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Plant functional composition influences rates of soil carbon and nitrogen accumulation

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Summary

1. The mechanisms controlling soil carbon (C) and nitrogen (N) accumulation are crucial for explaining why soils are major terrestrial C sinks. Such mechanisms have been mainly addressed by imposing short-term, step-changes in CO_2, temperature and N fertilization rates on either monocultures or low-diversity plant assemblages. No studies have addressed the long-term effects of plant functional diversity (i.e. plant functional composition) on rates of soil C accumulation in N-limited grasslands where fixation is the main source of N for plants.

2. Here we measure net soil C and N accumulation to 1 m soil-depth during a 12-year-long grassland biodiversity experiment established on agriculturally degraded soils at Cedar Creek, Minnesota, USA.

3. We show that high-diversity mixtures of perennial grassland plant species stored 500% and 600% more soil C and N than, on average, did monoculture plots of the same species. Moreover, the presence of C4 grasses and legumes increased soil C accumulation by 193% and 522%, respectively. Higher soil C and N accrual resulted both from increased C and N inputs via (i) higher root biomass, and (ii) from greater root biomass accumulation to 60 cm soil depth resulting from the presence of highly complementary functional groups (i.e. C4 grasses and legumes).

4. Our results suggest that the joint presence of C4 grass and legume species is a key cause of greater soil C and N accumulation in both higher and lower diversity plant assemblages. This is because legumes have unique access to N, and C4 grasses take up and use N efficiently, increasing below-ground biomass and thus soil C and N inputs.

5. *Synthesis.* We demonstrate that plant functional complementarity is a key reason why higher plant diversity leads to greater soil C and N accumulation on agriculturally degraded soils. We suggest the combination of key C4 grass–legume species may greatly increase ecosystem services such as soil C accumulation and biomass (biofuel) production in both high- and low-diversity N-limited grassland systems.

Key-words: biodiversity, C4 grasses, functional complementarity, functional groups, legumes, N_2-fixing, plant productivity, root biomass, soil C sequestration

Introduction

Reduced plant diversity has raised numerous concerns, including the possibility that the ecological services of terrestrial ecosystems may be negatively affected (Naeem *et al.* 1994; Chapin *et al.* 2000; Sala *et al.* 2000; Loreau *et al.* 2001; Hooper *et al.* 2005; Balvanera *et al.* 2006). Lower plant diversity could potentially weaken the ability of long-lived carbon (C) pools of terrestrial ecosystems to continue to act as C sinks of atmospheric CO_2 (Fan *et al.* 1998; Pacala *et al.* 2001). The soil C pool alone is 3.3 times the size of the atmospheric pool and 4.5 times the size of the terrestrial biotic pool (Lal 2004). Hence, from a greenhouse gas perspective, it is important to understand whether and how changes in plant species number and composition might influence rates of soil C accumulation.

Plant species number and/or composition are thought to influence ecosystem productivity, stability, nutrient dynamics and invasibility (Tilman *et al.* 1996, 2001, 2006b; Hector *et al.* 1999; Hooper *et al.* 2000, 2005; Levine 2000; Naeem *et al.*

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2000; Wolters *et al.* 2000; Spehn *et al.* 2005; van Ruijven & Berendse 2005), but attribution of causation in such experiments has been open to alternative explanations (e.g. Huston 1997; Wardle 1999). Many of these studies have been interpreted as suggesting that experimentally imposed differences in plant species number mattered because greater species number gave greater differences in functional traits, thus allowing species to exploit resources in different ways (e.g. Hector *et al.* 1999; Tilman *et al.* 2001, 2006b; Spehn *et al.* 2005).

Because biodiversity experiments manipulate both plant species numbers and functional composition, they have the potential to provide unique insights into the factors controlling C and nitrogen (N) levels in soils. However, accrual of soil C and N is a slow process, and biodiversity experiments are only now getting to be of sufficient duration to explore its dynamics and potential causes. Here we measured soil C and N accumulation for the full soil profile to 1 m soil-depth, using data from the 12-year-long biodiversity experiment on N-limited grasslands at Cedar Creek, Minnesota, USA.

Although it has been shown that greater plant species numbers is associated with soil C accumulation in N-limited grassland communities (Tilman *et al.* 2006a), it is not clear whether and to what extent C accumulation depends upon the presence of one or more key functional traits in the plant community. Here we report results based on additional sampling of soil C and N levels that we use to determine the potential role of functional composition on soil C and N accumulation through time.

Our main hypothesis is that the positive effects of greater species numbers on soil C and N accumulation depend on enhanced C and N inputs returned to the soil from the plant community (i.e. increased plant productivity). Plant productivity is, however, strongly positively affected by the presence of critical plant functional traits which are related to the acquisition, processing and use of key resources such as N (see Díaz & Cabido 2001; Lavorel & Garnier 2002; Hooper *et al.* 2005). Using the terminology of Loreau & Hector (2001), we call the positive effects that result from interspecific differences in traits 'complementarity.' Complementarity encompasses the effects of two underlying but difficult to distinguish processes, niche differentiation (Tilman 1999) and facilitation (Brooker *et al.* 2008). In our N-limited system, for example, plant productivity is greatly enhanced by complementarity between legume and C4 grass species (Lambers *et al.* 2004). Complementarity occurs because (i) N fixation by legumes facilitates growth of C4 grasses, which have high N use efficiency, and (ii) because legume N supply and its use by C4 grasses are differentiated in time (i.e. cool-season legume N fixation is followed by warm-season C4 grass N uptake).

