Divergent effects of elevated CO_2, N fertilization, and plant diversity on soil C and N dynamics in a grassland field experiment

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Abstract

While increased atmospheric CO_2 concentrations, increased N deposition, and changes in plant diversity have all been shown to significantly alter soil carbon (C) and nitrogen (N) dynamics, the effects of these factors have never been studied simultaneously and in combination. We studied the response of soil C and N dynamics to changes in atmospheric CO_2 (ambient, 560 ppm), N fertilization (0, 4 g N m^{-2} yr^{-1}), plant species number (1, 4 species), and plant functional group number (1, 4 groups; all with 4 species) in a grassland field experiment in Minnesota, USA. During the fourth season of treatments, we used laboratory incubations to assess soil C pool sizes and dynamics and net N mineralization, and determined microbial C and N and total soil C and N. Elevated CO_2 increased labile C and microbial biomass, but had no effect on net N mineralization, respiration of more recalcitrant C, or total soil C and N. Nitrogen fertilization increased net N mineralization, because of faster decomposition or less immobilization by litter with higher N concentrations. In the four species plots, N fertilization also increased total soil C and N, likely because greater litter production more than offset any increases in decomposition. Increasing the species number from one to four increased C respiration that could largely be attributed to greater soil C inputs from increased biomass accumulation, but reduced net N mineralization, likely because of greater immobilization in the more productive four-species plots. An increase in functional group number did not affect any of the soil parameters measured. While elevated CO_2, N fertilization, and increased species number all increased plant biomass accumulation, they had divergent effects on soil C and N dynamics.

Introduction

Human induced changes in atmospheric CO_2 concentrations, nitrogen (N) deposition, and biodiversity can have dramatic impacts on ecosystem functioning (Vitousek, 1994; Sala et al., 2000).

Elevated atmospheric CO_2 (DeLucia et al., 1999; Hungate et al., 1997; Niklaus et al., 2001), N addition (Vitousek and Howarth, 1991), and increased plant diversity (Hector et al., 1999; Tilman et al., 1997) all increase net primary productivity and biomass accumulation. Net primary productivity in turn constrains decomposition and microbial biomass because micro-organisms strongly depend on plant substrates to meet their energy requirements (Myrold et al., 1989;

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Schimel, 1986; Zak et al., 1994) and are further influenced by variation in the chemistry (i.e., quality) of those substrates (Eviner and Chapin, 2004). Variation in the amount and chemistry of plant litter inputs to soils caused by elevated CO_2, N fertilization, and increased plant diversity will therefore likely have different impacts on soil microbial activity and carbon (C) and N dynamics, perhaps contributing to uncertainty that remains about these effects and their interactions.

Elevated CO_2 concentrations often lead to increased inputs of C into soil, mainly through increased detrital production and root exudation (Cheng and Johnson, 1998; Hungate et al., 1997; Ineson et al., 1996; Pregitzer et al., 2000), potentially stimulating soil organic matter decomposition and microbial biomass. While some studies have suggested that elevated CO_2 increases lignin concentrations in litter that may reduce decomposition per gram litter and increase soil C sequestration (Cotrufo et al., 1994; Van Ginkel et al., 1996), little evidence for this mechanism exists (Cotrufo et al., 1998; Norby et al., 2001). More recent studies have shown that most of the additional C released into the soil in response to elevated CO_2 is labile and decomposes quickly, and therefore does not alter C storage significantly (Hungate et al., 1997; Norby et al., 2002; Schlesinger and Lichter 2001; Tate and Ross 1997; Van Kessel et al., 2000). Increased labile C inputs into the soil in response to elevated CO_2 could in turn stimulate microbial activity and increase soil N availability (Martín Olmedo et al., 2002; Zak et al., 1993) or decrease soil N availability via greater microbial N immobilization (Díaz et al., 1993; Gill et al., 2002).

The influence of N fertilization on soil microbial activity and C and N dynamics is similarly unclear. While total and mass-specific litter decomposition may increase with N fertilization because of increased plant litter inputs and increased N concentrations in plant litter (e.g. Melillo et al., 1982), additions of inorganic N can inhibit decomposition of soil organic matter where inorganic N is incorporated into recalcitrant compounds that are formed during lignin degradation (Berg and Matzner, 1997; Fog, 1988; Nömmik and Vahtras, 1982).

