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Dario A. Fornara · Richard Bardgett
Sibylle Steinbeiss · Donald R. Zak
Gerd Gleixner · David Tilman

Plant effects on soil N mineralization are mediated by the composition of multiple soil organic fractions

Abstract Despite the topic of soil nitrogen (N) mineralization being well-studied, very few studies have addressed the relative contribution of different plant and soil variables in influencing soil N mineralization rates, and thus the supply of inorganic N to plants. Here, we used data from a well-studied N-limited grassland to address the relative effects of six plant and soil variables on net and on gross rates of soil N mineralization. We also addressed whether plant effects on soil N mineralization were mediated by changes in C and N concentrations of multiple soil organic matter (SOM) fractions. Regression analyses show that key plant traits (i.e., plant C:N ratios and total root mass) were more important than total C and N concentrations of bulk soil in influencing N mineralization. This was mainly because plant traits influenced the C and N concentration (and C:N ratios) of different SOM fractions, which in turn were significantly associated with changes in net and gross N mineralization. In particular, C:N ratios of a labile soil fraction were negatively related to net soil N mineralization rates, whereas total soil C and N concentrations of more recalcitrant fractions were positively related to gross N mineralization. Our study suggests that changes in belowground N-cycling can be better predicted by simultaneously addressing how plant C:N ratios and root mass affect the composition and distribution of different SOM pools in N-limited grassland systems.

Keywords Ecosystem process · Nitrogen cycling · Soil density fractionation · Soil organic matter

D. A. Fornara (✉)
Environmental Sciences Research Institute,
University of Ulster, Coleraine BT52 1SA, UK
E-mail: d.fornara@ulster.ac.uk
Tel.: +44-2870-324658
Fax: +44-2870-324911

R. Bardgett · D. A. Fornara
Soil and Ecosystem Ecology Laboratory,
Lancaster Environment Centre,
Lancaster University, Lancaster LA1 4YQ, UK

S. Steinbeiss
Institute of Groundwater Ecology,
Helmholtz Centre Munich,
85764 Neuherberg, Germany

D. R. Zak
School of Natural Resources and Environment,
University of Michigan, Ann Arbor,
MI 48109-1115, USA

G. Gleixner
Max-Planck-Institute for Biogeochemistry,
100164-07701 Jena, Germany

D. Tilman
Department of Ecology, Evolution, and Behavior,
University of Minnesota, St. Paul, MN 55108, USA

Introduction

The functioning of many terrestrial ecosystems can be influenced strongly by how plant–soil interactions affect the supply of N for plant uptake (e.g., NH_4^+, NO_3^- and amino acids; Hart et al. 1994; Chapin et al. 2002; Bardgett 2005). A critical process controlling N availability to plants is the microbial assimilation (immobilization) of N, which is governed by the bioenergetics of microbial growth in soil and the resulting demand for N during biosynthesis. Thus, one way through which plants take up available soil N is when microbial energy requirements are met and microbes release NH_4^+ in the rhizosphere environment (Chapin et al. 2002). Two variables that have long been used to estimate microbial release (and recycling) of NH_4^+ from organic N compounds are net and gross N mineralization rates (net N mineralization = gross N mineralization − microbial immobilization; see Binkley and Hart 1989). Such variables are useful because they can be associated with critical ecosystem functions such as total plant production of N-limited grassland systems (Zak et al. 2003; Fornara and Tilman 2009).

It is well known that plant and soil C:N ratios are key variables affecting net soil N mineralization rates (Springob and Kirchmann 2003; Manzoni et al. 2008; Meier and Bowman 2009). Plant and soil C:N ratios may also be responsible for changes in gross N mineralization rates, but their effects could be less important (Booth et al. 2005) and total soil C and N concentrations (which depend strongly on plant production) seem to play a more significant role (Accoe et al. 2004; Booth et al. 2005).

Despite the subject of N mineralizaton being well-researched, surprisingly little is known about the relative contribution of different plant–soil variables to changes in net and gross mineralization rates. Questions remain about which plant–soil variable is more effective in influencing local rates of net and gross N mineralization and through which underlying mechanisms.