We first suggested whether the effects of plant diversity on the accumulation of soil C and N through time and at different soil depths could be attributable to the presence of critical functional groups (i.e. functional composition effect) within the plant assemblage. Second, we asked whether soil C and N accrual depend upon the accumulation of plant below-ground biomass across years and whether this could be explained by a complementarity effect between particular plant functional groups. We consider such questions as important for providing the context to understand and predict future trends in soil C accumulation in N-limited grasslands.

Methods

We analysed data from a large biodiversity experiment where we controlled the number of herbaceous perennial grassland species in 152 plots (as in Tilman *et al.* 2006a), each 9×9 m, at Cedar Creek Natural History Area, Minnesota, USA. In 1993, an abandoned ex-agricultural area was treated with herbicide, burned, bulldozed to remove the top 6–8 cm of a horizon soil to reduce the seed bank, ploughed and harrowed. Plots were established in 1994 and seeded to contain 1, 2, 4, 8 or 16 grassland savanna species. Composition of each plot was randomly chosen from a pool of 18 species, which included four C4 grasses, four C3 grasses, four legumes, four non-legume herbaceous forbs and two woody-savanna species (*Quercus* spp.). There were 28–35 replicates at each level of species diversity (a list of species included in this biodiversity experiment and more information about methods and archive samples can be found at <http://www.cedarcreek.umn.edu/research/exper/e120>). The 152 plots neither included woody monocultures nor low-diversity plots (two- and four-species plots) with woody seedlings represented (Tilman *et al.* 2006a). 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. Soil C and N samples were collected during summer (e.g. first 2 weeks of August) in 1994 and 2006 at 0–20, 20–40, 40–60 and 60–100 cm soil depth increments for each of nine sites per plot. Additional soil samples were collected with the same methodology in 2000 and 2004, but only for the 0–20 cm soil depth increment. Samples from each plot were then sieved to remove roots, combined by depth for each plot, mixed and ground. Soil samples were then dried at 40 °C for 5 days and stored in glass vials. Two soil samples for each depth increment per plot from the 1994 and 2006 soil collections, and one soil sample per plot from the 2000 and 2004 soil collections were analysed for total C and N by combustion and gas chromatography (Costech Analytical ECS 4010 instrument, Costech Analytical Technologies Inc., Valencia, CA). For the 1994 and 2006 soil collections, we used the average of the two measurements of C and N at each depth in all statistical analyses. Net soil C and N accumulation at each soil depth level after 12 years was calculated as the difference in soil C and N concentration measured in the 2006 and the 1994 soil. Soil bulk density was measured multiple times in natural and abandoned grassland fields at Cedar Creek on the same N-limited sandy soils (Grigal *et al*. 1974; Knops & Tilman 2000; Tilman *et al.* 2006a). The average bulk density to 60 cm soil depth from these measurements was 1.5 g cm^{-3}. We also measured bulk density in a subsample of plots to test for potential differences across the diversity gradient but found that the variability in bulk density between monoculture and 16-species plots was negligible (3%).

Plots were also sampled for above- and below-ground biomass in mid-August 2006. Above-ground living plant biomass, which is a measure of net primary productivity, was collected by clipping, drying and weighing four parallel and evenly spaced 0.1×3.0 m vegetation strips per plot in 1998, and four 0.1×6.0 m strips in 2000, 2004 and 2006, respectively. Plots were sampled for below-ground biomass in mid-August 2006 by collecting three evenly spaced

soil cores in each of the four clipped strips. Each core was 5 cm in diameter and was divided into three soil depths (0–30, 30–60 and 60–100 cm deep). Soil cores were washed with a gentle spray of water over a fine mesh screen until roots were free of soil. Roots were then dried; any soil residual was removed and then weighed. Biomass samples collected in 2006 were ground and analysed for total C and N following standard methods on a 1500 NA Carlo-Erba element analyser (Elan Technology, Lambertville, NJ).

Root production was measured in 60 plots (20 randomly chosen plots for each of the 1, 4 and 16 species treatments) during 2006 by using ingrowth soil cores. We removed roots from a soil volume of 251.2 cm^3 which was collected at three different soil depths (0–20, 20–40 and 40–60 cm deep) in two sites per plot using a metallic cylinder corer. Soil cores were extracted at the beginning of August 2006, and roots were sieved and removed from the soil samples. A hardware mesh wire (1 cm diameter) was shaped to fit into the hole until a soil depth of 30 cm, then the root-free soil was returned to the hole from which was collected. After 2 months, soil samples were extracted in the same place by coring within the mesh wire (total rainfall during this period was *c.* 220 mm). New ingrown roots were sieved, dried and weighed.