The effects of plant diversity on microbial activity and C and N dynamics remain controversial. Increased plant diversity has been shown to increase soil respiration and microbial biomass because of increased net primary productivity (and therefore greater C inputs, Craine and Wedin, 2002; Zak et al., 2003), but has also been shown to have no effect on soil respiration and microbial biomass (Wardle et al., 1999). Higher plant diversity could decrease soil N availability for plants because of increased N immobilization into accruing litter (Knops et al., 2001). Alternatively, higher diversity could reduce N losses through leaching (Tilman et al., 1996, 1997) or increase inputs through N fixation because of increased probability of the presence of N–fixing plants (Spehn et al., 2002; Zanetti et al., 1997), thereby increasing N availability.

Our study is part of a long-term grassland field experiment in Minnesota (Reich et al., 2001a, b) where interactive effects of plant diversity, elevated CO_2, and N fertilization on ecosystem functioning are examined in a factorial way. Here we report effects of atmospheric CO_2 concentration (ambient or 560 ppm), N fertilization (0 or 4 g m^{-2} yr^{-1}), species number (1 or 4 species), and functional group number (1 or 4 groups) on microbial activity and organic matter pools in laboratory-incubated soils. The treatments were designed specifically to allow the separation of species number and functional group number effects (see below), since these have been shown to be both independent and important (Reich et al., 2004). Effects of plant species and functional group identity and their interactions with the CO_2 and N treatment are reported elsewhere (Dijkstra et al., submitted). Here we determine if elevated CO_2, N fertilization, and increased plant species/functional group number have similar or disparate effects on soil organic matter decomposition, microbial biomass, net N mineralization, and soil organic matter pools. We use laboratory incubations because they enable us (1) to determine treatment effects on the size and dynamics of multiple soil organic matter pools and (2) to isolate treatment effects that arise from changes in the nature of organic matter inputs to soils from those that might arise from changes in the physical environment. Because we expected that differences in biomass accumulation due to the CO_2, N and diversity treatment would influence microbial activity, we assessed whether total plant biomass could explain treatment effects on microbial activities,

and whether other factors, such as substrate quality, influenced responses even once biomass differences were accounted for statistically.

Materials and methods

Study site

The experimental site is located on a sandy glacial outwash plain in the Cedar Creek Natural History Area, Minnesota, USA. Mean annual precipitation is 660 mm with mean monthly temperatures of $-11\ °C$ in January and $22\ °C$ in July. The vegetation is a mosaic of tall-grass prairie, wetland, oak savanna, hardwood forest and abandoned agricultural fields. Soils are sandy and extremely poor in organic matter content, and N deficiency limits plant growth (Tilman et al., 1996).

Site preparation of the BioCON (biodiversity, CO_2, and N) experiment (Reich et al., 2001a, b) started in 1997 in an abandoned agricultural field. From six circular areas (rings, diam. 28 m), previous vegetation (secondary successional grassland) was removed. The soil was uniformly tilled and fumigated with methyl bromide to eliminate the soil seed bank. Soil was reinoculated with microbes with an extract from the soil surrounding the plots, and in 2000 arbuscular mycorrhizal fungal communities (Wolf et al., 2003) and soil respiration (unpublished results) recovered to levels that were as high or higher than other old fields at Cedar Creek. In 20 m diameter inner circles, 61–62 2×2 m plots were established per ring where herbaceous perennial grassland species were planted (12 g m^{-2} of seed) from a pool of 16 species, equally divided over four functional groups (C$_3$ grasses, C$_4$ grasses, forbs, and legumes). Our study included 196 plots: 128 monoculture plots of each of the 16 species (8 replicates), and 68 plots planted with four species, either of one functional group (12 replicates of each functional group) or of all four functional groups (20 replicates). Not all possible combinations of the four species plots with four functional groups were planted, and therefore species in these plots were chosen at random. The subset of plots chosen for study here (out of 371 plots) allowed us to study contrasts between plots with one and four species, and between plots with one and four functional groups within the four species plots.

All plots in our study were distributed nearly equally among the six rings. Three rings (with a total of 98 plots) received elevated atmospheric CO_2 concentrations (560 ± 30 ppm) using the free-air CO_2 enrichment (FACE) system (Lewin et al., 1994), while the ambient atmospheric CO_2 concentration gradually increased from 366 ppm in 1998 to 372 ppm in 2001. Plots were treated with CO_2 only when plants were photosynthetically active during four growing seasons (April–November, 1998–2001). Half of the plots in each treatment were fertilized with 4 g N m^{-2} yr^{-1} (as NH$_4$NO$_3$), applied in three doses during the growing season (in May, June, and July). The experiment for our study was set up in a complete factorial design. The 16 species that were used for this experiment 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. Plots were regularly weeded to remove all species other than assigned, watered only in the first year, and burned in early spring of 2000.