We hypothesized that changes in plant C:N ratios and plant production (i.e. total above- and belowground plant mass) are more important than total C and N concentrations (and C:N ratios) of bulk soil in influencing both net and gross soil N mineralization rates. Our hypothesis is supported by previous experimental work that suggests: (1) changes in plant litter chemistry and plant biomass production have important cascading effects on the physical–chemical properties of different organic substrates returned to the soil, on the interactions between organic and inorganic substrates and on their accessibility by soil microbes (Sollins et al. 1996); (2) the biodegradability and bioavailability of C and N compounds is expected to change across different soil organic matter (SOM) fractions (Sollins et al. 1996; Sohi et al. 2005; Swanston et al. 2005; Flessa et al. 2008); and (3) the 'single soil pool' approach does not include complex dynamics of multiple SOM fractions that may provide a more mechanistic basis for understanding key ecosystem processes (see Neff et al. 2002) such as belowground N cycling.

Soil fractionation effects on N mineralization have been addressed across forest systems (Zhong and Makeschin 2006) and managed (e.g., fertilized) grasslands (Monaghan and Barraclough 1997; Whalen et al. 2000). However, potential linkages between plant substrate quality-quantity, SOM fractions, and net and gross mineralization rates have never been addressed in N-limited grassland communities.

Here we address this issue by using data collected over 12 years of a grassland biodiversity experiment established on N-poor, sandy soils in Minnesota, USA. We know from previous studies that increased plant diversity and the simultaneous presence of critical plant functional groups (i.e., legumes and C4 grasses) in this experiment, both have significant positive effects on plant productivity and on soil C and N sequestration (Lambers et al. 2004; Fornara and Tilman 2008, 2009). We also know that plant functional composition can strongly affect root detritus N dynamic and net soil N mineralization rates (Fornara et al. 2009). In these studies we found that total plant mass or plant C:N ratios were better predictors than plant diversity/composition per se in affecting soil N sequestration or net soil N mineralization rates. Thus, here we ask how plant quality (i.e., plant C:N ratios) and quantity (i.e., above-belowground plant mass) in this grassland system will affect the composition of different SOM fractions, and how this, in turn, could influence gross and net soil N mineralization rates.

We first address how the relative importance of six plant–soil variables, i.e., (1) aboveground plant mass, (2) belowground plant mass, (3) plant aboveground C:N ratios, (4) plant belowground C:N ratios, (5) total soil C concentrations, and (6) soil C:N ratios, could affect net and gross soil N mineralization rates. Secondly, we address whether changes in soil N mineralization rates could be mediated by plant effects on total C and N concentrations (as well as C:N ratios) of different SOM fractions.

Methods

We conducted our study at Cedar Creek Ecosystem Science Reserve (Cedar Creek, MN), where a grassland biodiversity experiment was established in 1994 on a sandy glacial outwash characterized by N-poor soils (see Tilman et al. 2001). We utilized 152 experimental plots (9 m × 9 m each) dominated by herbaceous perennials (Tilman et al. 2006) seeded to contain 1, 2, 4, 8 or 16 grassland and savanna species representing four plant functional groups: C4 grasses, C3 grasses, legumes and forbs. We used these experimental plots because the six plant–soil variables have been measured multiple times across years in each diversity plot and also because significant differences in plant mass, plant C:N ratios and soil C and N concentrations were found across the plant diversity gradient (Tilman et al. 2001, 2006; Fornara and Tilman 2008, 2009).

Plot compositions were maintained by manually weeding (three or four times annually) and plots were burned each year in spring before growth began to mimic natural fire occurrence in these grasslands (Tilman et al. 2006).

Measurement of rates of soil N mineralization

We used laboratory and field incubations as an index of net soil N mineralization rates (Binkley and Hart 1989). For the laboratory incubation, three soil samples were collected in July 2007 to 20 cm soil depth from each of the 152 plots, mixed, and sieved through a 2 mm size mesh. Because these plots are burned every year, before sampling we carefully checked that soil surface was clear of unburned residuals that could affect our measurements. Charcoal formation could increase C inputs to soil; we know, however, from published studies (see Tilman et al. 2007) that annual accumulation of charcoal carbon in frequently burned grasslands was < 1% of the observed rate of soil carbon accumulation in our grassland system (Tilman et al. 2006).

A subsample was extracted with 1 M KCl, shaken for 0.5 h, settled overnight at 4°C and analyzed for NH_4^+-N and NO_3^--N with a Bran-Luebbe AA3 auto analyzer. An additional 25-g subsample for each plot was incubated for 30 days in a dark room at 22°C. Sufficient water was added to each sample to reach the assumed field moisture capacity of 9%, and again after 2 weeks if necessary to keep moisture constant. After 30 days, soil samples were extracted and analyzed for NH_4^+-N and NO_3^--N as described above.

