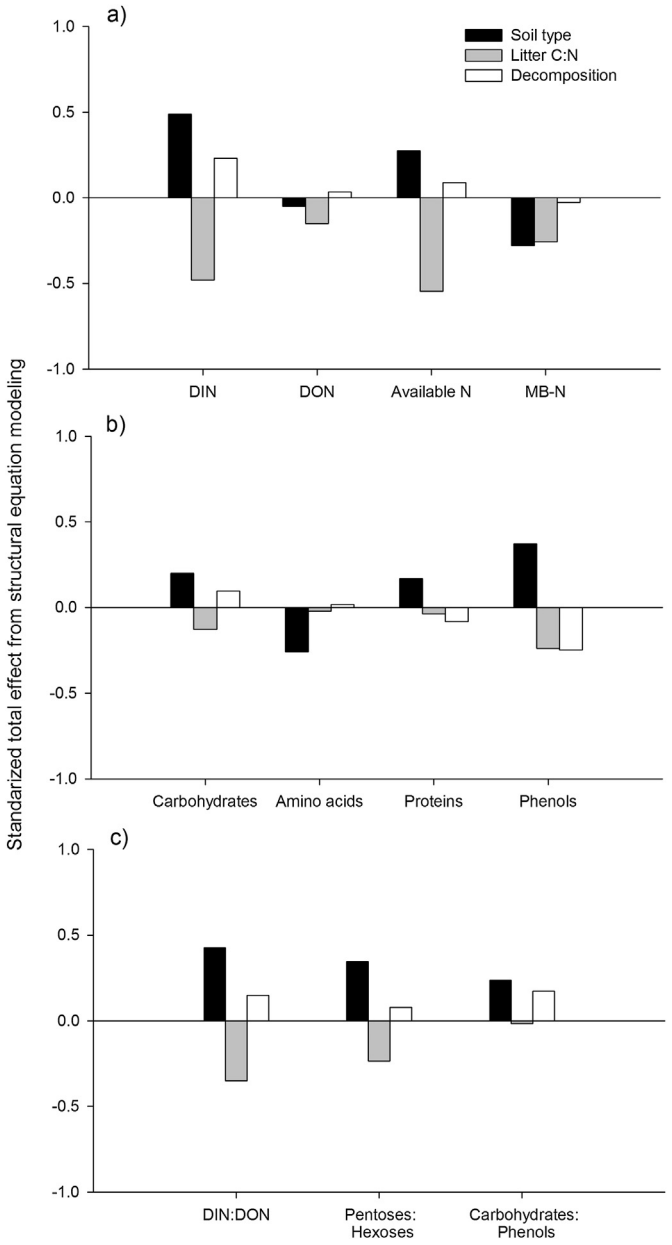

Fig. 4. Standardized total effects (direct plus indirect effects) derived from the structural equation modeling, including the effects of soil community, litter quality (litter C:N ratio) and litter decomposition (Decomp.) on the Ai of the N and C variables (salt extractable) studied.

microbial functional diversity (Table 1), which may enhance depolymerization and N mineralization processes (Schimel et al., 2005; Delgado-Baquerizo et al., 2013c). For example, the positive direct and total effects of the soil type on the DIN:DON ratio suggest that soils with fungal-dominated microbial communities (Soil B in our experiment) use N more efficiently, and thus accelerate mineralization compared to bacterial-dominated ones (Paul and Clark, 1996; Austin et al., 2004). The functional breadth hypothesis maintains that some microbial communities have a high capacity to deal with both labile and recalcitrant compounds than others (van der Heijden et al., 2008; Strickland et al., 2009; Keiser et al., 2011, 2013), holding a larger role in ecosystem processes such as litter decomposition than those with a lower functional range. In this respect, soils with high microbial biomass and functional diversity may improve the quality of available organic matter (e.g. higher carbohydrate: phenols ratio) and increase the availability of C and N in response to reductions in litter quality (e.g. high litter C:N ratio as a consequence of increasing atmospheric CO_2; Sardans et al., 2012). In addition, the positive direct and total effects of the soil type (e.g. microbial biomass and functional diversity) on the pentoses:hexoses ratio support results from García-Palacios et al. (2013b), as they suggested that well-developed microbial communities can enhance both litter decomposition and the inputs of plant-derived carbohydrates to the soil organic matter.

Remarkably, increasing litter decomposition (i.e. cumulative respiration) was only directly related to increments in soil N (DIN and available N), but not to labile C variables (e.g., carbohydrates,

