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Effects of plant species diversity, atmospheric [CO_2], and N addition on gross rates of inorganic N release from soil organic matter

JASON B. WEST*, SARAH E. HOBBIE* and PETER B. REICH†
*Department of Ecology, Evolution and Behavior, University of Minnesota, St. Paul, MN 55108, USA,
†Department of Forest Resources, University of Minnesota, St. Paul, MN 55108, USA

Abstract

A significant challenge in predicting terrestrial ecosystem response to global changes comes from the relatively poor understanding of the processes that control pools and fluxes of plant nutrients in soil. In addition, individual global changes are often studied in isolation, despite the potential for interactive effects among them on ecosystem processes. We studied the response of gross N mineralization and microbial respiration after 6 years of application of three global change factors in a grassland field experiment in central Minnesota (the BioCON experiment). BioCON is a factorial manipulation of plant species diversity (1, 4, 9 and 16 prairie species), atmospheric [CO_2] (ambient and elevated: 560 $\mu mol\, mol^{-1}$), and N inputs (ambient and ambient $+4\, g\, N\, m^{-2}\, yr^{-1}$). We hypothesized that gross N mineralization would increase with increasing levels of all factors because of stimulated plant productivity and thus greater organic inputs to soils. However, we also hypothesized that N addition would enhance, while elevated [CO_2] and greater diversity would temper, gross N mineralization responses because of increased and reduced plant tissue N concentrations, respectively. In partial support of our hypothesis, gross N mineralization increased with greater diversity and N addition, but not with elevated [CO_2]. The ratio of gross N mineralization to microbial respiration (i.e. the 'yield' of inorganic N mineralized per unit C respired) declined with greater diversity and [CO_2] suggesting increasing limitation of microbial processes by N relative to C in these treatments. Based on these results, we conclude that the plant supply of organic matter primarily controls gross N mineralization and microbial respiration, but that the concentration of N in organic matter input secondarily influences these processes. Thus, in systems where N limits plant productivity these global change factors could cause different long-term ecosystem trajectories because of divergent effects on soil N and C cycling.

Key words: atmospheric carbon dioxide, carbon mineralization, diversity, global change, gross nitrogen mineralization, microbial respiration, nitrogen deposition, nitrogen limitation, soil element cycling, soil organic matter

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Introduction

Human-induced changes to the global environment have important consequences for terrestrial ecosystem function (Chapin *et al*., 1997; Hungate *et al*., 1997a, b; Vitousek *et al*., 1997; Norby, 1998; Sala *et al*., 2000; Balmford & Bond, 2005). Some of these changes include altering plant species compositions and diversity, increasing atmospheric [CO_2], and increasing global fluxes of reactive nitrogen (N; Keeling *et al*., 1995; Vitousek *et al*., 1997; Loreau *et al*., 2001). Although certain aspects of terrestrial response to these changes are fairly well described (e.g. aboveground productivity, Hooper *et al*., 2005), belowground responses remain poorly understood (Hu *et al*., 1999; Loiseau & Soussana, 2000; Norby & Jackson, 2000; Ollinger *et al*., 2002; Shaw

Correspondence: Jason B. West, Department of Biology, University of Utah, Salt Lake City, UT 84112, USA, tel. +1 801 587 3404, fax: +1 801 581 4665, e-mail: jwest@biology.utah.edu

et al., 2002; Körner, 2003; Luo *et al*., 2004). In particular, because plants depend on the activities of soil microorganisms for much of their mineral nutrition, and since nutrient limitation of plant productivity may modulate responses to global environmental changes (Field *et al*., 1992; Rastetter *et al*., 1997), an understanding of global change effects on microbially mediated nutrient cycling is critical. This is especially true for N cycling, given the important role of N limitation to plants in many ecosystems worldwide (Vitousek & Howarth, 1991), and the central role microorganisms play in releasing plant-available inorganic N from unavailable N in soil organic matter.

Net N mineralization rates are often characterized in global change studies as an important metric of the response of N cycling to global change (Lee & Caporn, 1998; Zak *et al*., 2000, 2003a; Kirkman *et al*., 2001; Niklaus *et al*., 2001; Reich *et al*., 2001; Fenn *et al*., 2005; Hartley & Mitchell, 2005). However, net rates are the result of simultaneous gross fluxes (i.e. NH_4^+ mineralization from soil organic matter and NH_4^+ immobilization and nitrification by microbes). As such, quantifying net N mineralization alone cannot identify the mechanisms responsible for the observed changes to N cycling, limiting our understanding of the responses of plant N supply to global change (Hart *et al*., 1994a; Finzi & Schlesinger, 2003; Booth *et al*., 2005).