Sampling

In June and August of 1998, 1999, and 2000 we sampled total plant biomass (Reich et al., 2001b). Aboveground biomass was clipped in 10×100 cm strips just above the soil surface, all matter was collected, dried and weighed. Roots were sampled at 0–20 cm depth using three cores (diam. 5 cm) in the area used for the aboveground biomass clipping. Roots were washed, dried and weighed.

In June 2001 we sampled soils at 0–20 cm depth by taking four soil cores (diam. 2.5 cm) from each of the 196 plots (both root and soil samples were taken to represent the fairly homogenous plots well while avoiding too much disturbance in the plots). Composited soils from each plot were immediately sieved (2 mm) and

visible roots that went through the sieve were picked out by hand. We took sub-samples for analyses of C respiration, net N mineralization, microbial C and N, total C and N, and gravimetric water content.

Analyses

Total biomass (in $g\,m^{-2}$) was calculated as the sum of the aboveground and root dry matter from the destructive harvests. We used the average total biomass as a proxy for biomass accumulation. In this herbaceous system, aboveground biomass is a good estimate of aboveground litter inputs; because we do not have good estimates of root productivity, we use root standing stocks along with aboveground biomass as an estimate of total plant inputs (Zak et al., 2003).

To quantify soil C pools and their dynamics, we placed 35 g of moist soil into 120-mL polyethylene specimen cups and added de-ionized water to bring soils to 70% field capacity. Specimen cups were then placed into one-quart widemouth mason jars and covered with polyethylene film to allow CO_2 and O_2 exchange, but to prevent soils from drying out. Soils were incubated in the dark at room temperature for 74 days. Periodically we added de-ionized water to maintain constant water content throughout the incubation. Although temperature and soil moisture in the field fluctuated, we kept soils under optimum and constant temperature to separate treatment effects that might arise from changes in the nature of organic matter inputs to soils from those that might arise from changes in the physical environment. On days 2, 6, 11, 20, 41, and 74 after field sampling we measured the CO_2 production in the incubated soils. At each date, we removed the polyethylene film and replaced it with a mason jar lid fitted with a septum. Immediately after closing off the mason jar, we sampled the headspace gas with a syringe. After 24 h a final air sample was drawn from the jar. Jars were then covered with polyethylene film again until the next measurement date. Headspace samples were immediately analyzed for CO_2 on a gas chromatograph (Shimadzu GC14A, Shimadzu Scientific Instruments, Wood Dale IL) using a thermal conductivity detector and a Poropak N column.

Daily C respiration rates were calculated based on the difference in CO_2 concentration measured in the initial and final sample. Early during incubation, daily C respiration rates dropped rapidly and became almost stable after 74 days. We assumed that this pattern reflected a depletion of labile C early during the incubation and a constant C respiration rate of more recalcitrant C throughout the incubation (Townsend et al., 1997). In all plots all labile C was respired well before the end of the incubation. We calculated cumulative C respiration for each sample by multiplying the average daily C respiration rate between two measuring dates by the time interval between two measuring dates, and by adding the preceding C respiration. To estimate the pool size and respiration rate constant for the labile C pool we fit a simplified two-order model through cumulative C respiration data obtained from each individual sample (Bonde and Rosswall, 1987; Wedin and Pastor, 1993):

$$C_t = C_l(1 - e^{-kt}) + ct, \qquad (1)$$

where C_t is the cumulative amount of C respired at time t, C_l and k are the pool size and C respiration rate constant for the labile pool, and c is the constant C respiration rate of a more recalcitrant C pool. We refer to c as the respiration rate of the more recalcitrant C pool to indicate that the turnover of this pool is slower than that of the labile C pool. Because the curve fitting did not always converge, we improved the curve fitting by estimating c separately by fitting the daily C respiration rate measurements with the derivative of Equation (1):

$$R_t = ae^{-kt} + c, \qquad (2)$$

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

We measured potential net N mineralization during a 10-day period by placing 60 g of moist soil into separate specimen cups. Soils were brought to 70% field capacity, covered with a polyethylene film, and incubated in the dark at room temperature for 73 days. Water content was kept constant throughout the incubation. On

day 1 and 10 after field sampling we took a 10-g sub-sample from the incubated soil and extracted it with 25 mL of 1 M KCl solution. Filtered extracts were frozen until analyses for NO_3^- and NH_4^+ on an Alpkem auto-analyzer. We calculated net N mineralization as the sum of NO_3^--N and NH_4^+-N concentrations extracted at day 10 minus the concentrations extracted on day 1. Net N mineralization during a short-term incubation period such as ours has been shown to be a good indicator of how plant litter inputs affect net N mineralization rates in the field at Cedar Creek (Wedin and Pastor, 1993).