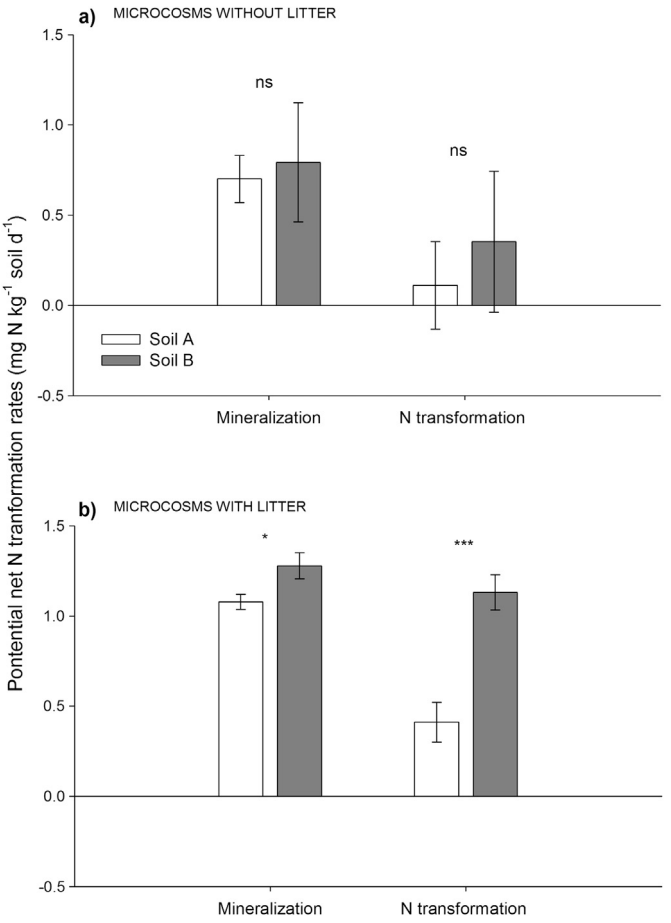

Fig. 5. Potential net N transformation and mineralization rates after the litter decomposability assay (once litter was removed) for the microcosms incubated with and without litter. Data from different litter qualities are collapsed to highlight the effects of the microbial community. Data are means ± SE ($n = 80$). Significance levels are as follows: ns = no significant, $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

amino acids and proteins). This result suggests that, because of the strict proportions of elements (C > N > P) required for all organisms to catalyze metabolic and anabolic reactions (Finzi et al., 2011; Sardans et al., 2012), the labile C present in litter may be immediately used by soil microorganisms for active microbial synthesis (e.g. phenols production linked to allelopathic effects; Chantigny et al., 2006), but can be also largely respired and lost as CO_2 during litter decomposition. In this direction, the soil characteristics had direct and total negative and positive effects on amino acids and proteins, respectively. This result suggests that amino acids may be used as energetic C sources (Chantigny et al., 2006), while the availability of proteins in soil (e.g. extracellular enzyme production; Sinsabaugh et al., 2002) may be the consequence of active microbial production (Fig. S3). In addition, our structural equation models indicate that the soil characteristics may be more important than litter quality (e.g. litter C:N ratio) controlling C than N availability, as showed by the consistently higher total effect of soil type on the C variables compared to the N variables. The higher control of the soil type on the C cycle matches with the idea that, in semiarid regions such as those we studied, soil microorganisms are highly limited by C availability due to the low C: N ratio of the organic matter (ca 9.5 in our study sites; Cookson et al., 2006, 2007). In this respect, the higher C and N availabilities observed in Soil B suggest that soil characteristics such as microbial abundance and functional diversity are key variables when it comes to evaluating the capacity of soils to store C and N.

It could be argued that the higher C and N availability in Soil B as compared to Soil A found at the end of our experiment is not the direct consequence of litter decomposition but the result of a higher priming effect on the soil organic matter decomposition (linked to the entrance of new C and N) during litter decomposition (Sullivan and Hart, 2013). The most prevalent view suggests that priming effect can be very important in soils with low nutrient availabilities, however, soil with high C and N availability tends to reduce priming effect on organic matter (Kuzyakov, 2002; Hagedorn et al., 2003; Hartley et al., 2010; García-Pausas and Paterson, 2011; Sullivan and Hart, 2013). This view is based on the idea that, under nutrient limitations, microbes may need an extra source of labile C to obtain the energy needed to mineralize the relatively-stable organic matter (Kuzyakov, 2002; Hagedorn et al., 2003; Hartley et al., 2010; García-Pausas and Paterson, 2011; Sullivan and Hart, 2013). In our case, the soil with the highest organic C and total N was also the soil with the highest C and N availability at the end of the study. In this respect, a likely priming effect should have reduced part of the differences between soils A and B, rather than enhancing them, because a higher priming effect is expected in the soils with lowest organic C and total N contents (Soil A in our case; Sullivan and Hart, 2013). Thus, we believe that the priming effect is not altering the conclusions that can be derived from this study.

Finally, it is interesting to note that we found differences in the potential net N transformation and mineralization rates between soils A and B after our litter decomposability assay, which were not found in the microcosms incubated without litter. These results suggest that changes in N availability linked to different microbial communities during litter decomposition can promote differences in the ability of microbial communities to carry out N processes (e.g. higher N mineralization), highlighting the importance of the nutrient legacy on subsequent mineralization processes (Keiser et al., 2011).

5. Conclusions

We have shown that soil characteristics such as a higher microbial biomass and functional diversity can increase the availability of C and N during litter decomposition regardless of the litter quality. In addition, our results indicate that changes in the N availability during litter decomposition, mostly linked to particular soil characteristics such as microbial communities, are able to promote dissimilarities in the capacity of the different soils to carry out mineralization. Overall, our study provides evidence that soil characteristics can be more important than the litter quality as a driver of soil C, and be equally important for N availability during the decomposition of leaf litter.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2014.11.009.

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