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Linkages between plant functional composition, fine root processes and potential soil N mineralization rates

D. A. Fornara[1,2]*, D. Tilman[1] and S. E. Hobbie[1]

[1]*Department of Ecology, Evolution and Behavior, University of Minnesota, St Paul, MN 55108, USA; and* [2]*Institute of Environmental and Natural Sciences, Lancaster University, Lancaster, LA1 4YQ UK*

Summary

1. Plant functional composition may indirectly affect fine root processes both qualitatively (e.g. by influencing root chemistry) and quantitatively (e.g. by influencing root biomass and thus soil carbon (C) inputs and the soil environment). Despite the potential implications for ecosystem nitrogen (N) cycling, few studies have addressed the linkages between plant functional composition, root decay, root detritus N dynamics and soil N mineralization rates.

2. Here, using data from a large grassland biodiversity experiment, we first show that plant functional composition affected fine root mass loss, root detritus N dynamics and net soil N mineralization rates through its effects on root chemistry rather than on the environment of decomposition. In particular, the presence of legumes and non-leguminous forbs contributed to greater fine root decomposition which in turn enhanced root N release and net soil N mineralization rates compared with C3 and C4 grasses.

3. Second, we show that all fine roots released N immediately during decomposition and showed very little N immobilization regardless of plant composition. As a consequence, there was no evidence of increased root or soil N immobilization rates with increased below-ground plant biomass (i.e. increased soil C inputs) even though root biomass negatively affected root decay.

4. Our results suggest that fine roots represent an active soil N pool that may sustain plant uptake while other soil N forms are being immobilized in microbial biomass and/or sequestered into soil organic matter. However, fine roots may also represent a source of recalcitrant plant detritus that is returned to the soil (i.e. fine roots of C4 and C3 grasses) and that can contribute to an increase in the soil organic matter pool.

5. *Synthesis*. An important implication of our study is that the simultaneous presence of different plant functional groups (in plant mixtures) with opposite effects on root mass loss, root N release and soil N mineralization rates may be crucial for sustaining multiple ecosystem services such as productivity and soil C and N sequestration in many N-limited grassland systems.

Key-words: C3 grasses, C4 grasses, fine root decomposition, forbs, legumes, multiple ecosystem services, plant functional groups, productivity, root biomass, soil carbon

Introduction

Fine roots (≤ 2 mm diameter) represent a large fraction of annual primary productivity in many terrestrial ecosystems (Jackson *et al.* 1997; Steinaker & Wilson 2005) and their quick turnover rate has important implications for soil organic matter formation and ecosystem nutrient cycling (McClaugherty *et al.* 1984; Aerts *et al.* 1992; Ruess *et al.* 2003). Although fine root processes may strongly influence nitrogen (N) and carbon (C) cycling, understanding of patterns of root decay and root detritus N dynamics is much more limited than it is for leaf litter decomposition.

Experimental evidence suggests that root decomposition processes are strongly influenced by root chemistry (Hobbie 1996; Mun & Whitford 1998; Silver & Miya 2001; Chen *et al.* 2002) and that root detritus N dynamics (i.e. root N immobilization and release) can be predicted by initial root N concentration because of fundamental constraints on decomposer physiologies (Parton *et al.* 2007). Environmental factors such as temperature and soil moisture may also affect root decay rates (Newbery 1979; Hobbie 1996; King *et al.* 1997).

*Correspondence author. Environmental Sciences Research Institute, School of Environmental Sciences, University of Ulster, Coleraine, Northern Ireland BT52 1SA. E-mail: d.fornara@lancaster.ac.uk

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Although root chemistry is known to be a primary controller of root decomposition (Silver & Miya 2001), the effects of root biomass on root decay rates remain uninvestigated. Increased root C inputs to the soil may increase soil moisture (Bardgett 2005) or stimulate soil N immobilization, both of which should affect root decomposition rates. For example, greater soil N immobilization may slow rates of decomposition of high C : N substrates whose decomposition requires associated immobilization of N supplied from soil.

However, previous studies suggest that, in contrast to leaf litter, fine roots may show little N immobilization during decomposition, even when they have C : N ratios comparable to those of leaf litter types that do exhibit immobilization (Seastedt *et al.* 1992; Moretto *et al.* 2001, Personeni & Loiseau 2005; Parton *et al.* 2007). The causes of such differences between leaf litter and roots in their N release are unclear. Thus, understanding of below-ground N cycling could improve through investigation of the linkages between plant functional composition, root decay, root detritus N dynamics and net soil N mineralization rates.

We hypothesize that because plant functional composition may strongly affect root chemistry (e.g. root N concentration of C4 grasses is lower than root N of C3 grasses and legumes; see Dijkstra *et al.* 2006; Vivanco & Austin 2006) and root biomass through time (Tilman *et al.* 2001; Fornara & Tilman 2008), the identity of different plant functional groups (i.e. C3 grasses, C4 grasses, legumes and non-leguminous forb species) should influence root decomposition and N dynamics. Because our study was conducted in an N-limited grassland system and because of the critical role of initial plant substrate N concentrations in affecting leaf and root N release through time (Parton *et al.* 2007), we focus on this particular aspect of root chemistry. The aim of our study was to investigate how plant functional composition affected root mass loss, root detritus N dynamics and soil N mineralization rates towards improved understanding of ecosystem N cycling.

