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Soil Processes Affected by Sixteen Grassland Species Grown under Different Environmental Conditions

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ABSTRACT

Plant species, and their interactions with the environment, determine both the quantity and chemistry of organic matter inputs to soils. Indeed, countless studies have linked the quality of organic matter inputs to litter decomposition rates. However, few studies have examined how variation in the quantity and chemistry of plant inputs, caused by either interspecific differences or changing environmental conditions, influences the dynamics of soil organic matter. We studied the effects of 16 grassland species from 4 functional groups (C3 and C4 grasses, forbs, and legumes) growing under ambient and elevated CO_2 (560 ppm) and N inputs (4 g m^{-2} yr^{-1}) on soil carbon (C) and nitrogen (N) dynamics after 4 yr in a grassland monoculture experiment in Minnesota, USA. Specifically, we related soil C and N dynamics to variation among species and their responses to the CO_2 and N treatments in plant biomass and chemistry of roots, the dominant detrital input in the system. The 16 species caused much larger variation in plant litter inputs and chemistry, as well as soil C and N dynamics, than the CO_2 and N treatment. Not surprising, variation in the quantity of plant inputs to soils contributed to up to a two-fold variation in microbial biomass and amount of respired nonlabile soil C. Root N concentration (across species and CO_2 and N treatments) was significantly negatively related to decomposition of nonlabile soil C and positively related to net N mineralization. Greater labile C inputs decreased rates of net N mineralization, likely because of greater N immobilization. Thus, of the traits examined, plant productivity, tissue N concentration, and labile C production such as from rhizodeposition were most important in causing variation in soil C and N dynamics among species and in response to altered atmospheric CO_2 and N supply.

Increased atmospheric CO_2 concentration and N deposition are potentially altering the structure and functioning of many terrestrial ecosystems (Vitousek, 1994). These global change factors influence the amount and chemistry of primary productivity as well as cause shifts in the relative abundances of species and functional groups that themselves differ from one another in their productivity and chemical composition (Reich et al., 2001a).

The amount (i.e., dry mass) and chemistry of plant litter inputs have long been known to affect fresh litter decomposition and nutrient release (e.g., Hobbie, 1992; Mack and D'Antonio, 2003; Melillo et al., 1982). Differences among and changes in plant communities have been shown to affect soil organic matter pools and dynamics through interspecific differences in litter quantity and chemistry (Eviner and Chapin, 2004; Finzi et al., 1998; Knicker et al., 2000), but also through interspecific differences in rhizodeposition of labile C compounds (Cheng et al., 2003; Fu and Cheng, 2002; Reid and Goss, 1982). Similarly, interspecific differences in litter quantity and chemistry and labile C production through rhizodeposition play an important role in soil N dynamics (Eviner and Chapin, 2004; Wedin and Tilman, 1990), but their relative importance remains unclear.

It is also unclear how important species-specific changes in litter quantity, chemistry and labile C production caused by elevated atmospheric CO_2 and N supply are on soil organic matter and N dynamics, compared with plant species or community effects. Although elevated atmospheric CO_2 and N supply can alter litter quantity, chemistry, and labile C production (e.g., Cheng and Johnson, 1998; Hobbie and Vitousek, 2000), their effect on soil organic matter and N dynamics may be limited compared with plant community effects (Aerts et al., 2003; Finzi and Schlesinger, 2002). Nonetheless, litter quantity and chemistry (including both nutrient and C chemistry) and labile C production may serve to integrate influences of species trait differences (both within and among functional groups) as well as trait responses to atmospheric CO_2 and N supply on soil organic matter dynamics.

The aim of this study was to assess how soil microbial biomass, soil organic matter, and N dynamics are affected by 16 grassland species grown under ambient and elevated atmospheric CO_2 (560 ppm) with 0 or 4 g m^{-2} yr^{-1} of N fertilizer. We also examined the degree to which the quantity and chemistry of litter inputs and labile C production influence soil C and N dynamics under the different treatments. In a companion paper, we have reported the main effects of CO_2, N, plant species richness, and their interactions on microbial biomass and activity (Dijkstra et al., 2005). Whereas the previous paper compared the monoculture plots to more species-rich plots to determine how increasing species richness (along with elevated CO_2 and N) affects soil processes, here we focus on comparisons among the different monocultures themselves (i.e., among different species) to determine the relationship between plant traits and aspects of soil C and N dynamics such as C and N mineralization and microbial C and N, under ambient and elevated CO_2, with and without N fertilization.

MATERIALS AND METHODS

Study Site

This research is part of the BioCON (Biodiversity, CO_2, and N) experiment (Reich et al., 2001a; Reich et al., 2001b)

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Abbreviations: ANOVA, analysis of variance; BioCON, Biodiversity, CO_2, and N; FACE, free-air CO_2 enrichment.