STATISTICAL ANALYSIS

To determine the effects of species number on the accumulation of C and N through time in the soil top 20 cm, we performed repeated measures analysis of variance (ANOVA) using data from 1994, 2000, 2004 and 2006. We also performed repeated measures analysis to determine the effects of functional composition on the same dependent variables where functional composition was expressed by four independent variables each describing each functional group (C4, C3, forbs and legumes) as either absent from a plot or represented by at least one species. We used simple linear regressions to determine the effects of plant species number and functional composition on net soil C and N accumulation for the full soil profile to 1 m soil-depth, 12 years after the grassland biodiversity experiment was established. Simple linear regressions were also used to address the relationship between above- and below-ground biomass as well as the effects of species number on total above- and below-ground plant biomass, and root production. We performed multiple regressions including backward/forward stepwise regression analyses to test for the effects of species number, soil depth level and functional composition on different ecosystem response variables. Multiple regression analyses, including stepwise backward/forward regressions, were performed using different predictor variables such as total below- and above-ground biomass, plant tissue N, plant C : N ratio, number and composition of plant species, number of functional groups in different combinations on net rates of soil C and N accumulation. Because the effects of species number and functional composition are likely to be correlated, these two variables (and other combinations of likely correlated variables) were simultaneously included in type III regressions, which are highly conservative. We then interpreted the results by making comparisons across multiple regressions so as to understand better the explanatory importance of each independent variable.

We also estimated the annual instantaneous rate of change in soil C ($r = dC/dt \times 1/C$) in the top 20 cm of soil for the time interval 1994–2006 (calculated as in Knops & Tilman 2000):

$$r = \left(\log e \frac{C_{2006}}{C_{1994}}\right) \cdot \frac{1}{12}.$$

This gives rates of change for C with units of per year. We similarly calculated rates of changes for soil N. We estimated these rates of change in other studies from literature in order to make independent comparisons regardless of differences in geographic location, climatic conditions or differences in initial soil C and N contents after agricultural abandonment. Data were analysed using JMP v. 6.0.2 (SAS Institute, Inc. 2006).

Results

PLANT DIVERSITY EFFECTS ON C AND N ACCUMULATION THROUGH TIME IN THE SOIL TOP 20 CM

Results from repeated measures ANOVA show that more soil C accumulates at higher diversity. In particular, there is a significant positive species number × time interaction on soil C (%) accumulation ($F = 5.04$, d.f. = 3, density d.f. = 148, $P = 0.0023$; Fig. 1a). Soil N accumulation is also greater at higher diversity, with a significant positive species number × time interaction ($F = 4.89$, d.f. = 3, density d.f. = 148, $P = 0.0028$; Fig. 1b) for the 0–20 cm soil depth interval. Repeated measures analysis also show a positive significant C4 grass × time

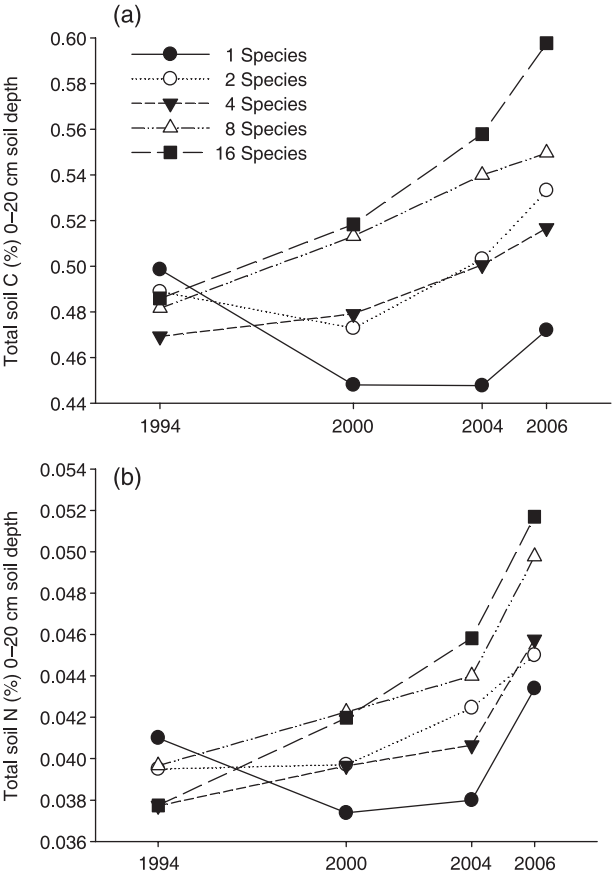

Fig. 1. Dependence of total soil C (%) (a), and total soil N (%) (b), measured between 0 and 20 cm soil-depth on the number of species planted in each plot and across the 12-year grassland biodiversity experiment.