Methods

We conducted our study at Cedar Creek Ecosystem Science Reserve, Minnesota, USA where a grassland biodiversity experiment (comprising 168, 9 m × 9 m plots) was established in 1994 on a sandy glacial outwash characterized by N-poor soils (see Tilman *et al.* 2001). We collected roots for a decomposition study from 28 monoculture plots planted with the following species (http://www.cedarcreek.umn.edu/flora/plantcover.html for more detailed taxonomic information): *Achillea millefolium*, *Asclepias tuberosa*, *Liatris aspera*, *Solidago rigida*, *Monarda fistulosa* (forb species); *Amorpha canescens*, *Lespedeza capitata*, *Lupinus perennis*, *Petalostimum purpureum* (legume species); *Poa pratensis*, *Agropyron smithii*, *Elymus canadensis*, *Koeleria cristata* (C3 grasses); and *Andropogon gerardi*, *Schizachyrium scoparium*, *Sorghastrum nutans* (C4 grasses). Each species had two replicate plots except for *E. canadensis*, *P. pratensis*, *S. rigida* and *M. fistulosa* for which we collected specimens from one plot each only. We also collected roots from 28 plots within the 4-species diversity treatment which contained random combinations of the above grassland species and from 28 plots at the highest diversity treatment which contained all 16 species. We refer to these 84 plots as 'donor plots' (see Fig. 1).

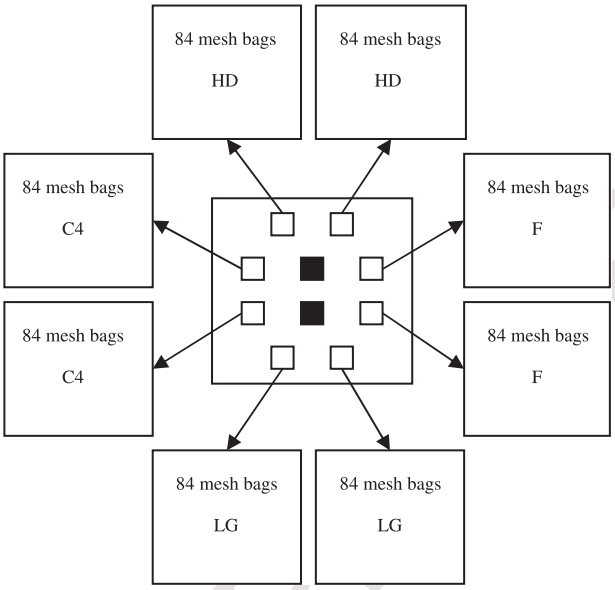

Fig. 1. Experimental diagram of the fine root decomposition study. Fine roots were collected from 84 plots (28 plots for each of 1, 4 and 16 species diversity level). From each of these 84 plots ('donor plots'), represented by the heavy line quadrat in the figure centre, 10 mesh bags were filled with 0.35 ± 0.02 g of root dry mass. We then performed two decomposition trials. In Trial 1 two mesh bags (represented by the two small black-filled quadrats in the figure centre) were relocated in each of the same plots where the roots were harvested. In Trial 2 each of eight mesh bags (represented by the small white-filled quadrats in the figure centre) was decomposed in various 'recipient' plots (i.e. in two high-diversity plots (HD), two forb monoculture plots (F), two legume monoculture plots (LG) and two C4 grass monoculture plots (C4).

ROOT SAMPLING

Plots were sampled for total root biomass using 12 soil cores per plot, 5 cm diameter each, 0–30 cm deep in mid-August 2006. We placed soil cores on a fine mesh screen and rinsed them under a gentle spray of water. We used roots with diameters < 2 mm (i.e. fine roots) with the following in mind: while leaf litter decomposition experiments utilize senescent leaves collected during peak litter fall, collecting senescent fine roots is more difficult because of inaccuracies in determining how recently a root may have senesced and the extent of its prior decomposition. Thus as done in previous root decomposition studies (e.g. McClaugherty *et al.* 1984; Hobbie 1996; Chen *et al.* 2002) we minimized these problems by performing a one time fine root collection and using roots that had a light colour and some structural flexibility within the < 2 mm diameter size range. This sorting excluded roots that were already partially decomposed. These criteria allowed us to use more homogeneous root samples (e.g. more similar in phenology) for comparing root decomposition rates across root functional types and sites of decomposition.

All root samples were dried (65 °C) until constant mass to estimate total plant below-ground mass in each plot. Fine roots from each plot were gently but thoroughly mixed to homogenize them, which is especially important for high-diversity plots which included various plant species. From the homogeneous root sample, *c.* 5 ± 1 g was ground to fine powder and analyzed for total C and N by combustion and gas chromatography (COSTECH Analytical ECS 4010). The remaining root mass was put into N-free polyester mesh

bags (5 × 10 cm each) with a pore size of 50 microns (ANKOM Technology, Macedon, NY). Each of 10 mesh bags from each of 28 'donor plots' at the 1-, 4- and 16-species diversity levels (840 bags in total) was filled with 0.35 ± 0.02 g of dry root mass (this corresponds to *c.* 0.07 g of root mass per cm^3 of soil) and allocated to two different decomposition trials as follows (see Fig. 1): in Trial 1 two mesh bags were placed back in the same plots where root samples were collected (small black-filled quadrats in Fig. 1); in Trial 2 each of eight mesh bags (represented by the small white-filled quadrats in the figure centre) was placed in one of the following decomposition locations: two high-diversity (16-species) plots (HD), two forb monoculture plots (F), two legume monoculture plots (LG) and two C4 grass monoculture plots (C4) which all represented the 'recipient plots'. All mesh bags were buried (inserted vertically in the soil) on 2 October 2006 between 5 and 10 cm soil depth and collected on 30 July 2007. Upon collection, roots were carefully removed from mesh bags. Visible fungal hyphae (see Discussion) were removed after drying roots at 65 °C for 1 day and each root sample was then weighed and analyzed for C and N content.