established in an old field in the Cedar Creek Natural History Area (CCNHA), Minnesota, USA (45°24′ N, 93°12′ W). Soils (Argic Udipsamments) in this nearly level area are very homogenous, sandy (93% sand, 3% silt, and 4% clay) and poor in soil organic matter and N content (on average 0.6% total C and 0.06% total N). Mean annual precipitation is 660 mm with mean monthly temperatures of −11°C in January and 22°C in July. Vegetation (secondary successional grassland) was removed from six circular rings (diam. 20 m) in 1997 and planted with perennial grassland species in 2 by 2 m plots. For our study, we used 128 monoculture plots of 16 different species, distributed nearly equally among the 6 rings. The 16 species that were used (all native or naturalized to the CCNHA) are the C_4 grasses *Andropogon gerardii* Vitman, *Bouteloua gracilis*, *Schizachyrium scoparium* (Michaux) Nash, and *Sorghastrum nutans* (L.) Nash; the C_3 grasses *Agropyron repens* (L.) Beauv., *Bromus inermis* Leysser, *Koeleria cristata* Pers, and *Poa pratensis* L.; the forbs *Achillea millefolium* L., *Anemone cylindrica* A. Gray, *Asclepias tuberosa* L., and *Solidago rigida* L.; and the legumes *Amorpha canescens* Pursh, *Lespedeza capitata* Michaux, *Lupinus perennis* L., and *Petalostemum villosum* Nutt. Three rings received elevated atmospheric CO_2 concentrations (560 ppm, a concentration expected to occur globally by 2050) during four growing seasons (1998–2001), using the free-air CO_2 enrichment (FACE) system. Half of the plots in each treatment were fertilized with 4 g N m^{-2} yr^{-1} (comparable with N deposition rates in industrialized areas) as NH_4NO_3, applied in three equal doses during the growing season. The experimental treatments of species (16 species), CO_2 (two levels), and N (two levels) were arranged in a complete factorial design with two replicates ($16 \times 2 \times 2 \times 2$). Plots were regularly weeded to remove all species other than assigned, watered only in the first year. Plots were also burned two out of every 3 yr, which is a common management practice at CCNHA. For a detailed description of the experimental design see Reich et al. (2001b).

Sampling and Analyses

We used total plant biomass (above- and belowground) sampled in June and August of 1998, 1999, and 2000 (see Reich et al., 2001b) to test for plant biomass effects on soil C and N dynamics (see below). Aboveground biomass was clipped in 10 by 100 cm strips just above the soil surface, all matter was collected, dried and weighed. Roots were sampled at 0- to 20-cm depth using three cores (diam. 5 cm) in the area used for the aboveground biomass clipping. Roots were washed, dried, and weighed. In this herbaceous system aboveground biomass is a good estimate of aboveground productivity (and litter inputs); because we do not have good estimates of root productivity, we used root standing stocks of root biomass along with aboveground biomass as an estimate of total plant inputs (Zak et al., 2003).

We analyzed roots sampled in August 1999 for chemical characteristics that we related to soil C and N dynamics. We focused on roots because they represent roughly three-fourths of plant biomass in these grassland ecosystems (Reich et al., 2001a) and are a critical source of litter inputs (especially given that residual aboveground litter is burned two out of every 3 yr). We analyzed roots for N on an Element Analyzer (ECS 4010, Costech Analytical Technologies, Valencia, CA) and cell soluble compounds, hemicellulose, cellulose, and lignin and related recalcitrant materials on an Ankom Fiber Analyzer (Ankom Tech., Fairport, NY) according to Van Soest (1994).

In June 2001, we sampled three soil cores (diam. 2.5 cm) at 0- to 20-cm depth from each of the 128 plots. We sieved soils (2 mm) and removed most of the roots. Subsamples were taken for measurements of gravimetric water content (105°C, 48 h), soil C respiration, microbial biomass, and net N mineralization.

We incubated soils for 74 d under optimum moisture conditions (70% of field capacity) at room temperature (22°C) to measure soil C respiration. By keeping temperature and moisture constant, we were able to separate the influence of plants on soil C respiration (and on net N mineralization, below) via litter quantity and chemistry from their effects on microclimate. Although plants can have large effects on microclimate in the field, potentially altering microbial processes, our objective in this study was to isolate litter quantity and chemistry effects. We measured soil C respiration from the incubated soils on Day 2, 6, 11, 20, 41, and 74 after field sampling by taking headspace samples from soil samples incubated in mason jars, and analyzing the CO_2 on a gas chromatograph (Shimadzu GC14A, see Dijkstra et al., 2005). Daily soil C respiration rates dropped rapidly and became almost stable after 74 d of incubation. We assumed that this pattern reflected a depletion of labile C early during the incubation and a constant soil C respiration rate of more recalcitrant or nonlabile C throughout the incubation (Townsend et al., 1997).

We separated soil C respiration of fast versus slow cycling C by fitting a two-order model through the cumulative soil C respiration over time (Bonde and Rosswall, 1987; Wedin and Pastor, 1993) for each sample:

$$C_t = C_l(1 - e^{-kt}) + ct \quad [1]$$

where C_t is the cumulative amount of C respired at time t, C_l and k are the fast or labile C pool size and its respiration rate constant respectively, and c is the constant nonlabile soil C respiration rate. Because of its short-lived nature, we assumed the labile C pool to be directly coming from recent plant inputs (e.g., labile C compounds in litter and root exudates). Because the curve fitting did not always converge, we improved the curve fitting by estimating c separately by fitting the daily soil C respiration rate measurements with the derivative of Eq. [1]:

$$R_t = ae^{-kt} + c \quad [2]$$

where R_t is the daily soil C respiration rate at time t, and a the daily soil C respiration rate at time 0 (equal to $C_l \times k$). All curve-fitting was performed with Sigmaplot (version 5.0; Systat Software Inc., Richmond, CA). The random pattern of residual plots indicated that the models used were adequately describing the data.