Potential net N mineralization rates were also estimated in situ in July 2007 (field incubation) using 1.9 cm diameter plastic (PVC) tubes that extended to a depth of 20 cm at three different locations within each plot; each tube was covered with a cap to prevent leaching losses. An initial soil sub-sample from each plot was mixed, sieved and analyzed for NH_4^+-N and NO_3^--N as described above. After 30 days, soils incubated within each PVC tube were also extracted and analyzed for NH_4^+-N and NO_3^--N. To determine net rates of N mineralization, initial extractable concentrations of NH_4^+ and NO_3^- were subtracted from final extractable concentrations at the end of the incubation (i.e., net mineralization rates = net ammonification + net nitrification).

We used data summarized in Zak et al. (2003) on gross rates of N mineralization that were determined using a ^{15}N isotope-pool dilution technique on soil samples collected in 2000 between 0–20 cm depth from 116 of the 152 diversity plots included in our study. The gross N mineralization rate was estimated from the rate at which the added ^{15}N isotope is "diluted" by influx of the natural ^{14}N isotope.

SOM fractionation

Soil samples from 55 experimental plots were collected in August 2006 between 0–20 cm soil depth for each of nine sites per plot. Plots were selected randomly within each of four different species diversity levels as follows: thirteen monoculture plots, fourteen 2-species plots, thirteen 4-species plots and fifteen 16-species plots. Soils were sieved to remove roots and combined to give one composite soil sample per plot; each sample was dried at 40°C for 5 days and stored in glass vials. We separated SOM fractions by floatation and sedimentation (density-based separation) using a sodium iodide (NaI) solution at a density of 1.80 g cm^{-3}. We followed a density based procedure according to Sohi et al. (2001, 2005); for more details see http://www.rothamsted.bbsrc.ac.uk/aen/CarbonCycling/pdf/Fractionation_manual.pdf). To separate light and heavier soil fractions we also used ultrasonic dispersion (sonication). We used an MSE Soniprep 150 sonicator (Sanyo Gallenkamp, Leicestershire, UK), fitted with a 9.5-mm probe submerged 15 mm into the soil suspension. Soil suspensions in the centrifuge bottles were contained in an ice-packed 500 mL beaker. Sonication treatments equated to an energy input of 1,500 J g^{-1} soil applied for 3 min.

This density-based separation method distinguishes between: (1) a free SOM fraction (FR-SOM) at density < 1.80 g cm^{-3}, which represents discrete free organic particles located between stable soil aggregates; (2) an intra-aggregate SOM fraction (IA-SOM) at density < 1.80 g cm^{-3}, which represents discrete organic particles located within stable soil aggregates; and (3) a residual heavy organo-mineral fraction at densities > 1.80 g cm^{-3} (see Sohi et al. 2001, 2005 for nomenclature). In terms of resistance to biodegradability, the organo-mineral fraction is considered more recalcitrant than the FR-SOM and IA-SOM fractions, whereas the IA-SOM contains a greater proportion of microbial products and more recalcitrant C as compared with the FR-SOM (Sohi et al. 2005). Both soil bulk samples and soil fractions were analyzed for total C and N by combustion and gas chromatography (COSTECH Analytical ECS 4010 instrument).

Data analysis

We performed linear and multiple regression analyses to test for potential relationships between the six plant–soil variables, and net and gross rates of soil N mineralization. Our first set of analyses included data on net soil N mineralization rates measured in July 2007, while plant and soil parameters were measured in 2006. Evidence suggests that plant and soil data collected in each experimental plot do not significantly differ ($P > 0.45$ for all paired t tests) between 2005 and 2007 (see also Tilman et al. 2006; Fornara and Tilman 2008). In our second set of analyses we addressed potential relationships between gross N mineralization rates and six soil–plant variables that were all measured in 2000 in the same biodiversity plots.