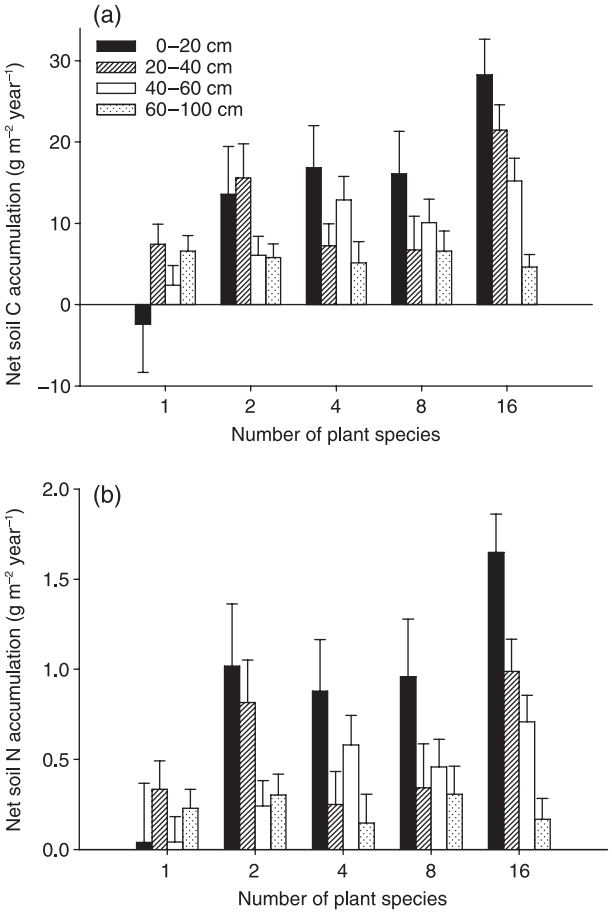

Fig. 2. Dependence of net soil C (a), and N (b), accumulation at different soil depth increments on the number of plant species (regression model has d.f. = 7, 608). SE bars indicate variability within each soil depth increment.

interaction ($F = 3.07$, d.f. = 3, density d.f. = 144, $P = 0.029$) as well as a legume × time interaction ($F = 5.58$, d.f. = 3, density d.f. = 144, $P = 0.0012$) on soil C (%) accumulation and a positive significant legume × time interaction on soil N (%) accumulation ($F = 4.49$, d.f. = 3, density d.f. = 145, $P = 0.0048$).

PLANT DIVERSITY EFFECTS ON SOIL C AND N ACCUMULATION FOR THE FULL SOIL PROFILE

Net soil C accumulation for the full soil profile to 1 m soil-depth was on average 69.5 ± 8.86 (mean + SE) g C m^{-2} year^{-1} in the 16-species diversity plots and 13.9 ± 9.57 g C m^{-2} year^{-1} in the monoculture plots (Fig. 2a). Net soil N accumulation was, on average, 3.5 ± 0.53 g N m^{-2} year^{-1} in the 16-species diversity plots and 0.59 ± 0.57 g N m^{-2} year^{-1} in the monoculture plots (Fig. 2b). This effect of species number on soil C and N accumulation from 1994 to 2006 seems to be related to higher diversity plant assemblages storing more C and N to 60 cm soil depth (Fig. 2a,b). We found a significant effect of soil depth ($F_{3,607} = 5.5$, $P = 0.0009$) and a significant species diversity by soil depth interaction ($F_{3,607} = 4.02$, $P = 0.007$) on net soil C accumulation (Fig. 2a). The 16-species plots gained more C than lower-diversity plots at 0–20 cm ($F_{1,152} = 12.9$, $P = 0.0004$), 20–40 ($F_{1,152} = 7.2$, $P = 0.008$) and 40–60 cm soil depth levels ($F_{1,152} = 9.8$, $P = 0.002$), but not for the 60–100 cm depth ($F_{1,152} = 0.18$, $P = 0.944$). We found that net soil N accumulation was similarly affected by soil depth ($F_{3,607} = 10.1$, $P < 0.0001$) and by a diversity–soil depth interaction ($F_{3,607} = 2.9$, $P = 0.03$; Fig. 2b). Higher diversity plots gained more N than lower-diversity plots at 0–20 cm ($F_{1,152} = 10.8$, $P = 0.0012$), 20–40 cm ($F_{1,152} = 4.15$, $P = 0.04$) and 40–60 cm soil depth levels ($F_{1,152} = 9.4$, $P = 0.0025$), but not for the 60–100 cm depth ($F_{1,152} = 0.16$, $P = 0.69$).

Multiple regression analyses using as predictor variables the presence/absence of legumes, C4 grasses, C3 grasses and forbs, showed that the presence of legumes and C4 grasses had significant positive effects on net soil C and N accumulation to 60 cm soil depth as measured for the interval from 1994 to 2006 (Table 1).

GREATER PLANT BELOW-GROUND BIOMASS INFLUENCES SOIL C AND N ACCUMULATION

For the 0–60 cm soil depth, net soil C and N accumulation from 1994 to 2006 was dependent on total root biomass (Fig. 3a,b). In a multiple regression with total root biomass,

Table 1. Dependence of different ecosystem variables, measured 12 years after the biodiversity experiment was established, on the presence or absence of the legume, C3 grass, C4 grass and forb functional groups as determined by six separate multiple regressions (one for each response variable). Regressions have d.f. = 4, 152 (d.f. = 4, 58 for root production). The coefficients as well as intercept values and R^2 of the multiple regressions refer to the final accepted model which only included the effects of the significant variables

	Regression parameters for presence of each functional group						
Response variable	Intercept	Legume	C3	C4	Forb	Overall R^2	Overall F value
Net soil C accumulation	24.8**** (g m^{-2} year^{-1})	18.8****	NS	10.9***	NS	0.19	17.3****
Net soil N accumulation	1.33**** (g m^{-2} year^{-1})	0.95****	NS	0.47*	NS	0.13	11.3****
Root biomass	749**** (g m^{-2})	229****	102***	243****	NS	0.54	59.2****
Root production	193**** (g m^{-2})	105***	NS	NS	NS	0.24	17****
Root C : N	39.2**** (C : N, g : g)	–10.5****	NS	3.9****	NS	0.58	51.2****
Above-ground biomass	134.7**** (g m^{-2})	59.8****	NS	17.5**	24.8****	0.54	57.4****