We measured microbial biomass C and N using the fumigation-extraction method (Brookes et al., 1985). Extracts (0.5 *M* K_2SO_4) from nonfumigated and fumigated soil samples were analyzed for total dissolved C and N on a total organic C analyzer with a N-measuring unit attached (Shimadzu TOC-V_{CPN}, Shimadzu Scientific Instruments, Columbia, MD). Microbial C and N were calculated as the difference in C and N between the extracts from the nonfumigated and fumigated soil samples, divided by 0.45 for C (Beck et al., 1997) and by 0.54 for N (Brookes et al., 1985).

Net N mineralization was measured during a 10-d period in soil laboratory incubations by extracting soil subsamples (moistened to 70% field capacity) at the beginning and end of incubation with 1 *M* KCl and analyzing extracts for NO_3^- and NH_4^+ on an Alpkem auto-analyzer (see Dijkstra et al., 2005). Net N mineralization during a short-term incubation period such as ours has shown to be a good indicator for how plant litter inputs affect net N mineralization rates in the field at Cedar Creek (Wedin and Pastor, 1993).

We used ANOVA to test for main effects of CO_2 (ambient or elevated), N (0 or 4 g m^{-2} yr^{-1}), and either species identity (among 16 species) or functional group identity (C_3 grasses, C_4

Table 1. Mean values ± standard error for total plant biomass, root chemistry, labile C, its respiration rate constant (k), nonlabile soil C respiration rate (c), net N mineralization and microbial biomass averaged by CO_2 (pooled across N, $n = 96$) and N treatment (pooled across CO_2, $n = 96$) both averaged across species with statistical probabilities (P-values) for CO_2 and N treatment effects from analysis of variance (ANOVA, $CO_2 \times N \times$ Species identity model). Species identity treatment effects are shown in Tables 2 and 3. There were no significant ($P < 0.05$) interactions between the CO_2, N, and species identity treatments, except for total plant biomass ($CO_2 \times$ species: $P = 0.05$, N × species: $P = 0.05$) and root N concentration (N × species: $P = 0.003$).

Parameter	Ambient CO_2	Elevated CO_2	P-value	Unfertilized	Fertilized	P-value
Tot. plant biomass, g m^{-2}	664 ± 37	751 ± 39	0.02	673 ± 35	742 ± 41	0.005
Root N, %	1.19 ± 0.07	1.15 ± 0.07	0.59	1.15 ± 0.08	1.19 ± 0.06	0.36
Root cell soluble, %	34.4 ± 1.6	33.9 ± 1.5	0.74	34.7 ± 1.6	33.6 ± 1.5	0.33
Root hemicellulose, %	24.7 ± 0.9	25.3 ± 0.9	0.44	25.0 ± 1.0	25.1 ± 0.9	0.87
Root cellulose, %	25.3 ± 0.6	25.8 ± 0.6	0.54	24.8 ± 0.5	26.2 ± 0.6	0.004
Root lignin, %	15.2 ± 0.5	14.6 ± 0.4	0.68	15.0 ± 0.5	14.7 ± 0.5	0.52
Root lignin/N	14.8 ± 0.9	14.7 ± 0.7	0.94	15.6 ± 0.8	13.8 ± 0.8	0.02
Labile C, mg kg^{-1}	90 ± 5	105 ± 6	0.08	111 ± 9	85 ± 8	0.01
K, d^{-1}	0.047 ± 0.002	0.048 ± 0.002	0.50	0.043 ± 0.002	0.052 ± 0.003	0.001
c, mg kg^{-1} d^{-1}	4.67 ± 0.16	5.03 ± 0.19	0.34	4.63 ± 0.15	5.08 ± 0.20	0.02
Net N min., mg kg^{-1} d^{-1}	0.17 ± 0.02	0.15 ± 0.02	0.34	0.13 ± 0.02	0.19 ± 0.02	0.02
Microbial C, mg kg^{-1}	116 ± 4	135 ± 4	0.15	130 ± 4	120 ± 5	0.07
Microbial N, mg kg^{-1}	14.1 ± 0.6	16.9 ± 0.7	0.16	16.9 ± 0.6	14.1 ± 0.7	0.0002

grasses, forbs, and legumes), and their interactions. Here we focus on species identity, functional group identity, and their interactions with the CO_2 and N treatment, while main effects of CO_2, N, and plant diversity and all interactions among these are reported elsewhere (Dijkstra et al., 2005). The effect of the CO_2 treatment was tested against the random effect of ring nested within the CO_2 treatment. All treatment effects were considered as fixed factors. We used simple and multiple regressions to relate total plant biomass, root tissue chemistry, and labile C (determined from incubations) to microbial biomass and net N mineralization, and to relate total plant biomass and root tissue chemistry to c. We could not test for the effect of labile C on c because they were not estimated independently. For multiple regressions all independent variables were standardized to unit-free variables with a mean equal to zero and a variance equal to one. All statistical analyses were done with JMP (version 4.0.4, SAS Institute, Cary, NC).

RESULTS

Elevated CO_2 and N fertilization effects on total plant biomass, root chemistry, labile C, k, c, microbial C and N, and net N mineralization averaged across all 16 species grown as monocultures are summarized in Table 1. Briefly, elevated CO_2 significantly increased total plant biomass (13%), and N fertilization significantly increased total plant biomass (10%), root cellulose concentration (6%), k (21%), c (10%), and net N mineralization (46%), and significantly decreased root lignin/N ratio (13%), labile C (31%), and microbial N (20%).