We also used regression analyses to test for plant C:N ratios and total plant mass effects on C and N concentrations of different SOM fractions. The effects of multiple plant and soil parameters on N mineralization were tested in type III multiple regressions, which are highly conservative. In regression analyses that included multiple independent variables, such as the C and N concentrations of the three different SOM fractions, we first checked whether some of these variables were correlated (by performing stepwise regressions) to avoid collinearity problems. In such cases, multiple regressions were performed after the removal of one or more correlated variables. For example, we removed the N (%) concentration of each SOM fractions from our regression analyses because N (%) was highly correlated with the C (%) concentration of each fraction. To estimate the relative importance of individual predictor variables (e.g., plant aboveground mass vs soil C content) in our multiple regression analyses we submitted each of the partial regression coefficients (β_i) to a two-tailed hypothesis ($H_0 = \beta_i = \beta_0$, where $\beta_0 =$ is most frequently zero; see Zar 1999). We then inferred the importance of each predictor variable according to the F statistic of this test and its P values.

Finally, because we collected data from a plant diversity experiment we also tested the potential effect of plant species diversity on C and N concentrations of the three different SOM fractions. Plant diversity was expressed either as number of species seeded in each plot or as the presence/absence of four plant functional groups among C3 grasses, C4 grasses, legumes and non-leguminous forbs in each plot. Data were analyzed using JMP v. 6.0.2 (http://www.sas.com/).

Results

Plant–soil effects on N mineralization rates

Three separate multiple regression analyses, each including six predictor variables (see Table 1), showed that: (1) potential net soil N mineralization rates measured in the laboratory incubation were significantly negatively related to plant above- and belowground C:N ratios. Net N mineralization rates were also negatively affected by bulk soil C:N ratios and positively affected by total C concentration of bulk soil (Table 1); (2) potential net soil N mineralization rates measured in the field incubation were significantly negatively affected by plant belowground C:N ratios (Table 1); and (3) gross N mineralization rates were significantly positively affected by total plant belowground mass and negatively affected by plant belowground C:N ratios (Table 1).

SOM fractions

Of the total soil mass recovered after fractionation ($96.4 \pm 2.2\%$ of initial mass), FR-SOM accounted for $0.81 \pm 0.04\%$, IA-SOM accounted for $0.78 \pm 0.15\%$, and the organo-mineral fraction accounted for $98.4 \pm 0.16\%$. Mean C (g C kg^{-1} soil) and N (g C kg^{-1} soil) concentrations were higher in the FR-SOM and IA-SOM soil fractions than in the organo-mineral fraction; this fraction, however, represented the main soil C and N pool

(Table 2). Separate linear regression analyses showed that the C:N ratio of the FR-SOM increased significantly with increasing plant aboveground C:N ratios ($R^2 = 0.17$, $F_{1,50} = 9.86$, $P = 0.002$; see Fig. 1a), as well as with increasing plant belowground C:N ratios ($R^2 = 0.22$, $F_{1,50} = 13.4$, $P = 0.0006$; Fig. 1b). We also found that both plant aboveground mass ($R^2 = 0.24$, $F_{1,53} = 16.1$, $P = 0.0002$) and plant belowground mass ($R^2 = 0.25$, $F_{1,53} = 17.1$, $P = 0.0001$) were significantly positively related to total C concentrations of the IA-SOM (Fig. 1c, d).

Plant qualitative/quantitative effects on SOM fractions

To compare the relative contribution of plant C:N ratios and total plant mass with the composition of the different SOM fractions, we performed nine multiple regression analyses, each including the following four predictor variables: plant aboveground C:N ratio, plant belowground C:N ratio, plant aboveground mass and plant belowground mass. These regressions showed the following significant relationships: (1) plant belowground C:N ratio was positively related to the C:N ratio of the FR-SOM ($F_{4,50} = 5$, $P = 0.03$), and negatively related to its N (%) concentration ($F_{4,50} = 6.81$, $P = 0.01$) and C (%) concentration ($F_{4,50} = 4.72$, $P = 0.03$); (2) plant aboveground mass was positively related to N (%) concentration of the IA-SOM ($F_{4,50} = 9.17$, $P = 0.004$) and negatively related to the C:N ratio of the IA-SOM ($F_{4,50} = 10.3$, $P = 0.002$); and (3) plant belowground mass was positively related to the C:N ratio of the IA-SOM ($F_{4,50} = 9.36$, $P = 0.003$) and to the C (%) concentration of the organo-mineral fraction ($F_{4,50} = 6.94$, $P = 0.01$).