Using data from Trials 1 and 2, we aimed to disentangle potential effects of root substrate chemistry (resulting from differences in plant functional composition of the 'donor plots') and the effects of decomposition location (resulting from differences in the plant composition of the 'recipient plots') on root decomposition and root detritus N dynamics.

POTENTIAL NET SOIL N MINERALIZATION RATES

We performed laboratory and field incubations of soils collected from each of the 84 'donor plots' in June–July 2007 to assess net N mineralization rates as indices of N availability for plant uptake (Schimel & Bennett 2004). Because both incubations involve some disturbances of key variables associated with below-ground processes such as soil temperature and moisture, root respiration and so on, they represent estimates of potential net soil N mineralization rates and cannot be used to estimate actual *in situ* annual rates. Hereafter we refer to them as laboratory incubation and field incubation. For the laboratory incubation soil samples were collected to 20 cm soil depth from three sites within each plot on 27 June mixed, 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 from each plot was incubated for 30 days in a dark room at 22 °C after roots were sieved out. Sufficient water was added to each sample to reach the assumed field moisture capacity of 9% and again after 2 weeks during the laboratory incubation if necessary to keep moisture constant. After 30 days soil samples were extracted and analyzed for NH_4^+-N and NO_3^--N as above. For the field incubation net N mineralization rates were estimated *in situ* using 1.9 cm diameter plastic tubes (PVC) sunk to a depth of 20 cm at three different locations within each plot and covered with caps to prevent leaching losses. On 3 July an initial set of soil subsamples from each plot were analyzed for NH_4^+-N and NO_3^--N as 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 N mineralization rates (for both laboratory and field incubations) final extractable concentrations of NH_4^+ and NO_3^- were subtracted from initial extractable concentrations.

DATA ANALYSIS

We first performed a two-way analysis of variance (ANOVA) using as predictor variables the fine root functional composition (i.e. C4 grass, C3 grass, forb or legume fine roots collected from monoculture 'donor plots' for which we knew with accuracy the origin of the root samples and species identities) and the site of decomposition (i.e. the different decomposition site offered by the 'recipient plots' in Trial 2). We used as response variable the proportion of initial fine root mass that was lost over the 10-month incubation (values were arcsine-transformed to homogenize variances). Moreover, to assess potential differences in root detritus N dynamics (i.e. root N release vs. root N immobilization) caused by root substrate chemistry and decomposition location, we calculated the Nutrient Accumulation Index (NAI) for each bag as follows (see Romero *et al.* 2005): $NAI = (W_t X_t)/(W_0 X_0)$, where W_t is the organic mass remaining in the mesh bag at time t, X_t is the root N concentration at time t, W_0 is the initial root mass in each mesh bag, and X_0 is the initial root N concentration of each mesh bag. Values of NAI < 1 would indicate net root N mineralization (i.e. net root N release) in the mesh bag and NAI > 1 would indicate net root N immobilization (i.e. net root N accumulation). We performed multiple regression analyses to test for the effects of initial root N concentration on root mass loss and NAI (using data from both Trials 1 and 2). We also performed multiple regressions to seek for significant effects of plant functional composition and plant species number on fine root decomposition, root detritus N dynamics, soil C : N, total root biomass and soil net N mineralization rates (using data from either Trial 1 or 2).

Results

EFFECTS OF ROOT FUNCTIONAL COMPOSITION AND SITE OF DECOMPOSITION ON FINE ROOT MASS LOSS AND ROOT DETRITUS N DYNAMICS

Results from a two-way ANOVA showed that fine root functional composition significantly affected both root mass loss and NAI (Table 1). Root mass loss of C3 grasses (23.8 ± 1.8%; mean ± SE) and C4 grasses (15.6 ± 1.47%) was lower than root mass loss of legumes (38.2 ± 1.36%) and forbs (39.1 ± 1.44%), and differences among root functional types were all significant (Tukey's HSD test, $P < 0.05$) except between forbs and legumes. NAI was significantly greater for fine roots of C4 grasses (0.84 ± 0.03; mean ± SE) than for those of legumes (0.66 ± 0.03; mean ± SE, Tukey's HSD test, $P < 0.05$), whereas forbs (0.76 ± 0.03) and C3 grasses (0.78 ± 0.04) were intermediate and did not differ significantly from the other functional groups. Interestingly all fine roots released N over the course of the study (NAI < 1). The site of decomposition significantly affected fine root mass loss after 10 months of incubation (Table 1). Specifically, root mass loss was significantly lower in the high-diversity plots (0.25 ± 0.03%; mean ± SE, Tukey's HSD test, $P < 0.05$) than in forb (0.33 ± 0.02%), legume (0.29 ± 0.02%) and C4 (0.32 ± 0.021%) monoculture plots. However, decomposition location did not affect NAI during the period of incubation (Table 1).