Total plant biomass and root chemistry showed large and significant differences among species (Table 2) giving rise to a large potential to alter soil C and N dynamics (see below). Differences in total plant biomass among species after 4 yr of treatment were similar to those reported for the first 2 yr of the experiment (Reich et al., 2001a). The C3 and C4 grasses were the functional groups with the greatest average biomass, with two C3 grasses, *A. repens* and *P. pratensis*, having the greatest and *A. tuberosa* (a forb) and *A. canescens* (a legume) having the smallest biomass of all the species. However, we found only weak evidence for significant differences

Table 2. Mean values ± standard error for total plant biomass and root chemistry of the 16 different species averaged over the CO_2 and N treatment ($n = 8$) with statistical probabilities (P-values) for species and functional group identity effects from analysis of variance (ANOVA, $CO_2 \times N \times$ Species identity and $CO_2 \times N \times$ Functional group identity models respectively). CO_2 and N treatment effects are shown in Table 1.

Functional group	Plant species	Tot. plant biomass	N	Cell soluble	Hemicellulose	Cellulose	Lignin	Lignin/N
		g m^{-2}	%					
C4 grasses	*A. gerardii*	857 ± 37	0.81 ± 0.09	26.4 ± 1.6	30.7 ± 0.8	27.0 ± 0.9	15.7 ± 0.5	20.8 ± 2.1
	B. gracilis	672 ± 37	1.06 ± 0.08	20.8 ± 0.9	34.5 ± 0.9	26.9 ± 0.7	17.5 ± 0.9	16.8 ± 0.7
	S. scoparium	632 ± 35	0.99 ± 0.09	24.5 ± 1.4	32.1 ± 1.0	29.1 ± 0.4	14.0 ± 0.5	21.0 ± 1.6
	S. nutans	734 ± 43	0.78 ± 0.03	22.9 ± 0.6	32.4 ± 0.4	29.1 ± 1.0	15.5 ± 0.8	19.8 ± 0.9
	Mean	724 ± 24	0.83 ± 0.04	23.6 ± 0.7	32.4 ± 0.5	28.0 ± 0.4	15.7 ± 0.4	19.6 ± 0.8
C3 grasses	*A. repens*	1147 ± 72	0.76 ± 0.05	45.3 ± 0.7	23.9 ± 0.4	21.7 ± 0.5	8.9 ± 0.4	11.8 ± 0.6
	B. inermis	960 ± 29	0.87 ± 0.04	26.9 ± 1.7	30.3 ± 0.5	28.9 ± 0.9	13.7 ± 0.8	15.6 ± 0.6
	K. cristata	796 ± 55	0.95 ± 0.03	22.2 ± 0.7	33.0 ± 0.5	28.6 ± 0.9	16.0 ± 0.8	17.0 ± 1.0
	P. pratensis	1054 ± 74	0.87 ± 0.05	25.4 ± 0.7	30.9 ± 1.1	25.5 ± 0.9	18.0 ± 2.3	21.7 ± 4.0
	Mean	989 ± 37	0.86 ± 0.02	30.0 ± 1.7	29.5 ± 0.7	26.2 ± 0.6	14.1 ± 0.9	16.5 ± 1.2
Forbs	*A. millefolium*	979 ± 76	0.86 ± 0.06	56.3 ± 1.6	12.5 ± 0.4	18.1 ± 0.6	12.9 ± 1.0	15.1 ± 0.5
	A. cylindrica	361 ± 49	1.33 ± 0.11	41.3 ± 4.2	19.9 ± 1.4	23.3 ± 2.0	14.9 ± 1.5	11.6 ± 1.4
	A. tuberosa	230 ± 28	1.37 ± 0.14	37.5 ± 4.2	22.1 ± 1.6	25.5 ± 1.3	13.7 ± 1.9	10.4 ± 2.0
	S. rigida	963 ± 59	0.98 ± 0.09	52.6 ± 2.8	13.6 ± 0.9	18.5 ± 0.9	14.9 ± 1.2	15.6 ± 1.1
	Mean	633 ± 67	1.13 ± 0.06	47.2 ± 2.1	16.9 ± 0.9	21.2 ± 0.8	14.1 ± 0.7	13.3 ± 0.7
Legumes	*A. canescens*	317 ± 41	1.63 ± 0.13	30.1 ± 1.6	22.6 ± 0.6	29.6 ± 1.1	17.2 ± 1.7	11.0 ± 1.3
	L. capitata	544 ± 90	2.03 ± 0.08	37.1 ± 1.2	23.7 ± 0.9	25.4 ± 0.8	13.4 ± 0.9	6.7 ± 0.6
	L. perennis	625 ± 42	2.33 ± 0.20	43.6 ± 3.1	15.3 ± 1.1	26.4 ± 1.9	14.3 ± 1.0	6.1 ± 0.7
	P. villosum	447 ± 62	1.41 ± 0.05	35.4 ± 3.2	21.6 ± 0.9	24.8 ± 1.2	17.1 ± 1.5	12.2 ± 1.1
	Mean	483 ± 36	1.85 ± 0.09	36.3 ± 1.4	21.0 ± 0.7	26.6 ± 0.7	15.6 ± 0.7	9.1 ± 0.7
P-value Species id.		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0004	<0.0001
P-value Funct. gr. id.		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.21	<0.0001

Table 3. Mean values ± standard error for labile C, its respiration rate constant (*k*), nonlabile soil C respiration rate (*c*), and microbial C and N of the 16 different species averaged over the CO_2 and N treatment ($n = 8$) with statistical probabilities (*P*-values) for species and functional group identity effects from analysis of variance (ANOVA, $CO_2 \times N \times$ Species identity and $CO_2 \times N \times$ Functional group identity models respectively). CO_2 and N treatment effects are shown in Table 1.