We measured microbial biomass C and N using the fumigation–extraction method (Brookes et al., 1985). We placed 12 g of moist soil into a specimen cup and another 12 g of moist soil into a 30-mL Pyrex glass beaker. The glass beakers were then put into a vacuum dessicator and were fumigated with chloroform for 5 days, as Davidson et al. (1989) showed that peak N-flush in grassland soils was achieved after 5–7 days of fumigation. We then transferred the fumigated soil samples into specimen cups. We added 50 mL of 0.5 M K_2SO_4 to both the fumigated and non-fumigated samples. Samples were gently shaken for 1 h and filtered through pre-leached (with 0.5 M K_2SO_4) Whatman No. 1 filter paper. Extracts were frozen until analyses for total C and N on a total organic C analyzer with an N measuring unit attached (Shimadzu TOC-V$_{CPN}$). Microbial C and N were calculated as the difference between C and N in the fumigated and non-fumigated samples divided by 0.45 for C (Beck et al., 1997) and by 0.54 for N (Brookes et al., 1985). These constants were not obtained from our soils and therefore caution should be taken when comparing our microbial C and N data with those from other sites. However, correcting our microbial C and N data with these values should not affect comparisons among our different treatments.

Soil sub-samples were dried at 65 °C, ground with a coffee mill, and analyzed for total soil C and N with a Costech 4050 Element Analyzer. Gravimetric water content was analyzed on a 10 g sub-sample by oven drying (105 °C, 48 h).

For all plots ($n = 196$) we used ANOVA to test for main effects of CO_2 (ambient or elevated), N (0 or 4 g m^{-2} yr^{-1}), and species number (1 or 4 species), and their interactions. Two secondary tests allowed separation of species and functional group number effects. First, for plots with only one functional group ($n = 176$), we used ANOVA to test for main effects of CO_2, N, and species number (1 versus 4) and their interactions. Second, in the four species plots ($n = 68$) we used ANOVA to test for main effects of CO_2, N, and functional group number (1 or 4 groups), and their interactions. The effect of CO_2 was tested against the random effect of ring nested within CO_2. Differences among the three rings within the ambient and elevated CO_2 treatment were sometimes significant and constrained the statistical significance of the CO_2 treatment. We also used total plant biomass (average of six harvests during 1998–2000) as a covariate in ANCOVAs to account for differences in biomass accumulation caused by the CO_2, N, and diversity treatment. All treatment effects were considered as fixed factors. All statistical analyses were done with JMP (version 4.0.4).

Results

For most parameters that we measured we found significant effects of CO_2, N, and species number. Only in a few occasions did we find significant interactions between CO_2 and N and between N and species number, and therefore we mostly

Table 1. Mean values ± standard error for total biomass (above- and belowground) based on two harvests per year in 1998–2000, averaged by CO_2, N and species number for all 196 plots, and averaged by functional group number within the 68 four species plots

Treatment	Total biomass (g m^{-2})	P-value
Ambient CO_2 ($n = 98$)	759 ± 37	0.05
Elevated CO_2 ($n = 98$)	843 ± 39	
Unfertilized ($n = 98$)	745 ± 34	0.02
Fertilized (n = 98)	857 ± 42	
1 Species ($n = 128$)	681 ± 32	< 0.0001
4 Species ($n = 68$)	1027 ± 38	
1 Functional group ($n = 48$)	956 ± 45	0.003
4 Functional group ($n = 20$)	1198 ± 51	

Main treatment effects with statistical probabilities from analysis of variance (ANOVA) are shown. No significant ($P < 0.05$) interactions were found.

present results separately for each treatment, pooled across the other treatments.

The average total biomass from six harvests was significantly higher (ANOVA, $P < 0.05$) with elevated CO_2, N fertilization and increased species number for the subset of 196 plots that we chose for this study (Table 1). Within our

selected four species plots, total biomass was also significantly higher ($P < 0.05$) with four functional groups than with one functional group.