EFFECTS OF INITIAL ROOT N CONCENTRATION ON ROOT MASS DECOMPOSITION AND ROOT DETRITUS N DYNAMICS

Results of a multiple regression showed that root functional composition of the monoculture 'donor plots' explained 62%

Table 1. Dependence of fine root mass loss and Nitrogen Accumulation Index (NAI) on fine root functional type (i.e. fine roots collected in either C3 grass, C4 grass, forb or legume monoculture 'donor plots') and on the site of decomposition (i.e. identity of the 'recipient plots') assessed using two-way ANOVA. Results refer to data collected from Trial 2

	Fine root mass loss (%)					NAI				
Predictor variables	d.f.	s.s.	m.s.	v.r.	F pr.	d.f.	s.s.	m.s.	v.r.	F pr.
Root functional type	3	2.47	0.82	65.6	**< 0.001**	3	0.97	0.32	5.58	**0.001**
Decomposition site	3	0.12	0.04	3.38	**0.02**	3	0.05	0.01	0.29	0.83
Interaction	9	0.11	0.01	1.01	0.43	9	0.33	0.03	0.64	0.76
Residual	190	2.38	0.01			183	10.1	0.05		
Total	205	4.92				198	11.2			

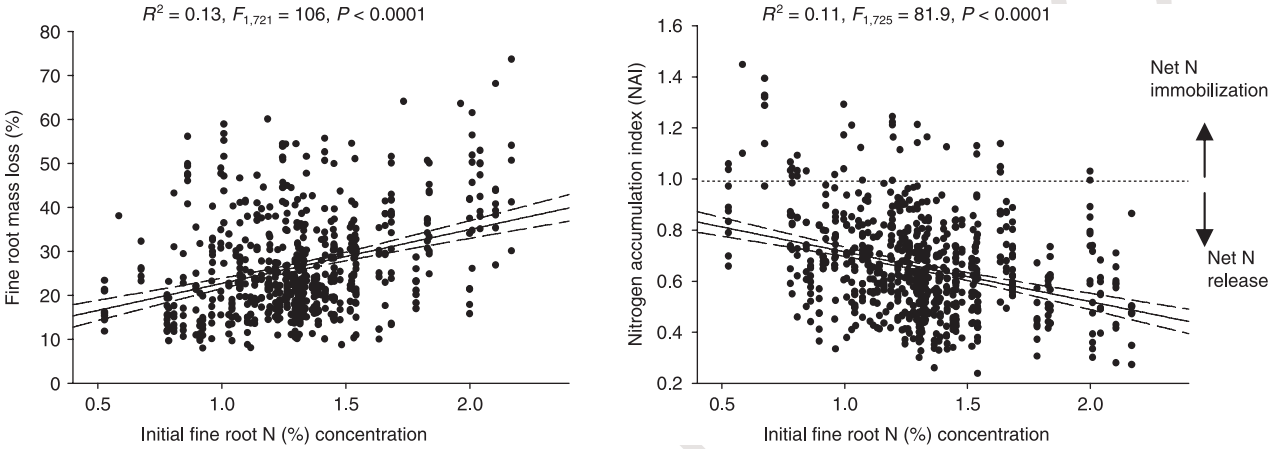

Fig. 2. Dependence of fine root mass loss (a) and nitrogen accumulation index (NAI; b) on initial root N concentration of each mesh bag buried in both Trials 1 and 2.

of the variability in initial root N and root N, in turn, was a significant predictor of both mass loss and NAI. Specifically, legumes had significantly higher root N concentrations (1.81 ± 0.1%, mean ± SE; Estimate = 0.52, P < 0.0001) than C4 grasses (0.89 ± 0.11%, mean ± SE; Estimate = –0.38, P < 0.0001). In a linear regression with initial root N concentration of each mesh bag included in both decomposition Trials 1 and 2, root N concentration alone explained 13% of the variability associated with root mass loss (Fig. 2a) and 11% of the variability associated with NAI (Fig. 2b) after 10 months of root incubation. Figure 2b also shows that there was an overall N release from decomposing roots during the 10-month incubation period regardless of fine root functional composition.

However, in a multiple regression of only the decomposition of roots collected from monoculture 'donor plots' (and decomposed in both Trials 1 and 2) which included root substrate functional composition and initial root N as predictors, root substrate functional composition significantly affected root decomposition ($F_{4,233}$ = 54.6, P < 0.0001) but initial root N did not ($F_{4,233}$ = 2.29, P = 0.0131). This suggests that other differences in root chemistry likely contribute to variation in decay rates and while some plant functional groups have distinctive and relatively predictable root N concentrations and decomposition rates (i.e. low root N content in C4 grasses led to low decay rates; Fig. 3a) other groups such as non-legume forbs show greater variability in intrinsic root N concentrations and decomposition rates (Fig. 3a). Similarly, in a multiple regression that included the identity and initial root N of each monoculture species as predictors, species identity significantly affected root decomposition ($F_{16,233}$ = 14.8, P < 0.0001; Fig. 3b) but initial root N did not ($F_{16,233}$ = 0.31, P = 0.57; Fig. 3b). Finally, we found a significant negative relationship between fine root decomposition and NAI (R^2 = 0.11, $F_{1,724}$ = 79.5, P < 0.0001) with more root mass loss being associated with greater root N release.

EFFECTS OF TOTAL ROOT BIOMASS ON ROOT MASS LOSS, ROOT DETRITUS N DYNAMICS AND POTENTIAL NET SOIL N MINERALIZATION RATES

In a multiple regression which included the presence/absence of each functional group in the 84 'donor plots' we found that total root mass to 30 cm soil depth was greater when plots included C3 grasses ($F_{4,84}$ = 15.5, P = 0.0002), C4 grasses ($F_{4,84}$ = 24.7, P < 0.0001) and legumes ($F_{4,84}$ = 19.4, P < 0.0001).