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

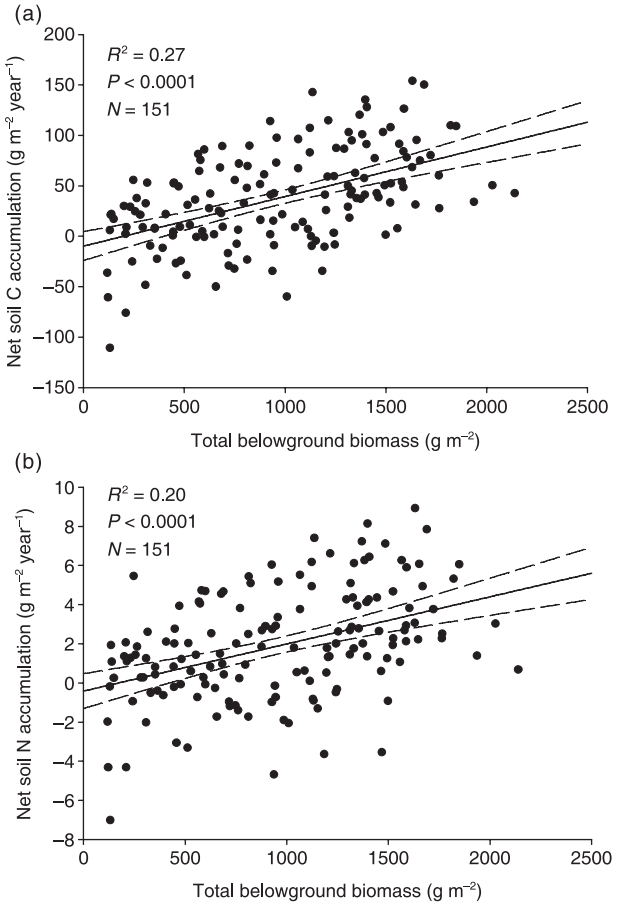

Fig. 3. Dependence of net soil C (a), and N (b), accumulation between 0 and 60 cm soil-depth on total plant below-ground biomass as measured in 2006. Regressions have d.f. = 1.151. Dashed lines represent 95% confidence bands.

the number of species and the presence/absence of each functional group as predictor variables, we found that only root biomass positively and significantly affected net soil C accumulation (overall $R^2 = 0.30$; for root biomass $F_{6,152} = 10.9$, $P < 0.0001$; $P > 0.05$ for other variables) and net N accumulation (overall $R^2 = 0.23$; for root biomass $F_{6,152} = 13.24$, $P < 0.0001$; $P > 0.05$ for other variables) over the 12 years of the experiment. This suggests that high plant species number and functional composition may enhance soil C accumulation via increasing total root biomass. Moreover, a multiple regression comparing the effects of total root biomass and total root production, both measured in 2006, on net soil C and N accumulation ($n = 60$), showed that only root biomass significantly affected net soil C (overall $R^2 = 0.26$; for root biomass $F_{2,59} = 10.7$, $P = 0.0018$) and net soil N accumulation (overall $R^2 = 0.19$; for root biomass $F_{2,59} = 7.7$, $P = 0.007$). Below- (root) and above-ground plant biomass were highly correlated ($R^2 = 0.55$; $F_{1,152} = 187$, $P < 0.0001$). Consistent with this, both net soil C and net soil N accumulation after 12 years were strongly positively dependent on total above-ground biomass ($P < 0.0001$ for both analyses), and, in a separate analysis, on total plant biomass (the sum of above- and below-ground biomass; $P < 0.0001$ for both analyses).

PLANT FUNCTIONAL COMPLEMENTARITY INCREASES SOIL C AND N ACCUMULATION BY ITS EFFECTS ON ROOT BIOMASS

Because total root biomass was so strongly associated with soil C and N accumulation, we investigated whether and how species number and functional composition affected plant below-ground biomass accumulation over 12 years. Total below-ground biomass to 60 cm soil depth was positively and significantly affected by the experimentally imposed number of plant species ($R^2 = 0.45$; $F_{1,152} = 121.8$, $P < 0.0001$). The presence of C4 grasses, C3 grasses and legumes significantly positively affected total root biomass (Table 1). However, while a C4 grass × legume interaction had also a significant effect on total root biomass in 2006 ($F_{10,151} = 19.8$, $P = 0.007$), a C3 grass × legume interaction did not ($F_{10,151} = 19.8$, $P = 0.551$).