Functional group	Plant species	Labile C	*k*	*c*	Microbial C	Microbial N
		mg kg^{-1}	d^{-1}	mg kg^{-1} d^{-1}	mg kg^{-1}	
C4 grasses	*A. gerardii*	91 ± 19	0.050 ± 0.004	5.2 ± 0.4	111 ± 13	13.5 ± 1.6
	B. gracilis	92 ± 12	0.058 ± 0.007	5.4 ± 0.3	103 ± 7	9.6 ± 1.2
	S. scoparium	91 ± 16	0.048 ± 0.006	4.9 ± 0.3	131 ± 9	17.8 ± 1.6
	S. nutans	135 ± 31	0.046 ± 0.007	5.5 ± 0.4	138 ± 9	17.1 ± 1.4
	Mean	102 ± 10	0.050 ± 0.003	5.3 ± 0.2	121 ± 5	14.5 ± 0.9
C3 grasses	*A. repens*	110 ± 12	0.066 ± 0.008	6.2 ± 0.5	153 ± 10	19.3 ± 1.2
	B. inermis	76 ± 13	0.046 ± 0.007	5.8 ± 0.4	135 ± 12	20.0 ± 2.5
	K. cristata	111 ± 23	0.058 ± 0.006	5.2 ± 0.4	128 ± 11	16.8 ± 2.1
	P. pratensis	96 ± 16	0.058 ± 0.004	4.9 ± 0.3	115 ± 10	12.1 ± 1.4
	Mean	98 ± 8	0.057 ± 0.003	5.5 ± 0.2	133 ± 6	17.1 ± 1.0
Forbs	*A. millefolium*	246 ± 44	0.033 ± 0.006	6.3 ± 0.8	155 ± 12	16.0 ± 1.5
	A. cylindrica	50 ± 10	0.042 ± 0.004	3.8 ± 0.3	107 ± 13	12.5 ± 1.7
	A. tuberosa	89 ± 44	0.034 ± 0.008	3.0 ± 0.5	105 ± 9	14.1 ± 1.2
	S. rigida	124 ± 30	0.043 ± 0.004	5.7 ± 0.3	137 ± 13	16.1 ± 1.6
	Mean	131 ± 22	0.038 ± 0.003	4.7 ± 0.3	126 ± 7	14.7 ± 0.8
Legumes	*A. canescens*	46 ± 8	0.036 ± 0.005	3.3 ± 0.2	106 ± 9	12.6 ± 0.6
	L. capitata	40 ± 6	0.056 ± 0.008	4.5 ± 0.2	110 ± 11	13.1 ± 0.8
	L. perennis	113 ± 25	0.044 ± 0.003	4.4 ± 0.2	160 ± 12	22.7 ± 2.0
	P. villosum	48 ± 7	0.042 ± 0.004	3.6 ± 0.3	111 ± 18	14.8 ± 1.7
	Mean	62 ± 8	0.044 ± 0.003	4.0 ± 0.1	122 ± 7	15.8 ± 1.0
P-value Species id.		<0.0001	0.0003	<0.0001	0.0005	<0.0001
P-value Funct. gr. id.		0.01	0.0002	0.0002	0.45	0.11

among plant species in terms of total plant biomass and root chemistry responses to elevated CO_2 or N fertilization. That is, we only found significant interactions between species identity and the CO_2 and N treatments for total plant biomass ($CO_2 \times$ species: $P = 0.05$, N $\times$ species: $P = 0.05$) and root N concentration (N $\times$ species: $P = 0.003$). Most of the C3 plants increased in plant biomass with elevated CO_2, but not the C4 grasses *S. scoparium* and *S. nutans*, while plant biomass and root N concentration of the four legumes did not respond to N addition as much as the other species. Plant species identity interactions with elevated CO_2 and N fertilization in terms of plant biomass and root N concentration are discussed in detail by Reich et al. (2001a).

We observed large differences in labile C, *k*, *c*, and microbial C and N among soils associated with different plant species and functional groups (Table 3). Labile C, *c*, and microbial C were highest and *k* lowest in soils of the forb *A. millefolium* while microbial N was highest in soils of the N-fixing *L. perennis*. Soils of the C4 grasses as a group had the highest labile C, soils of the C3 grasses had the highest *k* and *c* values, soils of the forbs had the lowest *k* value, and soils of the legumes had the lowest labile C and *c* values. We found no CO_2 or N treatment interactions with species or functional groups for labile C, *k*, *c*, and microbial C and N.