We then investigated the potential relationships among total root biomass and root mass loss and NAI. In two separate multiple regression analyses that included root substrate functional composition (of the donor plots) and total root mass of the 'recipient plots' (Trial 2), total root biomass was

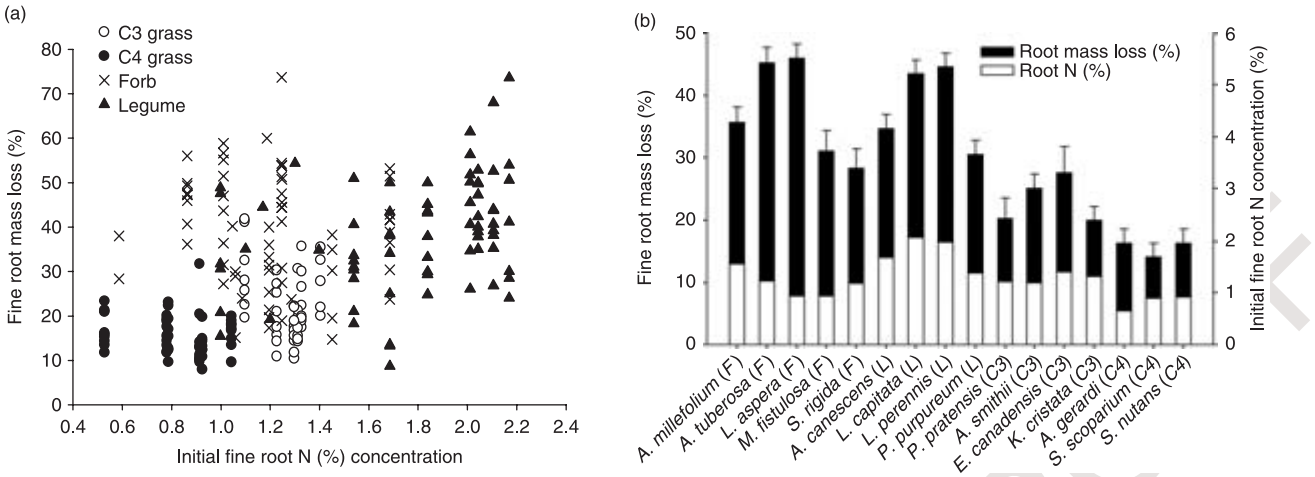

Fig. 3. Dependence of fine root mass loss on initial root N concentration in both Trials 1 and 2 only showing plant functional composition of the monoculture 'donor plots' (a). Mean fine root mass loss and initial root N concentration of each species (b).