The high complementarity between C4 grasses and legumes greatly increased total root biomass across different species diversity plots (see the 'C4L' combinations in Fig. 4a). Indeed, plots containing at least one C4 grass and one legume species had significantly greater root biomass than any other two-species functional group combination ($F_{1,26} = 23.7$, $P < 0.0001$), or four-species ($F_{1,28} = 9.89$, $P = 0.004$) or eight-species diversity plots ($F_{1,29} = 6.9$, $P = 0.01$) that lacked the presence of both a C4 grass and a legume species (Fig. 4a). Total root biomass associated with the joint presence of at least one C4 grass and one legume species within the two species plots ($N = 7$; Fig. 4a) was slightly less than, but not significantly different from, the root biomass associated with the high-diversity plots ($F_{1,42} = 1.78$, $P = 0.23$). High-diversity plots showed, however, greater below-ground biomass than C4L combinations (see Fig. 4a) within the four species plots ($F_{1,49} = 10.4$, $P < 0.0016$) as well as within the eight species plots ($F_{1,54} = 5.76$, $P < 0.021$).

We also found that legume and C4 grass presence significantly affected root production (Table 1); plots with at least a C4 grass and a legume species produced on average 333.7 ± 37.9 g m^{-2} of roots, twice as much as the average root biomass produced by all other functional group combinations that lacked C4 grasses and legumes (145.2 ± 41.9 g m^{-2}).

Finally, we found that total above-ground biomass was positively affected by the number of species ($R^2 = 0.46$; $F_{1,152} = 125.6$, $P < 0.0001$) and, in a separate analysis, it was also dependent on the presence of C4 grasses, forbs and legumes (Table 1). The joint presence of C4 grasses and legumes within two species plots increased above-ground biomass production, which was not significantly different from the 16 species plots ($F_{1,42} = 0.86$, $P = 0.35$; Fig 4b). However, total above-ground biomass was not significantly different between diversity plots that included at least one C4 grass and one legume species vs. other functional combinations which did not include any C4L combinations within the four and eight species plots (see Fig. 4b; $P > 0.05$ for all analyses).

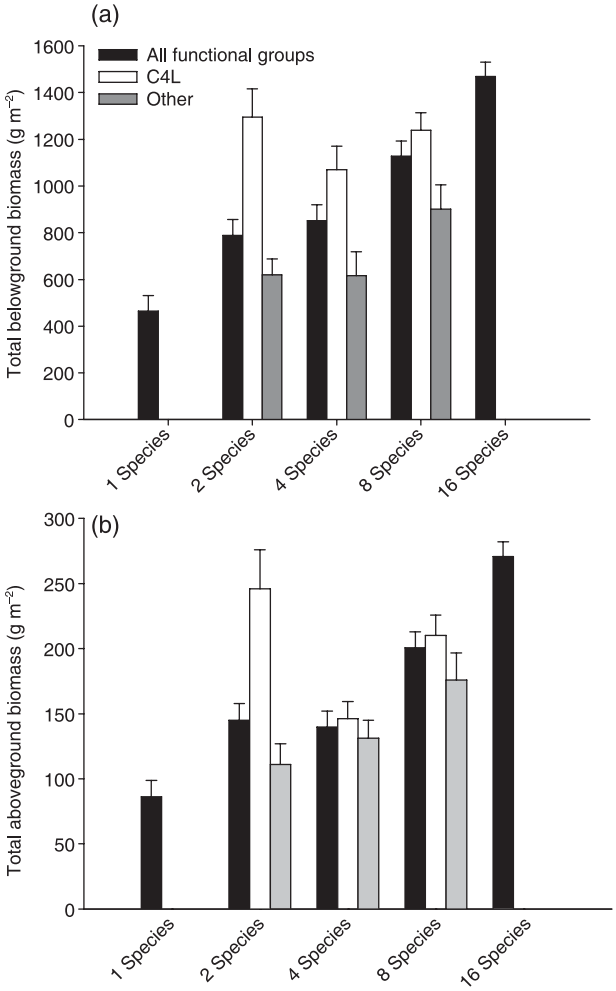

Fig. 4. Dependence of total plant below-ground biomass measured between 0 and 60 cm soil-depth (a), and total plant above-ground biomass (b), as both measured in 2006 on the number of plant species and functional composition. Black bars show plant biomass averaged for all functional groups within each species diversity level; white bars show plant biomass averaged only for those functional combinations that included at least one C4 grass and one legume within each species diversity level; finally, grey bars show plant biomass averaged for those functional combinations that did not include C4 grasses and legumes together.