Net N mineralization differed significantly among plant species and functional groups (Fig. 1). Net N mineralization was on average 40 times higher for *L. perennis* than for *A. millefolium*. The C4 grasses had on average the lowest and the legumes the highest net N mineralization. As with soil C respiration parameters

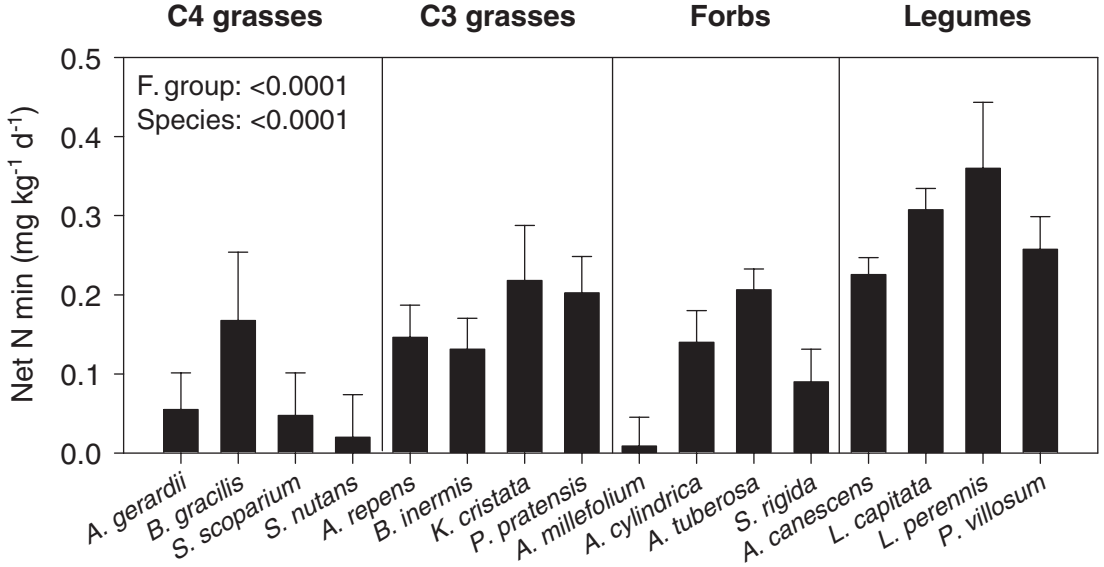

Fig. 1. Average net N mineralization rate separated by species (pooled across CO_2 and N treatment). Error bars show standard error ($n = 8$).

Table 4. Summary of multiple regressions for nonlabile soil C respiration rate (*c*), microbial C and N, and net N mineralization using all 128 plots. *P*-values are shown for each independent variable and adjusted R^2 values for the whole regression.

Independent variable	*c*	Microbial C	Microbial N	Net N min.
Tot. plant biomass	<0.0001	<0.0001	<0.0001	0.07
Root N conc.	0.08	0.63	0.63	<0.0001
Root cell soluble conc.	0.27	0.31	0.34	0.18
Root hemicellulose conc.	0.34	0.19	0.23	0.91
Root cellulose conc.	0.17	0.39	0.45	0.73
Root lignin conc.	0.32	0.09	0.09	0.96
Labile C	–†	0.02	0.03	0.05
R^2_{adj}	0.46	0.30	0.18	0.33

† Not included in multiple regression.

and microbial biomass, we found no CO_2 or N treatment interactions with species or functional groups for net N mineralization.

Between 18 and 46% of the variation in *c*, microbial C and N, and net N mineralization among species could be explained by interspecific differences in total plant biomass, root chemistry, and labile C inputs (Table 4). Total plant biomass had the largest effect on *c* and microbial C and N. Simple regressions between total plant biomass and *c* and microbial C and N all showed significant positive relationships (*c*: $P < 0.0001$, $R^2 = 0.45$; microbial C: $P < 0.0001$, $R^2 = 0.13$; microbial N: $P = 0.01$, $R^2 = 0.05$, Fig. 2).

The multiple regression analyses showed that labile C had a significant positive effect on microbial C and N, and a negative effect on net N mineralization (Table 4). Simple regressions between microbial biomass (C and N) and labile C showed several outliers from *A. millefolium* plots. When we excluded *A. millefolium* plots from the simple regression analyses the relationships were highly significant ($P < 0.0001$, $R^2 = 0.24$ for microbial C and $P < 0.0001$, $R^2 = 0.26$ for microbial N). Similarly, the relationship between net N mineralization and labile C showed outliers from *L. perennis* plots. Removing *L. perennis* plots from the simple regression analysis with net N mineralization showed a significant negative relationship with labile C ($P < 0.0001$, $R^2 = 0.19$).

Root N concentration was highly significantly positively related to net N mineralization (Table 4, Fig. 2). Although only marginally significant in the multiple regression analysis, root N concentration was significantly negatively related to *c* ($P < 0.0001$, $R^2 = 0.13$, Fig. 2) in a simple regression.

Root hemicellulose, root cellulose, and root lignin concentration could not explain any of the variation in *c*, microbial C and N, or net N mineralization among species in the multiple regressions. Excluding these variables from the multiple regressions did not substantially alter the effects of the other variables (not shown).