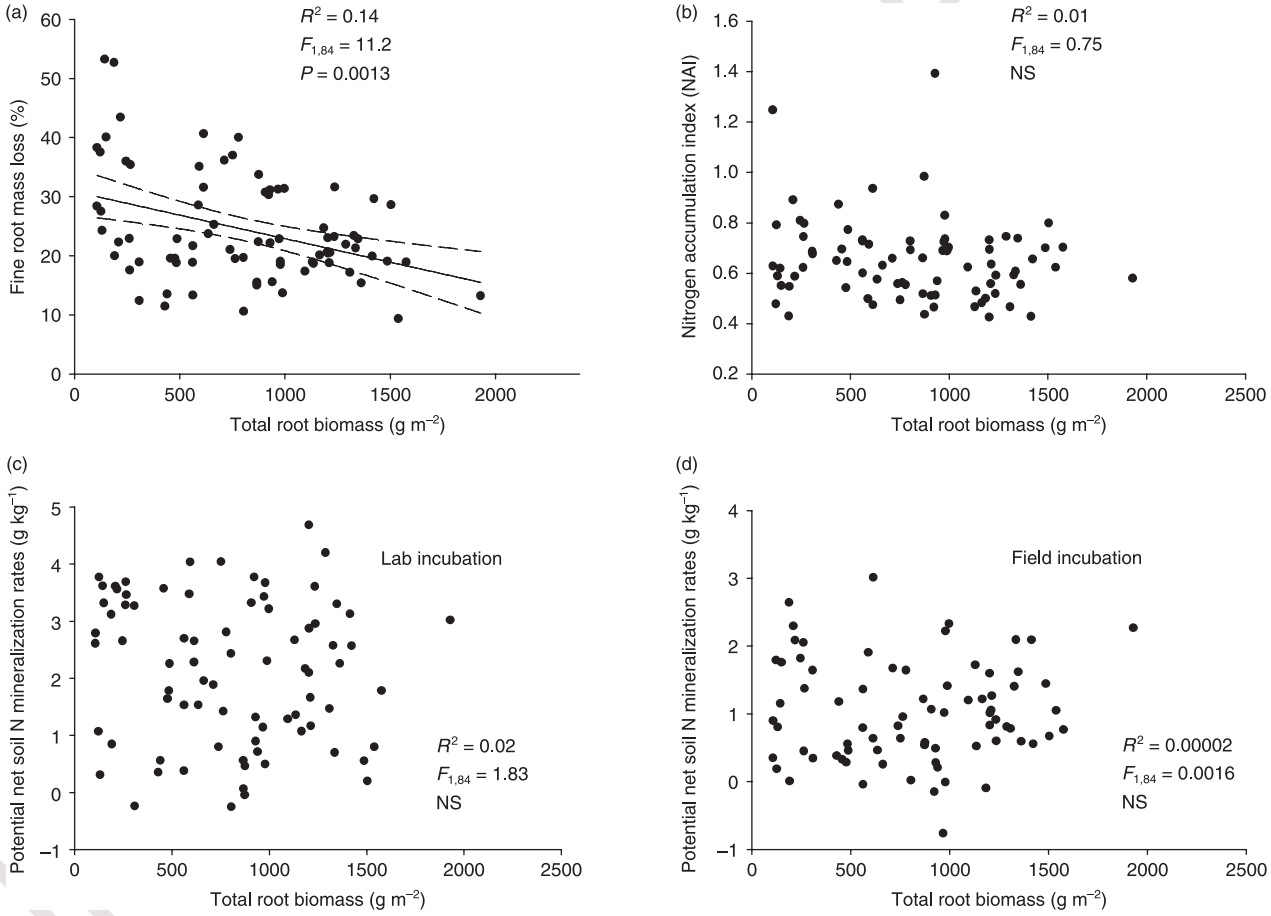

Fig. 4. Dependence of fine root mass loss after 10 months incubation period (a), nitrogen accumulation index (NAI; b) and net soil N mineralization rates as either measured in the laboratory incubation (c) or in the field incubation (d) on total root biomass as measured in 2006 to 30 cm soil depth. Regression analyses were performed on data collected from Trial 1.

significantly negatively related to root mass loss (Estimate = –0.004, $F_{4,205}$ = 4.90, P = 0.01) but was not related to NAI (P = 0.08) while root functional composition of the recipient plots had significant effects on both root decomposition and NAI (P < 0.01 for both analyses). We found a similar result analyzing data of decomposition Trial 1, where total root biomass (Fig. 4a) had significant negative effects on root mass loss but not on NAI (Fig. 4b).

Besides being negatively related to root decomposition, total root biomass was positively related to soil C : N

ratio : soil C : N ratio was positively related to total root biomass ($P < 0.005$) and negatively related to root mass loss in both Trial 1 (Estimate = −4.7, $F_{1,83} = 8.25$, $P = 0.005$) and Trial 2 (Estimate = −1.4, $F_{1,637} = 5.8$, $P = 0.014$).

A multiple regression testing for the effects of the presence/absence of each of the four plant functional groups in the 84 'donor plots' showed that the presence of C4 grasses was strongly negatively related to potential net soil N mineralization rates in both the laboratory incubation (Estimate = −0.86, $F_{4,84} = 40.9$, $P < 0.0001$) and the field incubation (Estimate = –0.33, $F_{4,84} = 17.4$, $P < 0.0001$), whereas the presence of legume species was positively related to net N mineralization rates in both the laboratory incubation (Estimate = −0.53, $F_{4,84} = 16.1$, $P = 0.0001$) and the field incubation (Estimate = –0.39, $F_{4,84} = 24.6$, $P < 0.0001$). In addition, potential net soil N mineralization rates measured in both laboratory (Estimate = 3.46, $F_{1,84} = 21$, $P < 0.0001$) and field incubations (Estimate = 0.027, $F_{1,84} = 10.7$, $P = 0.0016$) increased with greater root mass loss. Surprisingly total root biomass and NAI were unrelated in Trials 1 and 2 ($P > 0.07$) as were total root biomass and potential net soil N mineralization rates (in both laboratory and field incubations) in the 84 donor plots (Fig. 4c,d). Rates of soil N mineralization in laboratory and field incubations were positively significantly correlated ($r = 0.37$, $P = 0.001$).

Finally, in two separate multiple regressions that included initial fine root N concentration of each mesh bag from each 'donor' plot and total root biomass in each 'recipient' plot we found that root N concentration was positively related to fine root mass loss and negatively related to NAI after 10 months (Table 2), whereas total root biomass was negatively related to fine root decay rates but not to NAI (Table 2).

PLANT DIVERSITY, NET SOIL N MINERALIZATION RATES, FINE ROOT MASS LOSS AND ROOT DETRITUS N DYNAMICS

The negative effects of total root biomass on root mass loss partly explain the lower root decomposition rates in the high-diversity plots when used as recipient plots. Indeed, both total root biomass (1-species plot = 381 ± 59.8 g m^{-2}, 4-species plot = 754 ± 54.2 g m^{-2}, 16-species plot = 1218 ± 54.3 g m^{-2}) and soil C : N ratio (1-species plot = 10.9 ± 0.12, 4-species plot = 11.2 ± 0.1, 16-species plot = 11.6 ± 0.1) increased with increasing species numbers (Table 3). However, plant species number had only a weak negative effect on fine root mass loss in the 84 donor plots and plant diversity was unrelated to fine root C : N ratios, NAI and potential net soil N mineralization rates (Table 3). Thus below-ground N cycling may not be negatively affected by increasing species numbers (i.e. by greater soil C inputs). Indeed in a multiple regression including initial root N concentration, total root biomass and soil C : N ratio, only root N concentration was positively associated with potential net soil N mineralization rates measured in both laboratory and field incubations (Table 4). In addition, fine root production measured during 2006–2007 in the first top 20 cm soil depth (see Fornara & Tilman 2008 for more detailed information and presentation of data) in the 84 donor plots increased with plant species numbers (Table 3) and was negatively and significantly related with NAI (Estimate = −0.0004, $F_{1,84} = 11.2$, $P = 0.0013$). This suggests that greater root production at higher species numbers may enhance root N release and partly offset potential negative effects of greater C inputs on below-ground N cycling.