Because plant species diversity and composition are known to affect plant substrate C:N ratios and plant production, we performed further analyses to test for indirect effects of plant species numbers and composition on C and N concentrations of different soil fractions. In separate linear regression analyses, we found that the number of

Table 1 Results of three multiple regressions showing the dependence of net soil N mineralization rates measured in both laboratory and field incubations and of gross N mineralization rates on six plant–soil variables

	Net soil N mineralization in lab incubations			Net soil N mineralization in field incubations			Gross N mineralization using a ^{15}N isotope-pool dilution study		
	$R^2 = 0.49$, $F = 22.7$, $P < 0.0001$			$R^2 = 0.26$, $F = 8.12$, $P < 0.0001$			$R^2 = 0.43$, $F = 10.7$, $P < 0.0001$		
	Estimate	F	P	Estimate	F	P	Estimate	F	P
Plant aboveground mass (g m^{-2})	0.002	2.44	0.119	−0.0005	0.280	0.597	−0.00009	0.037	0.846
Aboveground plant C:N ratio	−0.04	9.1	0.002*	−0.0157	2.459	0.119	0.002	0.287	0.593
Plant belowground mass (g m^{-2})	−0.0003	1.50	0.222	−0.00009	0.170	0.680	0.0004	18.8	<0.0001*
Belowground plant C:N ratio	−0.055	18.7	<0.0001*	−0.0348	13.08	0.0004*	−0.009	5.44	0.022*
Total soil C (g m^{-2})	1.411	4.12	0.044*	0.383	0.568	0.452	−0.02	0.007	0.933
Soil C:N ratio	−0.375	5.68	0.018*	−0.018	0.023	0.878	−0.018	0.133	0.715

Laboratory and field incubations were performed using soils from 152 plots ($df = 6.151$). For the ^{15}N isotope study we used soil samples from 116 plots ($df = 6.115$)
*$P \leq 0.05$

Table 2 Results of density fractionation analyses performed on soil samples collected from 55 experimental plots in August 2006

	C (%)	N (%)	C:N	C (g C kg^{-1} soil)	N (g N kg^{-1} soil)
Bulk soil	0.40 ± 0.01	0.03 ± 0.001	11.1 ± 0.91	4.01 ± 0.18	0.30 ± 0.01
FR-SOM fraction	2.76 ± 0.20	0.18 ± 0.01	15.6 ± 0.42	0.23 ± 0.021	0.015 ± 0.001
IA-SOM fraction	1.86 ± 0.13	0.09 ± 0.007	21.3 ± 1.22	0.092 ± 0.01	0.005 ± 0.0005
Organo-mineral fraction	0.29 ± 0.01	0.026 ± 0.001	11.2 ± 2.29	2.84 ± 0.17	0.25 ± 0.01
All fractions				3.16 ± 1.14	0.27 ± 0.01
Recovered (%)				78.8 ± 2.54	90 ± 4.35

Total C and N are expressed both as percentage and as unit weight per kilogram of soil (mean ± SE). "Recovered" indicates the percentage of total C and N recuperated at the end of the density fractionation normalized to the initial mass of soil fractionated

FR-SOM Free aggregate SOM fraction, *IA-SOM* intra-aggregate SOM fraction

Fig. 1 Dependence of the C:N ratios of the free-aggregate SOM fraction (FR-SOM) on plant aboveground (**a**) and plant belowground (**b**) substrate C:N ratios. Also dependence of total soil C (%) content of the intra-aggregate SOM fraction (IA-SOM) on total plant aboveground mass (**c**) and on total plant belowground mass (**d**) in each of the 55 experimental plots
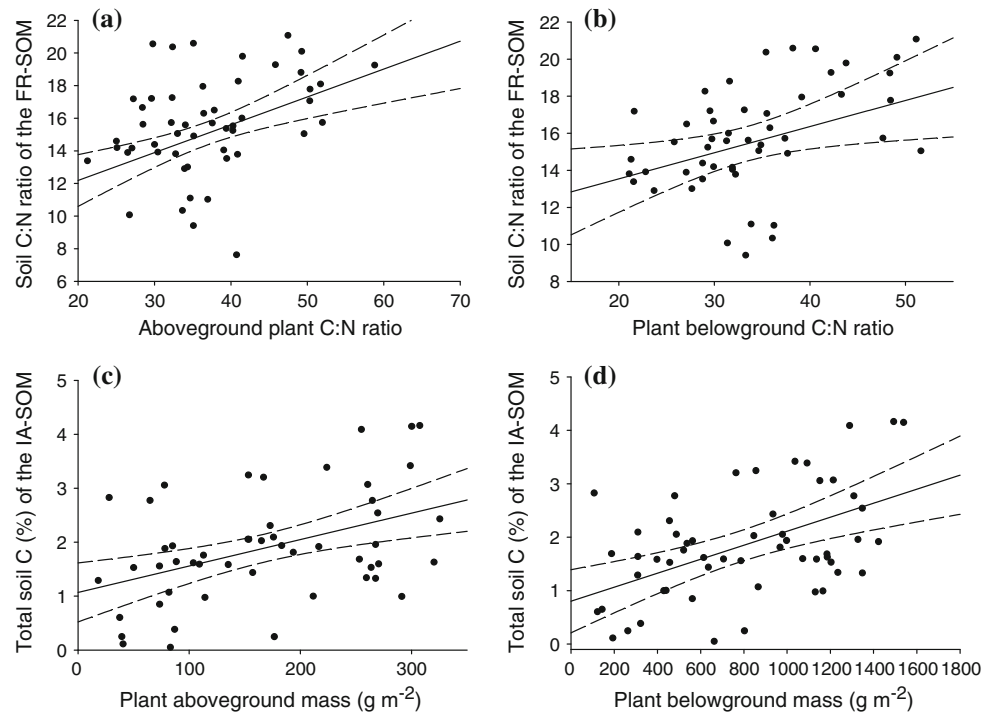