Although important to formulating mechanistic understandings of N cycle changes, gross rates of N mineralization are seldom quantified in global change studies and, when they are, they are typically examined for single global change factors in isolation. Zak *et al*. (2000) reviewed the responses of soil microorganisms to elevated [CO_2], and of the 10 studies that reported gross N mineralization rates, none of them observed statistically significant effects, with the overall coefficient of variation at 800%, well above the variation observed in other microbial responses (e.g. biomass or microbial respiration). In that same review, 95% of the 20 studies that reported microbial respiration reported a stimulatory response to elevated [CO_2], although in only three of those studies were the responses statistically significant. A more recent review and reanalysis also found that elevated [CO_2] had no effect on gross N fluxes in soils (Booth *et al*., 2005).

Surprisingly, Booth *et al*. (2005) also found no significant effect of N fertilization across studies on gross N fluxes (with the exception of gross nitrification). They suggested that direct application of inorganic N may not stimulate gross N mineralization, but that treatments that resulted in organic matter with higher N concentration would.

Because loss of diversity from terrestrial ecosystems is another important ongoing global change, the effects of plant species diversity on productivity and other ecosystem functions have been intensively studied over the past 10 years or so (Hooper *et al*., 2000, 2005; Loreau *et al*., 2001; Tilman *et al*., 2001; Catovsky *et al*., 2002; Gastine *et al*., 2003; Hedlund *et al*., 2003; Porazinska *et al*., 2003; Zak *et al*., 2003b; De Deyn *et al*., 2004; Wardle *et al*., 2004; Spehn *et al*., 2005), but again only rarely were gross N fluxes quantified in these studies. Zak *et al*. (2003b) reported a significant stimulation of gross N mineralization in response to increasing plant species diversity that was only partially explained by increased plant productivity. Microbial biomass and respiration were also stimulated by increasing supplies of organic matter from plants as diversity increased in that study.

Natural systems are experiencing numerous global changes simultaneously, rather than in isolation as they are often studied. Thus, in addition to the significant challenges that remain in attempts to understand microbial responses to these individual global changes, much more work is needed to understand whether the effects of these changes on microbial activity significantly interact. We studied the response of gross N mineralization rates after 6 years of simultaneous manipulation of three major global change factors in a relatively natural field setting in east-central Minnesota (Reich *et al*., 2001, 2004). In this experiment, plant species biodiversity (1, 4, 9, and 16 species), atmospheric [CO_2] (ambient and 560 $\mu mol\,mol^{-1}$), and N addition (ambient and ambient $+4\,g\,N\,m^{-2}\,yr^{-1}$) were varied in a full-factorial combination (BioCON). Previous reports have shown that all three factors stimulated plant productivity individually in this experiment, and diversity increased the productivity response to elevated atmospheric [CO_2] and added N (Reich *et al*., 2001, 2004). Although initial results did not indicate an N limitation to elevated [CO_2] growth stimulation (Reich *et al*., 2001), by 4–6 years of treatment, elevated atmospheric [CO_2] stimulated productivity more in elevated than in ambient N plots (Reich *et al*., 2006), consistent with the development of progressive N limitation over time (Luo *et al*., 2004). After 6 years, *in situ* net N mineralization rates decreased in response to elevated [CO_2] under ambient N (from 0.08 to 0.06 $mg\,N\,kg\,soil^{-1}\,day^{-1}$), but were stimulated by [CO_2] under enriched N (from 0.05 to 0.10 $mg\,N\,kg\,soil^{-1}\,day^{-1}$; Reich *et al*., 2006). The treatments have also significantly altered the characteristics of soil organic matter (Dijkstra *et al*., 2004, 2005). For example, elevated [CO_2] has increased labile C and microbial biomass, but did not significantly change total soil C or N, at least for the 1 and 4 species treatments (Dijkstra *et al*., 2005). Dijkstra *et al*. (2005) also attributed increased total soil C and N pools in response to N fertilization to proportionally higher increases in