Elevated CO_2 significantly increased the size of the labile C pool in all plots by 32% on average, but had no effect on its decay rate (k) or on the constant respiration rate of the more
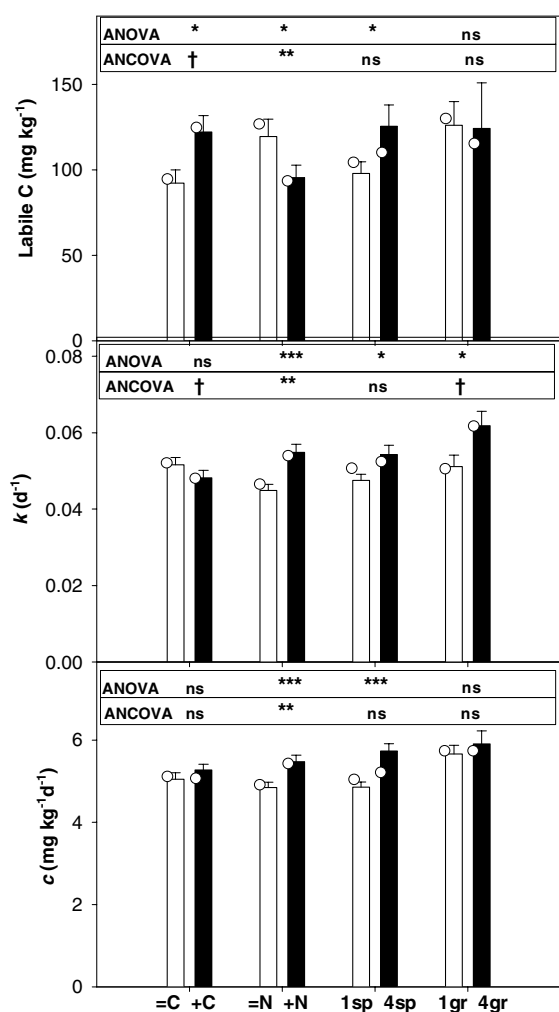

Figure 1. Labile C, its respiration rate constant (k), and the respiration rate of the more recalcitrant soil C (c) affected by CO_2 (=C: ambient CO_2, +C: elevated CO_2), N fertilization (=N: unfertilized, +N: fertilized), species and functional group number. The CO_2, N and species number effects were pooled across all other treatments ($N = 196$), while the functional group number effect was pooled across the CO_2 and N treatment in the four species plots only ($N = 68$). Error bars are standard errors. †$P < 0.1$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (shown for both ANOVA and ANCOVA with total plant biomass as covariate). No significant ($P < 0.05$) interactions were found. Least-square means adjusted for variation in total plant biomass (ANCOVA) are indicated with open circles.
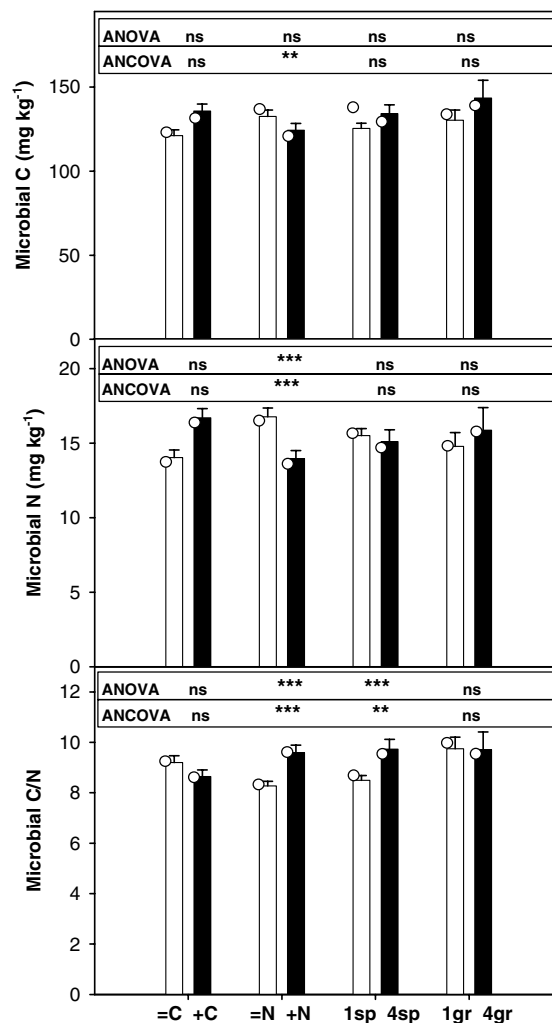
Figure 2. Microbial C, N, and C/N ratio affected by CO_2 (=C: ambient CO_2, +C: elevated CO_2), N fertilization (=N: unfertilized, +N: fertilized), species and functional group number. The CO_2, N and species number effects were pooled across all other treatments ($N = 196$), while the functional group number effect was pooled across the CO_2 and N treatment in the four species plots only ($N = 68$). Error bars are standard errors. **$P < 0.01$; ***$P < 0.001$ (shown for both ANOVA and ANCOVA with total plant biomass as covariate). $CO_2 \times N$ interactions for microbial C and N are mentioned in the text. No other significant ($P < 0.05$) interactions were found. Least-square means adjusted for variation in total plant biomass (ANCOVA) are indicated with open circles.