plant species seeded in each diversity plot had a positive significant effect on total C ($F_{1,54} = 8.35$, $P < 0.005$) and on total N concentrations ($F_{1,54} = 22.1$, $P < 0.0001$) of the IA-SOM. In separate multiple regression analyses that included as predictor variables the presence/absence of the four plant functional groups, we found that the presence of legumes had positive significant effects on N (%) concentrations of the FR-SOM and IA-SOM fractions ($P < 0.001$ for both analyses). The presence of forb species also had a positive significant effect on the N (%) concentrations of the IA-SOM ($F_{4,54} = 6.15$, $P = 0.01$).

SOM fractions and net soil N mineralization rates

We repeated two of the three multiple regressions shown in Table 1 (including as response variables net soil N mineralization rates measured in both laboratory and field incubations) after we replaced total C concentration

and C:N ratio of the bulk soil with C and C:N ratios of the three soil fractions. We found that the ten predictor variables accounted for 62% of the variability associated with changes in net soil N mineralization rates in the laboratory incubation ($R^2 = 62$, $F_{10,49} = 6.05$, $P < 0.0001$). Potential net soil N mineralization rates measured in the laboratory incubation were still significantly negatively related to plant belowground C:N ratios (estimate = -0.05, $F_{10,49} = 4.46$, $P = 0.04$), but were also negatively related to the C:N ratio of the FR-SOM (estimate = -0.21, $F_{10,49} = 11.1$, $P = 0.002$; Fig. 2a). We also found that 41% of the variation in field rates of net soil N mineralization could be attributed to ten predictor variables ($R^2 = 41.3$, $F_{10,48} = 2.53$, $P = 0.01$). Potential net soil N mineralization rates measured in the field incubation were significantly negatively related to plant belowground C:N ratios (estimate = 0.063, $F_{10,48} = 6.07$, $P = 0.01$), the C:N ratio of the FR-SOM (estimate = -0.14, $F_{10,48} = 4.28$,
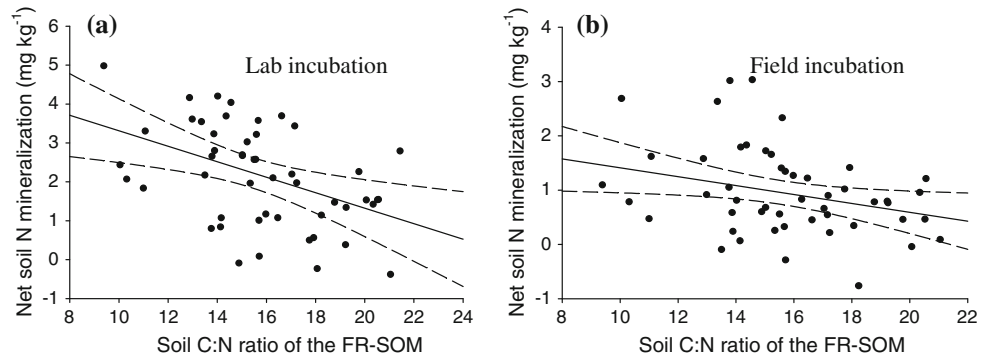
$P = 0.04$; see Fig. 2b), and the C concentration of the organo-mineral fraction (estimate $= -2.97$, $F_{10,48} = 5.33$, $P = 0.02$).