litter production relative to decomposition. Increasing diversity from 1 to 4 species also increased microbial respiration but decreased laboratory rates of net N mineralization, an effect hypothesized to be the result of a proportional increase in immobilization (Dijkstra *et al*., 2005). For the present study, we predicted that plant species diversity, elevated [CO_2], and added N would all increase gross N mineralization, along with microbial respiration, because each of these treatments increases plant productivity (Reich *et al*., 2001) and, therefore, the supply of organic matter to soils (Dijkstra *et al*., 2004, 2005), thus, increasing the substrate available for decomposition and gross N mineralization (Booth *et al*., 2005). However, we predicted that divergent treatment effects on the chemistry (e.g. C : N ratio) of organic matter inputs would cause differences in the magnitude of microbial responses among treatments. Specifically, we hypothesized that the response to added N would be large relative to responses to elevated [CO_2] and diversity because of the concurrent increase in plant N inputs (caused by enriched plant N concentrations; Reich *et al*., 2001) in that treatment. Because both elevated [CO_2] and greater diversity reduced plant N concentrations (Reich *et al*., 2001), we expected the decline in tissue N concentrations relative to C to constrain the N mineralization response in those treatments. Related to that, we further hypothesized enhanced effects of elevated [CO_2] and diversity with added N, where those N constraints should be alleviated. Our approach to understanding the microbial response to these treatments was to isolate microbial activity from the effects of field conditions (e.g. soil moisture, temperature), as well as from the confounding effects of plant uptake and root respiration, by conducting short-term laboratory incubations of field-collected soils. Here, we report results from soils collected after 6 years of treatment application, and, although there is a temporal trend to the ecosystem response to these treatments (Reich *et al*., 2006), the responses we report here should represent microbial responses relevant to understanding longer-term ecosystem responses to these global changes.

Methods

Experimental design

This study was conducted as part of a larger experiment designed to determine how biodiversity, elevated atmospheric [CO_2], and N deposition affect ecosystem function (BioCON; http://www.lter.umn.edu/biocon; see Reich *et al*., 2001 for detailed methodological description). It is located at the Cedar Creek Natural History Area in east central Minnesota, USA (45°N, 93°W) with a mean annual temperature of 5.7 °C and mean annual precipitation of 660 mm. BioCON is located on sandy, nutrient poor soils (Typic Udipsamments, on part of the Anoka sand plain, Grigal *et al*., 1974). Before installation of the experiment, the site was an abandoned agricultural field dominated by grasses and forbs (primarily *Bromus* spp.), with average organic matter content in the top 20 cm of 0.57 g N and 6.32 g C kg soil^{-1}. Average soil pH in the experiment was 5.41. Briefly, BioCON consists of six 20 m diameter 'rings,' three at ambient [CO_2] and three at elevated [CO_2] (560 μmol mol^{-1}) using a free-air CO_2 enrichment (FACE) system (Lewin *et al*., 1994). Each ring contains 61 individual 2 m × 2 m plots. The main experiment includes 296 plots, including four levels of diversity: 1, 4, 9, or 16 species chosen at random for each plot from a pool of 16 species (except for the monocultures, which are represented by two replicates per species per treatment). In total there are 32, 15, 15, and 12 replicates of plots with 1, 4, 9, and 16 species respectively, at each of the four combinations of CO_2 and N. The 16 species were drawn from four functional groups: C_3 grasses (*Agropyron repens* (L.) Beauv., *Bromus inermis* Leysser, *Koeleria cristata* Pers, *Poa pratensis* L.), C_4 grasses (*Andropogon gerardii* Vitman, *Bouteloua gracilis* (H.B.K.) Lag. ex Steud., *Schizachyrium scoparium* (Michaux) Nash, *Sorghastrum nutans* (L.) Nash), C_3 nonleguminous forbs (*Achillea millefolium* L., *Anemone cylindrica* A. Gray, *Asclepias tuberosa* L., *Solidago rigida* L.), and legumes (*Amorpha canescens* Pursh, *Lespedeza capitata* Michaux, *Lupinus perennis* L., *Petalostemum villosum* Nutt. (= *Dalea villosa* (Nutt.) Spreng.)). Half of those plots, selected at random, receive the equivalent of 4 g N (NH_4NO_3) m^{-2} yr^{-1}. Total sown seed mass was constant across all plots. Seeds were planted in 1997, and CO_2 and N treatments were initiated in 1998.

Gross NH_4^+ fluxes and microbial respiration. In June 2003, we sampled soils at 0–20 cm depth by taking two soil cores (diameter 2.5 cm) from each of the 296 plots. The two cores from each plot were composited and immediately sieved (2 mm) and visible roots that went through the sieve were picked out by hand. Sieved soils were stored overnight in coolers with ice. Soils were ^{15}N labeled, extracted, and incubated in two, 2-day rounds. Each round consisted of samples from three of the six field CO_2 rings and, therefore, incorporated any effect of day of incubation into the random block effect of 'ring' (see Statistical analyses).