Table 2. Mean values ± standard error for total soil C and N averaged by CO_2 (pooled across N) and N treatment (pooled across CO_2) for monoculture and four species plots

	Soil		Soil (N%)	
Monoculture plots ($n = 128$)				
Ambient CO_2 ($n = 64$)	0.62 ± 0.02		0.058 ± 0.002	
Elevated CO_2 ($n = 64$)	0.57 ± 0.01		0.054 ± 0.001	
Unfertilized CO_2 ($n = 64$)	0.60 ± 0.02		0.056 ± 0.001	
Fertilized CO_2 ($n = 64$)	0.59 ± 0.02		0.056 ± 0.001	
Four species plots ($n = 68$)				
Ambient CO_2 ($n = 32$)	0.66 ± 0.03		0.061 ± 0.002	
Elevated CO_2 ($n = 32$)	0.60 ± 0.02		0.055 ± 0.002	
Unfertilized ($n = 32$)	0.60 ± 0.02		0.056 ± 0.002	
Fertilized ($n = 32$)	0.65 ± 0.03		0.060 ± 0.002	
	ANOVA	ANCOVA	ANOVA	ANCOVA
CO_2	0.65	0.60	0.63	0.59
N fertilization (N)	0.03	0.14	0.04	0.14
Species number (sp #)	0.09	0.81	0.19	0.85
N × sp#	0.03	0.04	0.19	0.16
Total plant biomass (covariate)		< 0.0001		0.0005

Statistical probabilities for main treatment effects and N × sp. # interaction terms from analysis of variance (ANOVA) and covariance (ANCOVA with total plant biomass as covariate) are shown. All other interactions were not significant ($P < 0.05$).

recalcitrant soil C (c, Figure 1). The increase in labile C was partly the result of increased biomass accumulation ($P < 0.0001$, ANCOVA), but the size of the labile C pool tended to be larger ($P = 0.08$) in the elevated CO_2 plots even after accounting for biomass accumulation. Despite the increase in labile C, elevated CO_2 did not significantly alter microbial biomass (C, N, and C/N) or net N mineralization (Figures 2 and 3). However, we found significant $CO_2 × N$ interactions in the ANOVA and ANCOVA for microbial C ($P = 0.007$ and 0.003 respectively) and N ($P = 0.05$ and 0.04 respectively). Both microbial C and N increased (by 21 and 25% respectively) in the elevated CO_2 plots, but only when they were not fertilized. Elevated CO_2 had no effect on total soil C and N (Table 2). However relatively large differences among the three rings within each CO_2 treatment made it difficult to detect significant CO_2 effects. For instance, average total soil C ranged between 0.46 (± 0.02 se, $n = 31$) and 0.78% (± 0.02 se, $n = 34$) among the three rings within the ambient CO_2 treatment, and between 0.49 (± 0.01 se, $n = 31$) and 0.65% (± 0.01 se, $n = 34$) among the three rings within the elevated CO_2 treatment.

In contrast to CO_2, N fertilization significantly decreased the size of the labile C pool (by 20%) and significantly increased k and c (by 22
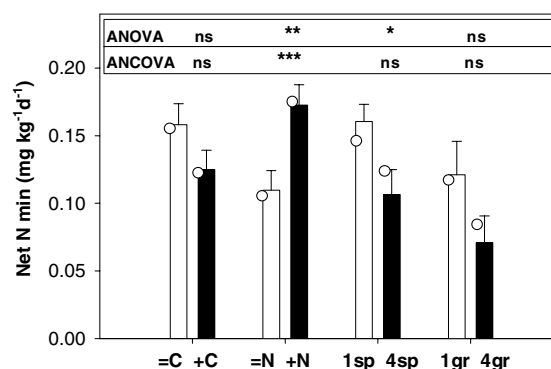