SOM fractions and gross N mineralization rates

We repeated the multiple regression shown in Table 1 for gross N mineralization rates in which we included the same four plant parameters measured in 2000 and the total C concentrations and C:N ratios of the soil organo-mineral fraction. We included only this recalcitrant fraction in our analyses, on the basis that its C content is unlikely to have changed substantially between 2000 and 2006; this notion was supported by the finding that the C content of the organo-mineral fraction measured in 2006 was highly correlated with the C concentration of bulk soil in 2000 ($r = 0.65$; Spearman's correlation coefficient), and that the C content of the recalcitrant organo-mineral fraction accounted for > 98% of the total soil C pool. The multiple regression analysis, which included four plant parameters and the C content and C:N ratios of the organo-mineral fraction, still showed the significant relationships between plant belowground mass and plant C:N ratios and gross N mineralization rates (see Table 1). However, gross N mineralization rates were also positively related to the soil C concentration of the organo-mineral fraction ($F_{6,39} = 2.45$, $P = 0.02$; Fig. 3).
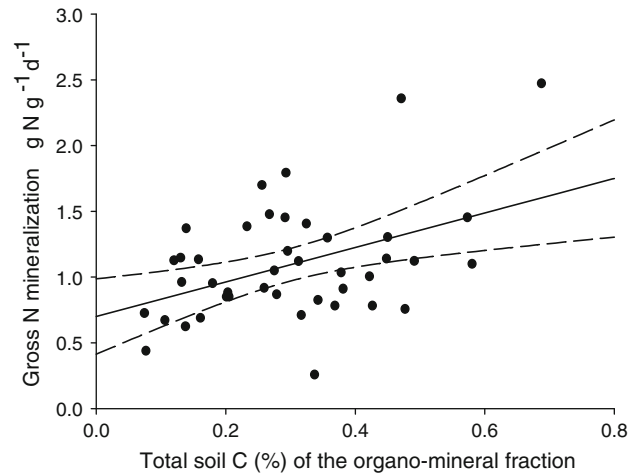
Discussion

Our results demonstrate that plant C:N ratios (both above- and belowground) and total plant belowground mass are more important than total C and N concentrations of bulk soil (and soil C:N ratios) in influencing net and gross rates of soil N mineralization. Our evidence is that such plant effects on net and gross N mineralization rates were mediated by changes in C and N concentrations (and C:N ratios) of multiple SOM fractions. In particular we found that the C:N ratios of the most labile soil fraction (i.e. FR-SOM) were highly related to the C:N ratios of plant above- and belowground tissues (Fig. 2a, b), and this is mainly because

Fig. 3 Dependence of gross N mineralization rates (as measured by Zak et al. 2003) on total soil C (%) of the organo-mineral fraction (the linear regression has: $R^2 = 0.22$; $F_{1,39} = 10.7$, $P = 0.002$)

decomposing plant tissues are primary sources of new C and N inputs to the soil. Not surprisingly, the C:N ratios of the FR-SOM were negatively related to net soil N mineralization rates. This is mainly because, in the early stages of decomposition, soil microbes are often N-limited and need to acquire and immobilize N from available soil N sources making the FR-SOM more a sink rather than a source of bioavailable N forms for plant uptake (Sollins et al. 1984).

We also found that total plant belowground mass was positively related to the C (%) and N (%) concentrations of more recalcitrant fractions, such as the IA-SOM and the organo-mineral fraction. Interestingly, the C and N concentrations of these recalcitrant fractions were positively related to gross N mineralization rates, suggesting that the bioavailability of soil N forms could be greater in heavier, more recalcitrant SOM fractions. This seems peculiar because recalcitrant fractions usually include a higher proportion of metabolites and microbial-derived material than labile fractions (Sollins et al. 1996; Sohi et al. 2001, 2005), and often show stable aggregates in which soil C and N forms could be more protected against further decomposition (Swanston et al. 2005; Flessa et al. 2008). Nevertheless,

our results, albeit based on correlations, agree with previous studies that demonstrate that heavier soil fractions act as the main sources of mineral N in both grassland and forest systems (Sollins et al. 1984; Whalen et al. 2000; Zhong and Makeschin 2006). Moreover, Accoe et al. (2004) observed positive significant relationships between gross N mineralization rates and total C and N concentrations of heavier soil fractions (density > 1.37 g cm^{-3}), as also found in our study.