Table 2. Dependence of fine root mass and Nitrogen Accumulation Index (NAI) on initial root nitrogen concentration (%) and total root biomass measured to 30 cm soil depth in August 2006. Results refer to two separate multiple regression analyses including data from Trials 1 and 2 (d.f. = 2721)

	Response variables					
	Fine root mass loss (%)			NAI		
Regression parameters	Estimate	SE	*F*	Estimate	SE	*F*
Initial root N (%)	11.5	1.24	86.7****	–0.2010	0.021	86.5****
Total root mass (g m^{-2})	–0.005	0.0009	27.4****	0.00002	0.0001	3.1 NS

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. NS, not significant ($P = 0.08$).

Table 3. Dependence of eight ecosystem variables on the number of plant species planted in each of the 84 'donor plots' as determined by eight separate linear regressions

	Species number			
Variable analyzed	Intercept	Estimate	R^2	*F*
Total root biomass (g m^{-2})	441****	49.9	0.54	92.8****
Soil C : N	10.9****	0.04	0.17	15.9****
Fine root mass loss (%)	27.2****	–0.33	0.067	4.26*
Fine root C : N ratios	35.6****	–0.18	0.012	1.26 NS
NAI	0.68****	–0.004	0.04	3.38 NS
Potential net soil N mineralization rates (Laboratory incubation; g kg^{-1})	2.15****	–0.004	0.0005	0.039 NS
Potential net soil N mineralization rates (Field incubation; g kg^{-1})	1.39****	–0.02	0.025	1.91 NS
Total fine root production (g m^{-2})	110****	8.34	0.21	20.2****

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$; NS, not significant.

Table 4. Dependence of potential net soil N mineralization rates on initial root N concentration, total root biomass and soil C : N ratio measured in the 84 'donor plots' from which roots were collected and decomposed. Results refer to two separate multiple regressions one for the laboratory incubation and one for the field incubation

	Potential net soil N mineralization rates (g kg^{-1})					
	Laboratory incubation overall $R^2 = 0.27$, $F_{3,84} = 9.23$, $P < 0.0001$			Field incubation overall $R^2 = 0.26$, $F_{3,84} = 9.05$, $P < 0.0001$		
Predictor variables	Estimate	*F*	*P*	Estimate	*F*	*P*
Initial root N (%)	1.96	26.5	**< 0.0001**	1.16	26.2	**< 0.0001**
Total root biomass (g m^{-2})	–0.0003	1.13	0.29	0.0004	0.06	0.801
Soil C : N	0.07	0.35	0.726	–0.039	0.09	0.763

Discussion

PLANT FUNCTIONAL COMPOSITION EFFECTS ON FINE ROOT DECAY

Plant functional composition had a strong effect on fine root decomposition with positive effects of N-fixing legumes and non-leguminous forbs and negative effects of graminoid species on root decay rates. These results are consistent with those of previous studies that compared leaf litter decomposition across plant functional groups in grassland systems at different latitudes (Cornelissen & Thompson 1997; Cornelissen *et al*. 1999; Quested *et al.* 2003). Thus, intrinsic differences in substrate chemistry among grassland plant functional groups affect above- and below-ground decomposition similarly. For example, low initial root N concentrations were clearly associated with low root mass loss in C4 grasses, whereas high initial root N concentrations were associated with high root mass loss in legumes (Fig. 3a). Differences in root chemistry not measured in this study could also contribute to differences in root decay rates among functional groups. For example, the production of secondary metabolites such as phenols in response to herbivory (Bardgett 2005) or variations in Ca concentrations may have contributed to variation in root decomposition (Silver & Miya 2001). Also, lignin : N ratio differences (Dijkstra *et al.* 2006) among the four functional groups (i.e. C4 grasses = 19.6 ± 0.8 (mean ± SE), C3 grasses = 16.5 ± 1.2, forbs = 13.3 ± 0.7 and legumes = 9.1 ± 0.7) may partly explain the variation in fine root decomposition in our study. Additionally, within functional groups, interspecific differences in lignin : N ratios may help explain differences in fine root decomposition among forb species not explained by initial root N concentration alone. For example, fine roots of *A. tuberosa* had lower initial N concentrations but also lower lignin : N ratios (10.4) and decomposed faster than roots of *S. rigida* which have a lignin : N ratio of 15.4 (Dijkstra *et al.* 2006; Fig. 3b).

An additional factor that may affect fine root decomposition is the presence of mycorrhizal symbionts which may slow (Langley *et al.* 2006) or accelerate (Hodge *et al.* 2001) the decay of organic plant material returned to the soil. Although we did not identify mycorrhizal fungi on roots collected for decomposition, recent studies at Cedar Creek show that all our species are mycorrhizal hosts except *L. perennis* (Johnson *et al.* 2005; Antoninka *et al.* in press). However, despite most of our species being mycorrhizal hosts, we observed high variability in fine root decomposition rates at the plant species level, especially among non-leguminous forbs and C3 grasses (see Fig. 3b), which makes it difficult to interpret any species-specific mycorrhizal effects on fine root decay. More importantly previous studies at Cedar Creek that addressed the composition of the below-ground microbial community through phospholipid fatty acid analyses (i.e. PLFA; see Zak *et al*. 2003; Chung *et al*. 2007) showed that a biomarker (i.e. 16 : 1ω5 c) used to infer the biomass and relative abundance of arbuscular mycorrhizal (AM) fungi was significantly greater at higher plant species richness. This together with the fact that we also found layering of hyphae (presumably from saprophytic fungi) in > 50% of the mesh bags retrieved from the high-diversity plots supports the argument that fungal abundance tends to increase with plant production (e.g. increased organic substrates in detritus) at greater species numbers in our experimental plots (Zak *et al*. 2003). The increased supply of organic substrates may alter competitive interactions between different microbial functional groups (e.g. fungi vs. bacteria; see Zak *et al.* 2003; Waldrop *et al.* 2006) and this in turn may have contributed to the slightly lower root decomposition rates we found in the high-diversity plots.