Figure 3. Net N mineralization affected by CO_2 (=C: ambient CO_2, +C: elevated CO_2), N fertilization (=N: unfertilized, +N: fertilized), species and functional group number. The CO_2, N and species number effects were pooled across all other treatments ($N = 196$), while the functional group number effect was pooled across the CO_2 and N treatment in the four species plots only ($N = 68$). Error bars are standard errors. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (shown for both ANOVA and ANCOVA with total plant biomass as covariate). No significant ($P < 0.05$) interactions were found. Least-square means adjusted for variation in total plant biomass (ANCOVA) are indicated with open circles.

and 14% respectively, Figure 1). Although biomass accumulation was positively related to k (P = 0.0005) and c (P < 0.0001) in the ANCOVA, differences in k and c remained significant after controlling for increased biomass accumulation with N fertilization. Microbial C and N decreased by 6 and 17% (significant for microbial N) with N fertilization, and became more significant after biomass accumulation was taken into account (Figure 2). Surprisingly, microbial N decreased more than microbial C, which caused a significant increase in microbial C/N (16%) with N fertilization. As anticipated, net N mineralization increased significantly (by 57%) with N fertilization (Figure 3). Accounting for biomass accumulation increased the significance of N fertilization on net N mineralization, because biomass accumulation was higher in the N-fertilized treatment, but had a negative effect on net N mineralization (P = 0.01, ANCOVA). Among all 196 plots total soil C and N increased slightly but significantly with N fertilization (both by 3%), apparently largely due to increased biomass accumulation, since this effect disappeared when biomass accumulation was taken into account (Table 2). Total soil C increased particularly in the four species plots with N fertilization (significant species by N interaction). Interestingly, this interaction between N fertilization and species number remained significant after accounting for biomass accumulation. We found similar interactions for total soil N, although they were not significant.

Increasing the species number from one to four significantly increased labile C (28%), k (14%), and c (18%). Comparisons of species number effects when four species plots were constrained to one functional group ($n = 176$) were in all cases similar to those for all plots ($n = 196$), except for k, where the significant increase disappeared. Increases in labile C and c could be largely ascribed to increased biomass accumulation with increased species number, since none of the species number effects remained significant in the ANCOVA (Figure 1). Increased species number had no effect on microbial C and N, but significantly increased microbial C/N ratio (15%), even after controlling for biomass accumulation (Figure 2).

Net N mineralization significantly decreased (34%) with increased species number, but this effect disappeared when biomass accumulation was taken into account (Figure 3), likely because of the negative effect of biomass accumulation on net N mineralization. Increased species number only marginally increased total soil C that could be ascribed to increased biomass accumulation (Table 2). In contrast to our expectations, increasing the number of functional groups from one to four while keeping species number constant at four had no significant effects on any of the parameters that we measured (e.g. Figures 1–3).

Discussion

After 4 years of treatment, we observed significant and divergent effects of elevated CO_2, N fertilization, and plant diversity on soil organic matter decomposition, microbial biomass, net N mineralization, and soil organic matter pools. In most cases, responses to increased plant diversity (i.e., species number) could largely be attributed to greater C inputs associated with increased biomass accumulation. Responses to the CO_2 and N treatment were often still significant after accounting for biomass accumulation and often showed opposite effects.

Elevated CO_2 increased labile C pools in soils, likely because of increased above- and belowground plant productivity (Reich et al., 2001a, b), but perhaps also because of increased root exudation with elevated CO_2 (Cheng and Johnson, 1998; Hungate et al., 1997). Because decomposition of more recalcitrant soil C (i.e., c) and net N mineralization were not affected by elevated CO_2, our results do not support the idea that an increase in labile C pools in the soil with elevated CO_2 will increase decomposition of more recalcitrant soil organic matter and net N mineralization (Martín-Olmedo et al., 2002; Zak et al., 1993), or that elevated CO_2 will increase microbial demand for N, increasing N immobilization and thereby reducing net N mineralization (Díaz et al., 1993). Instead, increased plant productivity with elevated CO_2 mainly resulted in increased production and decomposition of labile C without causing a significant change in total soil C, similar to what others have found (Hungate et al., 1997; Schlesinger and Lichter, 2001; Tate and Ross, 1997).

Despite increased biomass accumulation, labile C and microbial C and N pools decreased

with N fertilization. While an increase of the decay rate constant of the labile C (k) can explain the reduced labile C pool, N fertilization may also have reduced root exudation supporting significantly lower microbial C and N pools. It has also been suggested that N addition in the form of nitrate inhibits the activity of β-glucosidase in the soil, which causes reduced microbial C acquisition (DeForest et al., 2004). Our results contrast other studies that found significant increases in microbial N with N addition and no effect with elevated CO_2 (Niklaus and Körner, 1996; Zak et al., 2000).