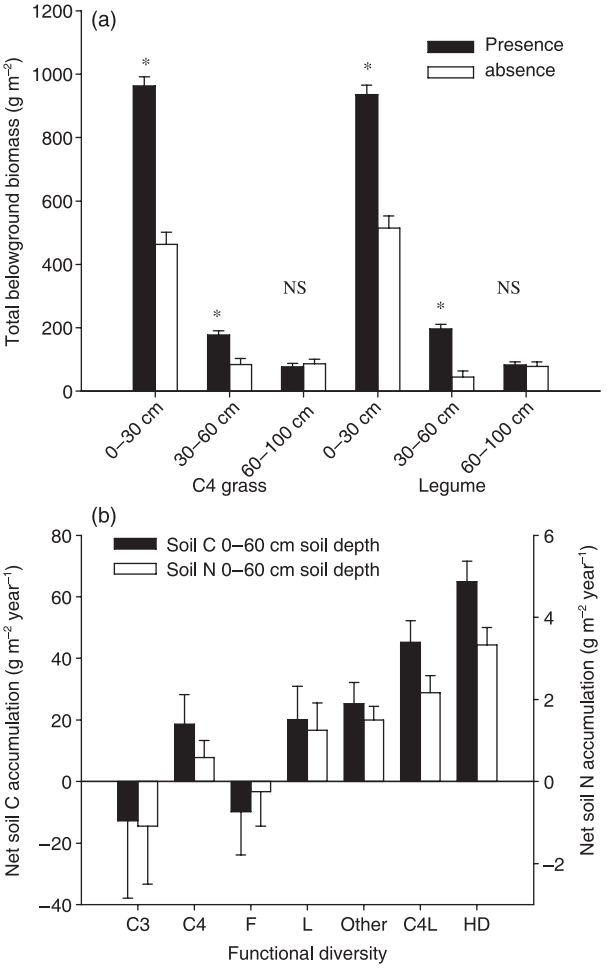

Fig. 5. Dependence of total below-ground biomass as measured in 2006 at different soil depth levels on the presence/absence of C4 grasses and legumes (a; $*P < 0.001$). Dependence of soil C and N sequestration to 60 cm soil depth on functional composition. (b) Monoculture plots (C3, C3 grasses; C4, C4 grasses; L, legumes and F, forbs); plots with at least one C4 grass and one legume species planted within the two, four and eight-species diversity plots, C4L; other plant functional combinations within the two, four and eight-species plots, Other, and high-diversity plots (HD, 16-species plots, which generally have three or four species, each, of C4's and legumes).

COMPLEMENTARITY DETERMINES SOIL C AND N ACCUMULATION BY INCREASING ROOT BIOMASS TO 60 CM SOIL DEPTH

Multiple regression shows that the presence of C4 grasses ($P < 0.0009$ for all analyses) and legumes ($P < 0.0001$ for analyses) significantly increased total root biomass between 0–30 and 30–60 cm soil depth (see Fig. 5a), whereas C3 grasses did not ($P = 0.82$). Moreover, C4 grass × legume interactions significantly increased root biomass between 0–30 and 30–60 cm soil depth ($P < 0.01$ for both analyses), whereas C3 grasses × legume interactions were not significant ($P > 0.39$ for both analyses). Finally, neither C4 grasses nor legumes significantly affected root biomass between 60 and 100 cm soil depth ($P > 0.5$ for all analyses; Fig. 5a).

The strong C4 grass × legume interaction on net soil C accumulation between 0 and 60 cm soil-depth is shown in Fig. 5b, where the presence of at least one C4 grass and one legume species within the two, four and eight species plots significantly increased soil C accumulation relative to monocultures and to all other combinations of functional groups ($P < 0.04$ for all analyses), but not relative to the 16 species plots ($F_{1,76} = 2.33$, $P = 0.13$). The 16 species plots usually contain three or four species of both legumes and C4 grasses. Similar patterns occurred for soil N accumulation (Fig. 5b).

Discussion

Our results demonstrate that a key variable associated with higher rates of soil C and N accumulation in N-limited

grasslands is greater root biomass accumulation (i.e. high plant C and N inputs in the soil) from the joint presence of C4 grasses and legumes. Soil C accumulation occurred despite annual burning of above-ground biomass, which thus limited the amount of above-ground organic matter that could move into the soil. The observed rate of C accumulation within the soil top 20 cm for the 16-species plots is 28.3 g C m^{-2} $year^{-1}$, which is similar to the average rate of soil C accumulation averaged from many grassland studies and calculated at varying years after agriculture abandonment (33.2 g C m^{-2} $year^{-1}$; see Post & Know 2000 for review).

Moreover, the instantaneous rate of change in soil C for the 16-species plots calculated in our experiment (1.8% per year) is similar to that estimated in a North American N-limited grassland system after 12 years (1.67% per year; Knops & Tilman 2000), and in the midwestern United States 40 years (2.04% per year; McLauchlan *et al.* 2006) after agricultural abandonment. The rate of change in our monoculture plots, however, was negative (–0.2%) and that of our four-species plots was just 1.15%, showing that plant diversity can greatly influence C accumulation rates. Rates also vary regionally, with early successional old fields in Michigan, USA, having higher rates of change in soil C 12 years after agricultural abandonment (3.9% per year; Grandy & Robertson 2007), whereas semi-arid grasslands in northeastern Colorado, had much lower rates of instantaneous change in soil C 50 years after agricultural abandonment (0.4% per year; Burke *et al.* 1995). Such differences may depend on the role of many soil-forming factors, including climate and parent material, and on the constancy of soil organic matter accumulation which is likely to be affected by root biomass accumulation through time and at different soil depths.

In our study, the presence of both C4 grasses and legumes increased root biomass in the upper 60 cm of soil (Table 1; Fig. 4a). Their interaction strongly affected overall root biomass accumulation when compared with monoculture plots or functional combinations, where C4 grasses and legumes were absent (Fig. 4a). This complementarity likely occurred because of both facilitation and niche differentiation (Hooper & Vitousek 1997; Tilman 1999; Lambers *et al.* 2004; Spehn *et al*. 2005; Brooker *et al.* 2008), especially among these two functional groups. Previous studies in the same biodiversity experiment at Cedar Creek showed that the effects of species number on productivity likely came via diversity-associated changes in functional composition and the number of functional groups present (Tilman *et al.* 1997, 2001). Moreover, a recent analysis demonstrates that complementarity effects on plant productivity become positive and increasingly strong through time (Fargione *et al.* 2007).