ROOT DETRITUS N DYNAMICS

Fine roots exhibited net N release regardless of plant functional composition and location of decomposition. Low capacity for N immobilization by fine roots was observed in previous studies under various climatic conditions (Seastedt *et al.* 1992; Moretto *et al.* 2001; Personeni & Loiseau 2005; Parton *et al.* 2007) and may have multiple causes. First, it may arise because of the relatively high N content of fine roots. For instance, fine root C : N ratios in this study varied from 48.1 ± 1.13 (mean ± SE) in C4 grass monocultures to 24.7 ± 1.062 (mean ± SE) in legume monocultures. These values fall on the lower end of the range of fine root C : N ratios measured for different plant growth forms (Silver & Miya 2001), as well as for their above-ground leaf litter counterparts (Wedin & Tilman 1990; Murphy *et al.* 2002) and for grass and tree species used in different decomposition

trials (Hobbie *et al.* 2006; Moore *et al.* 2006). A recent study of leaf litter decomposition in Canadian forests showed that net N release occurred at C : N ratios ranging between 37 and 71 (Moore *et al.* 2006), whereas a study that used leaf litter from different biomes showed a net N release at C : N ratios between 31 and 48 (Parton *et al.* 2007), consistent with results presented here.

Additionally, roots may release more N per unit of mass loss or for a given initial N concentration than leaf litter (Parton *et al*. 2007). We did not decompose leaf litter in our experiment to allow a direct comparison between fine root and leaf litter dynamics. However, for a variety of leaf litter types ranging in initial N concentration that were decomposed in two nearby successional old fields, N immobilization was greater at a given initial N concentration and for a given unit of mass loss (Hobbie 2005) than for the roots decomposed in this study. Greater relative N release by roots may occur because the below-ground environment gives decomposers greater access to moisture, soil organic matter and mineral N (Silver & Miya 2001) and buffers them from environmental changes compared to the soil surface (King *et al.* 1997). For example, leaf litter of *P. ligularis* decomposed faster when incubated below-ground than above-ground (Vivanco & Austin 2006).

Although greater total root biomass should increase soil C inputs, stimulating N immobilization and reducing net rates of N mineralization, the rapidity with which fine roots release N may lessen the overall depressing effects of increased root biomass on N cycling. The presence of decomposing fine roots that release N may partly explain why potential net soil N mineralization rates (Fig. 4c,d) did not decline with increasing root biomass. Interestingly, even though plots with greater root biomass exhibited slower root decomposition, and slower decomposition was in turn associated with higher NAI, NAI did not increase with increasing root biomass (Fig. 4b). Thus, other factors associated with high root biomass (e.g. plant diversity and functional composition, environmental and/or microbial factors such as the diversity and abundance of microbial functional groups at increasing root mass accumulation) may stimulate N release from roots. For example, we found that the increased probability of including more legume species at greater species numbers in the 84 donor plots significantly contributed to decrease NAI (Estimate = –0.058, $F_{1,84} = 4.3$, $P = 0.04$) whereas the number of C4 grasses ($P = 0.38$), C3 grasses ($P = 0.44$) and forbs ($P = 0.35$) did not. Thus the presence and number of legume species at greater species diversity may partly explain why root decay was only weakly negatively affected in the 16-species plots although such plots showed the highest root biomass.

LINKAGES BETWEEN PLANT FUNCTIONAL COMPOSITION, FINE ROOT PROCESSES AND ECOSYSTEM FUNCTIONING

Our results suggest how fine root processes may simultaneously promote soil C sequestration and above-ground primary productivity along a gradient of plant species diversity (Tilman *et al.* 2006). In our N-limited grassland system, soil C and N storage tended to increase at higher species diversity because of greater plant below-ground (and above-ground) C and N pools (Fornara & Tilman 2008). However, although total root biomass and soil C : N ratios increased in more diverse plots (Table 3), fine root C : N ratios, NAI and net soil N mineralization rates showed no relationship with plant species number (Table 3). This suggests below-ground N cycling in our N-limited system may not be negatively affected by increasing soil C inputs because of the rapid rate of N release from decomposing fine roots.

The presence and number of different plant functional groups (i.e. functional diversity; Tilman *et al.* 1997) with opposite effects on root mass loss, root N release and soil N mineralization rates is crucial for enhancing both ecosystem services. The presence of highly complementary plant functional groups such as C4 grasses and legumes (and of C3 grasses) enhances root biomass and soil C and N sequestration. The presence of fine roots of forb and legume species enhanced root decomposition, root N release and soil N mineralization rates. Also, because fine root longevity is positively related with root C : N ratios (Craine *et al*. 2002) it is likely that fine roots of legumes (and forbs) have a higher turnover rates than fine roots of C4 grasses and their presence contributes to enhance N cycling and fine root production at greater species numbers (Fornara & Tilman 2008). This together with low capacity for N immobilization by fine roots could contribute to support an active soil N pool (i.e. decaying fine roots) and sustain plant productivity under increasing soil C and N accumulation. However, more field studies are needed to compare the absolute amounts of N released from soil organic matter vs. decomposing roots in affecting below-ground N cycling along plant diversity gradients and under different conditions (e.g. grazed, burrowed or trampled grassland systems).

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