DISCUSSION

The 16 species caused much larger variation in plant litter inputs and chemistry, as well as soil C and N dynamics, than the CO_2 and N treatment in our study. Interspecific variation in plant litter inputs (plant productivity) and chemistry (root tissue N concentration) grown under different atmospheric CO_2 and N supply explained to a large degree the variation in soil C and N dynamics in this grassland system. Our results further show that plant species grown under different atmospheric CO_2 and N supply can also affect microbial biomass and net N mineralization through production of labile C, likely from rhizodeposition. Despite significant CO_2 or N treatment interactions with plant species for total plant biomass and root N concentration, there were no significant treatment interactions for microbial biomass, C and N mineralization. This suggest that in this system 4 yr of elevated CO_2 and N supply does not favor growth of certain plant species over others through feedback mechanisms between plant productivity and soil organic matter dynamics under monoculture. Studies addressing interactive effects of plant species, atmospheric CO_2 and N supply, on soil C and N dynamics are scarce, and much of our understanding of how microbial biomass and activity are affected by different plant species under changing atmospheric CO_2 and N supply remain incoherent (Bardgett et al., 1999; Zak et al., 2000). Our results indicate that the integrated effects of plant species and their responses to the CO_2 and N treatment on plant productivity, tissue N concentration, and labile C production, can to a large degree explain variation in soil C and N dynamics in this system. It remains to be seen if elevated CO_2 and N deposition show species-specific effects on microbial activity and soil organic matter dynamics in the long-term, and how this will affect plant community and soil organic matter dynamics in more diverse systems.

The substantial divergence in soil microbial biomass (up to twofold) and nonlabile soil C respiration (up to twofold) among the 16 species could in large part be explained by interspecific variation in total plant biomass and presumably the associated variation in plant C inputs to soils, but not by interspecific variation in root C chemistry such as cell solubles and lignin concentration. The positive relationship between nonlabile soil C respiration and total plant biomass indicates that a significant part of the nonlabile soil C respiration stemmed from soil C formed during the experiment. Greater nonlabile soil C respiration with greater total plant biomass may therefore have stemmed from greater litter inputs since the experiment started, which possibly may also have stimulated decomposition of pre-experiment soil C (Broadbent, 1947; Bingeman et al., 1953). Other studies have also shown that the quantity of plant C inputs can have a large influence on microbial biomass C and soil C respiration (Bardgett et al., 1999; Zak et al., 2003; Zak et al., 1994). Our results suggest that decomposition of soil organic matter (nonlabile soil C respiration) is sensitive to variation in the quantity of organic matter inputs, but that differences among species in root C chemistry largely disappear as microbes process plant litter. However, these results are in contrast with a study where we observed a significant negative effect of root lignin concentration on decomposition of old C (older than 5 yr) in elevated CO_2 and N fertilized plots, estimated from C isotope analyses (Dijkstra et al., 2004). Our 74-d soil laboratory incubations may not have been sensitive enough to detect significant long-