The C4 grass–legume effect thus results from complementarity and from the greater probability of jointly 'sampling' both C4 grasses and legumes in high-diversity plots (C4 and legume species together comprised *c.* 85% of the 2006 above-ground biomass of the 16-species plots, but only comprised 44% of the species in these plots). Non-legume forbs and C3 grasses accounted for *c.* 10% and *c.* 5%, respectively, of above-ground biomass in the 16-species plots; their presence had positive effects, respectively, on above- and below-ground biomass (see Table 1), and contributed to the significant diversity effect on total below- and above-ground biomass ($P < 0.03$ for all analyses) in those plots planted with two, four and eight species that lacked the simultaneous presence of C4 grasses and legumes (see grey bars in Fig. 4a,b).

Our C4 grasses have high root biomass, roots with high C : N ratios (Table 1), soils with low mineralization and decomposition rates, high N immobilization rates and high nutrient use efficiency (Pastor *et al.* 1987; Wedin & Tilman 1990). Although this may potentially lead to greater soil C accumulation rates over time (Knops & Tilman 2000), we found that net soil C accumulation to 60 cm soil depth in the C4 monocultures (and in two-species plots containing just C4 grasses) was on average less than one-third (18.7 ± 13.6 g C m^{-2} $year^{-1}$) the soil C stored in the 16-species plots within the same soil depth (64.9 ± 7.6 g C m^{-2} $year^{-1}$) after 12 years. C4 grasses also reduce the concentration of soil NO_3 to lower levels than other species, and thus may reduce leaching loss of N from the soil.

Conversely, legumes have high litter quality (low C : N), high litter decomposition rates, low nutrient use efficiency and, because of symbiotic relationships, have large effects on N availability and N supply rates in many N-limited natural and agricultural systems (Chapin *et al.* 1986; Vitousek & Howarth 1991; Vitousek 2004) including the N-limited grassland communities of Cedar Creek (Tilman & Wedin 1991). Legume-only plots (mainly monocultures) also stored one-third (20.2 ± 15.8 g C m^{-2} $year^{-1}$) the soil C stored to 60 cm soil depth of the high-diversity plots after 12 years. However, the presence of legumes significantly increased root biomass production as well as net soil N accumulation after 12 years (Table 1), and it is likely that legume-derived N is qualitatively important for building up soil organic matter and storing more C (see Drinkwater *et al.* 1998; Resh *et al.* 2002; Christopher & Lal 2007). It is nevertheless interesting that C3 grass and forb monocultures showed a net release in soil C and N after 12 years (Fig. 5b) perhaps from low root biomass accumulation (especially for forbs) and from processes involved in soil organic matter formation and their linkages with plant tissue chemistry.

The C4–legume interaction (i.e. the 'complementary effect') could be attributable to: (i) enhanced C inputs in the soil through cool-season legume N fixation followed by warm-season uptake of this N and its conversion into high C : N below- and above-ground biomass by C4 grasses; or (ii) the low soil NO_3 levels created by C4 grasses, which might cause legumes to fix more N, which in turn enhances soil N supply rates and sustains C4 grass below-ground growth in a positive feedback loop. This seems plausible for N-limited systems where warm-season C4 grasses are among the best competitors for N, whereas the access of legumes to atmospheric N_2 plays a critical role in sustaining below-ground plant productivity. The complementary effect may occur through mineralization of legume N and its uptake by grasses, a process that is well known in agroecosystems (Viera-Vargas

et al. 1995; Haby *et al.* 2006; Christopher & Lal 2007). These studies suggest that both N_2-fixation by legumes and grass biomass increase when grown together. By using ^{15}N concentrations in plant tissues, further studies could determine the rate of N transfers from legumes to C4 grasses and their dependence on the species involved (Spehn *et al.* 2002). Other underlying mechanisms responsible for the C4–legume effect may involve soil–plant–microbial community interactions as well as seasonal and spatial differences in N uptake and supply. Despite this uncertainty on the cause of the observed C4 grass and legume interactions, our results confirm that both plant diversity and plant identity matter and that both affect ecosystem processes (Hooper & Vitousek 1997), including net soil C accumulation.

Conclusion

Our study shows that the presence of species with differing functional traits increased soil C and N accumulation over the 12 years of this experiment. Greater C accumulation was associated with greater root biomass (i.e. greater C and N inputs in the soil) which, itself, resulted from positive interactions among legumes and C4 grasses and the greater soil depths through which their roots were located at higher diversity. It remains unclear, however, whether and how diversity or different combinations of functional traits, especially for cases where legumes are not present (see van Ruijven & Berendse 2005) might affect below-ground productivity and soil C accumulation. Further studies should also address whether C is accumulated into the heavy-recalcitrant or light-labile fraction of soil organic matter in order to predict the potential long-term efficacy of soils to remove and sequester atmospheric CO_2 (Sollins *et al.* 2007). Finally, our results suggest that key combinations of grass and legume species may improve ecosystem services such as soil C accumulation and possibly biofuel production at low N-inputs in both high- and low-diverse grassland systems.

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