There are multiple mechanisms that could explain why recalcitrant soil fractions, and in particular the organo-mineral fraction that represents the main soil C and N pool, are potential sources of available N forms for plant uptake. First, N becomes available to microbial groups that 'specialize' in extracting energy and thus in mineralizing N (and C) from more recalcitrant soil fractions (Noguez et al. 2008). This may occur through the activities of extracellular enzymes that are associated closely with soil particles (see Schulze et al. 2005), and also through the microbial recycling of organic C (Schimel and Bennett 2004; Flessa et al. 2008). In our N-limited grassland, increases in plant production are associated with increases in microbial biomass and in fungal abundance within the microbial community (Zak et al. 2003; Chung et al. 2007). These changes were also accompanied by greater cellulolytic and chitinolytic enzyme activities, which, for example, could have contributed to increase N release from more recalcitrant organic fractions (Noguez et al. 2008).

Second, the organo-mineral pool may act as a source of available N forms when new plant detritus inputs into the soil (via the FR-SOM) stimulate microbial activity and increase C and N mineralization rates within this recalcitrant soil pool (process referred to as 'priming effect'; Kuzyakov et al. 2000; Fontaine et al. 2004). In our N-limited system, 'priming effects' could be associated with increased fine root production and turnover. Indeed, in a similar study carried out within the same plant community, Fornara and Tilman (2009) found that soil NH_4^+ concentrations (before and after an aerobic laboratory incubation) were strongly positively related to fine root production. This suggests that fine roots may contribute to increase soil NH_4^+ concentrations by providing readily decomposable labile substrates (i.e. increased C inputs) that could stimulate NH_4^+ release from more recalcitrant organic fractions (but also that decomposing fine roots per se may represent an important source of NH_4^+ in the soil; see Fornara et al. 2009).

Third, the large size of the organo-mineral fraction pool could have a sink effect for available N forms previously released by microbial processes. Indeed, because NH_4^+ ions adsorb readily to the negatively charged surfaces of soil minerals and SOM (Chapin et al. 2002), a large SOM pool could act as an important source/sink of available N forms for plant uptake. The organo-mineral fraction usually represents the largest SOM pool, and its C and N concentrations tend to increase with greater plant belowground mass accumulation in our N-limited system (Fornara and Tilman 2008). These three potential mechanisms should not be considered mutually exclusive, and we may expect each to contribute to explaining the potential for N release from soil organic fractions that are considered recalcitrant based on their chemical and physical composition.

Our results suggest, however, that belowground N-cycling could be influenced greatly by plant-mediated effects on the composition of multiple soil organic matter fractions. While it is intuitive that labile soil organic fractions with low C:N ratios could contribute to the formation of small labile soil N pools important for plant uptake (Parton et al. 1987; Wedin and Pastor 1993), the term 'recalcitrant' used for heavier soil organic fractions could be misleading when associated with their potential for releasing mineralized soil N forms. Because labile and more recalcitrant soil organic fractions could simultaneously enhance belowground N-cycling, it is crucial that we understand the potential linkages between plant functional traits and the spatial/temporal distribution of both labile and recalcitrant soil organic fractions across diverse plant communities.

We found evidence in our study that both plant functional composition and plant species diversity have significant effects on the composition of different soil fractions. For example, the presence of legume species significantly increased the N (%) concentration of the FR-SOM fraction. Thus legumes, by reducing the C:N ratio of labile soil fractions, will contribute to increase N release in this N-limited grassland soils. We also found that greater plant species diversity contributed to increase total root mass which, in turn, was positively related to gross N mineralization rates. Finally, we might expect that the presence of highly complementary plant functional groups (i.e., legumes and C4 grasses) will contribute to increase both net and gross N mineralization rates in this N-limited system. This is mainly because the association between legumes and C4 grasses contributes to increase root mass (Fornara and Tilman 2008), while the presence of legumes provides good quality litter with low C:N ratios and with high decomposition rates. Further studies could address whether and how the functional diversity and composition of a plant community affect the distribution and size of (1) recalcitrant soil organic fractions at different soil depths, and (2) labile organic fractions at different seasonal intervals under different grassland management, etc.

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