The significant increase in respiration of more recalcitrant C (c) with N fertilization, even after accounting for biomass accumulation effects, contrasts results where we observed slower decomposition of old C estimated from C isotope analyses in N fertilized plots treated with elevated CO_2 (Dijkstra et al., in press), as well as other studies that found reduced decomposition of more recalcitrant soil C with N fertilization (Berg and Matzner, 1997; Neff et al., 2002). However, the strong positive relationship between c and total plant biomass ($P < 0.0001$, ANCOVA) that we found here does suggest that the respired CO_2 associated with c predominantly came from recent plant litter inputs, rather than from older soil organic matter. Therefore, increased c values with N fertilization after accounting for biomass accumulation may have been caused by increased substrate quality of the incoming litter. The small but significant increase in total soil C and N suggests however, that biomass accumulation increased more than soil organic matter decomposition with N fertilization. Consistent with this interpretation, increased total soil C and N with N fertilization were largest in the four species plots where in general biomass accumulation was highest.

The significant increased net N mineralization in the soil with N fertilization was likely a result of increased plant litter N concentrations. Above- and belowground plant tissue N concentrations were significantly higher in the N fertilized plots (Reich et al., 2001a, b). Biomass accumulation had a negative effect on net N mineralization ($P = 0.02$, ANCOVA), likely because of increased N immobilization with increased productivity (i.e. increased C inputs). However, at any given level of biomass accumulation, net N mineralization rates were higher in N-fertilized plots, because higher substrate N concentrations with N fertilization increased decomposition (and gross N mineralization) rates and/or reduced N immobilization.

In contrast to the CO_2 and the N treatment, significant changes in soil C and N dynamics caused by increased species number could largely be attributed to increased biomass accumulation. The increase in respiration of the more recalcitrant soil C with increased species number may have been of similar magnitude as the increase in biomass accumulation causing no or only marginal increases in total soil C after 4 years of treatment. Observing significant changes in total soil C may take longer than the duration of our experiment and it is unclear if in the long term an increase in species or functional group number will result in greater soil C storage.

Increased biomass accumulation with increased species number may have transferred N from soil to accruing litter reducing net N mineralization. A higher likelihood of occurrence of N-fixing legumes in the four species plots (Spehn et al., 2002; Zanetti et al., 1997) or potentially greater ecosystem N retention (Tilman et al., 1996, 1997) did not significantly increase total soil N or net N mineralization. Others found increased net N mineralization with increased plant diversity and it was suggested that the altered microbial community composition with plant diversity was responsible for the greater rates of N mineralization (Zak et al., 2003). The increased microbial C/N ratio with increased species diversity suggests that species diversity may also have changed the microbial community composition in our study, but apparently not in such a way to cause greater net N mineralization rates.

Plant species richness effects on labile C, decomposition of the more recalcitrant soil C, and net N mineralization were unrelated to functional group diversity. We observed species richness effects in plots containing only one single functional group, but increasing the functional group diversity from one to four groups, while keeping species richness constant at four, had no significant effect, despite a significant increased biomass accumulation with increased functional group diversity. However, the increase in biomass accumulation was less pronounced with increased functional group diversity than with species rich-

ness, perhaps explaining the lack of functional group diversity effects on decomposition and net N mineralization.

Conclusions

Elevated CO_2, N fertilization, and increased plant diversity all increased plant productivity but these treatments differed significantly in their effects on C and N dynamics in the soil. Increased biomass accumulation with elevated CO_2 mostly affected soil organic matter pools with fast turnover rates (labile C, microbial biomass), but had no significant effect on total soil C and N pools, or the decomposition of the more recalcitrant C. Nitrogen fertilization and increased plant diversity increased both biomass accumulation and decomposition, but apparently litter input rates increased more than decomposition rates, especially when these two treatments were combined, thereby increasing total soil C and N. Nitrogen fertilization increased net N mineralization, possibly because of reduced N immobilization or increased decomposition of litter with higher N concentration. Increased species number decreased net N mineralization, likely because greater C inputs increased N immobilization. Although elevated CO_2, N fertilization, and increased plant diversity all increase biomass accumulation, they have divergent effects on decomposition of rapid- and slow-cycling soil organic matter pools, microbial biomass, and net N mineralization rates that are potentially important in altering long-term soil C and N storage.

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