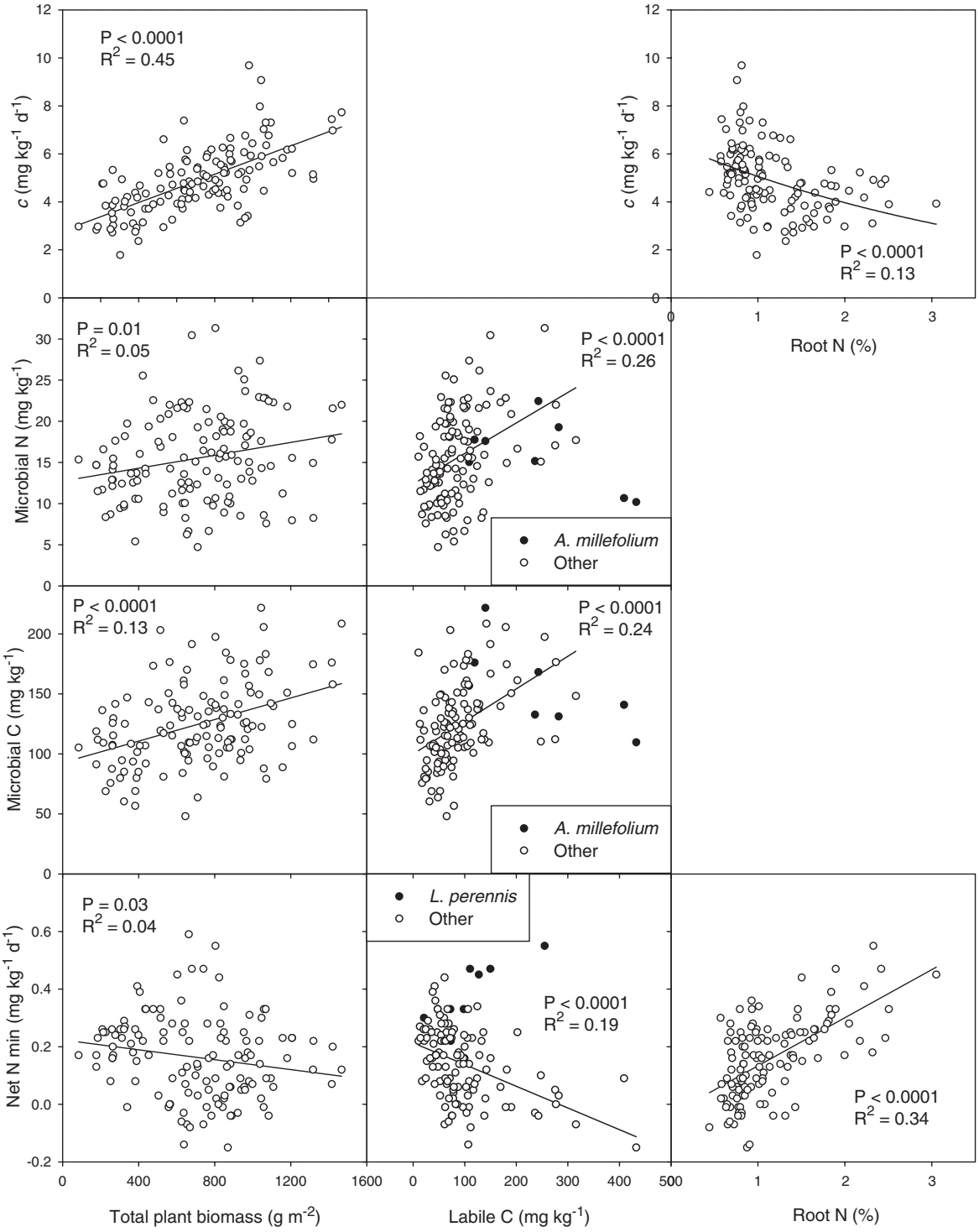

Fig. 2. Nonlabile soil C respiration (*c*), microbial C and N, and net N mineralization rate plotted against total plant biomass, labile C, and root N concentration, with regression lines. For the relationship between labile C and microbial C and N *A. millefolium* plots were excluded in the regression analyses, while for the relationship between labile C and net N mineralization *L. perennis* plots were excluded.

term effects of root C chemistry on decomposition of non-labile soil C, also because we assumed that non-labile soil C respiration rates were constant (Eq. [1]) after 74 d. Further, under field conditions living plant roots may also have affected non-labile soil C decomposition through rhizosphere processes (e.g., Cheng et al., 2003; Kuzyakov et al., 2001), which were excluded in our laboratory incubations.

In contrast to root C chemistry, we observed a significant root N concentration effect on nonlabile soil C decomposition. The significant negative relationship between root N concentration and nonlabile soil C respira-

tion suggests that increased N concentration in roots (e.g., in forbs and especially legumes) slowed down the decomposition of more recalcitrant soil organic matter. While others have reported increased stabilization of litter at later stages of decomposition for Norway spruce litters with initially higher N concentrations (Berg and Matzner, 1997; Matzner, 2002), to our knowledge, our results are the first showing that variation in root N concentration among grassland species under different atmospheric CO_2 and N supply can cause similar negative effects on soil organic matter decomposition.

Microbial biomass was positively related to labile C inputs (across species, CO_2 and N treatment). Much of the labile C was likely from rhizodeposition (e.g., exudates, mucilages, and lysates) rather than solely from leaching and decomposition of dead root cell soluble contents because root cell soluble concentration alone was not significantly related to microbial biomass. Since soil microbes are usually C limited (Michelsen et al., 1999; Smith and Paul, 1990; Van de Geijn and van Veen, 1993), plant C inputs, especially in the form of labile C such as root exudates, play an important role in supporting microbial biomass and activity (Kuzyakov, 2002), that could further obscure possible C litter chemistry effects on microbial biomass and nonlabile soil C decomposition. One plant species, the forb *A. millefolium*, showed very high labile C pools that did not correspond to similar increases in microbial biomass C and N, suggesting that this species produced labile C compounds that did not stimulate microbial growth. *Achillea millefolium* plants are rich in essential oils (terpenes) that in fact have been shown to reduce microbial activity (Candan et al., 2003; Fiori et al., 2000).

While total plant biomass (of all traits examined) showed the greatest effect on nonlabile soil C decomposition, labile C and root N concentration explained more of the variation in N dynamics. Low rates of net N mineralization were associated with large amounts of labile C, likely because stimulation of microbial growth caused greater microbial N immobilization (Jonasson et al., 1996; Schmidt et al., 1997), reducing N availability for plant growth (Hu et al., 2001). An exception was the legume *L. perennis*, which showed high net N mineralization rates despite high amounts of labile C, likely because of its high root N concentrations. Indeed, root N concentrations, and not root lignin or lignin/N ratio, explained most of the variation in net N mineralization rate. Legume species (and also forbs and C3 grasses) with the highest root N concentration also exhibited high net N mineralization rates. In a study with fewer species that overlapped those studied here, net N mineralization rates also appeared to be best related to plant litter N concentrations (Wedin and Tilman, 1990). Across a range of forest sites, litter lignin/N ratios showed a better relationship with net N mineralization rates than N alone (Scott and Binkley, 1997). As most of the forest sites had higher lignin/N ratios than our grassland species, the effect of lignin on net N mineralization may be more pronounced when relatively more lignin is produced in litter.

Our results suggest that variation in soil organic matter decomposition across species in our grassland system under different atmospheric CO_2 and N supply is more affected by variation in the amount of litter inputs (positive effect) and in root N concentration (negative effect), than by root C chemistry. In contrast, net N mineralization was not as sensitive to variation in the amount of litter inputs but highly sensitive to variation in the chemistry of plant inputs, increasing with root N concentration. Our results also indicate that, besides detrital quantity and chemistry, variation in labile C production, likely from rhizodeposition, can significantly affect C and N dynamics, increasing microbial biomass and reducing net N mineralization. We conclude that the large interspecific variability in plant productivity, tissue N concentration, and labile C production such as from rhizodeposition, and their alteration by changing atmospheric CO_2 and N supply can cause large variation in soil C and N dynamics in a grassland ecosystem.

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