For each sample, we simultaneously determined rates of microbial respiration and gross N mineralization by quantifying CO_2 accumulation in sealed chambers and dilution of added $^{15}NH_4^+$ through production of new $^{14}NH_4^+$ by microbial activity (^{15}N pool dilution, Davidson *et al*., 1991; Hart *et al*., 1994b). Before

incubation, each sieved, homogenized soil sample (≈100 g) received 10 mL of a 0.8 mg 99 atom% $(^{15}NH_4)_2SO_4\,L^{-1}$ solution (approximately 10% of the background inorganic N pool) by spraying the solution into a plastic bag containing the soil. After addition of the solution, the soil contained on average 14.5% water by weight (standard deviation = 3.4, $n = 293$; three gravimetric soil moisture samples lost during weighing process), which is close to field capacity for these soils. The soil was mixed gently to distribute the solution evenly through the soil. After approximately 15 min, a 50 g subsample was extracted with 50 mL of 2 M KCl (actual initial extraction time recorded). This extraction was filtered through preleached paper filters. A 5 mL subsample of the leachate was removed and frozen until it could be analyzed for NH_4^+ colorimetrically (Alpkem autoanalyzer; OI Analytical, College Station, TX, USA). The remaining extract solution was left in the specimen cups and stored at 3 °C until the ^{15}N diffusions could be done. The remaining 50 g of labeled soil was transferred to a specimen cup that was placed inside a glass jar (≈1 L headspace). In order to prevent water loss, all specimen cups were left capped until the glass jars could be simultaneously covered. After all specimen cups with soil samples were placed in the jars, the cups were uncapped and allowed some time to equilibrate with the atmosphere in the room (≈5 min). The jars were then simultaneously capped with lids that allowed an airtight seal and that were fitted with septa to allow gas sampling with syringes. Several gas samples were taken of the room air at that point and were used as the 'initial' CO_2 concentration in the jars. The soils were incubated for approximately 20 h at room temperature (≈22 °C). At the end of the incubation, syringes were used to withdraw 9 mL gas samples from the headspace of the jars (actual time recorded). These samples were injected into preevacuated gas-tight vials. The CO_2 concentration of all gas samples was analyzed using a Tekmar Dohrmann Headspace Autosampler 7000 (Teledyne Tekmar, Mason, OH, USA) connected to a Hewlett-Packard 5890A gas chromatograph (TCD detector Hewlett-Packard, Palo Alto, CA, USA). Following gas sampling, the specimen cups were retrieved, a 5 g subsample was removed to estimate gravimetric soil water content, and the remaining soil was extracted with 2 M KCl as described above.

In order to determine the ratio of $^{15}NH_4^+$ to $^{14}NH_4^+$ in the pre- and postincubation soil extractions, we employed a modification of the acidified disk/diffusion assay (Stark & Hart, 1996). We added ≈ 0.2 g of MgO to each KCl extract solution while simultaneously adding a teflon strip containing two sealed acidified (2.5 M $KHSO_4$) paper disks (7 mm diameter) and then quickly capped the cup securely. This method oxidizes the NH_4^+ in solution to NH_3 gas, which diffuses through the Teflon, and is then 'trapped' on the acidified disks. The cups were allowed to diffuse for 6 days at room temperature, and the cups were inverted daily to prevent the formation of competing acid droplets on the cup walls. After 6 days, the Teflon strips were removed and rinsed with 'Nanopure' water. The disks were then removed and impaled individually on wires and allowed to dry in a desiccator containing a vial of concentrated H_2SO_4. After the disks had dried, they were analyzed for $^{15}N/^{14}N$ by a stable isotope mass spectrometer (Europa Integra, University of California, Davis Stable Isotope Facility).

Although it is possible to calculate consumption of NH_4^+ based on these data (Kirkham & Bartholomew, 1954; Hart *et al*., 1994b), the addition of NH_4^+ to the pool of inorganic N could potentially artificially stimulate NH_4^+ uptake, nitrification and other loss pathways (Hart *et al*., 1994b), an artifact that should not occur by labeling the 'product pool' of mineralization. As a result, we present and discuss only the gross N mineralization results here. Microbial respiration was calculated as the production rate of CO_2-C over time per gram dry weight of soil. Gross N mineralization was calculated as follows (Kirkham & Bartholomew, 1954; Hart *et al*., 1994b):

$$m_{NH_4^+} = \left(\frac{[NH_4^+]_0 - [NH_4^+]_t}{t}\right)\left(\frac{\log APE_0/APE_t}{\log [NH_4^+]_0 \big/ [NH_4^+]_t}\right),$$

where $m_{NH_4^+}$ is the gross N mineralization rate ($\mu g\,N\,soil^{-1}\,day^{-1}$), $[NH_4^+]_0$ the ammonium-N concentration at time 0 ($\mu g\,N\,g\,soil^{-1}$), $[NH_4^+]_t$ the ammonium-N concentration at time t ($\mu g\,N\,g\,soil^{-1}$), t the time (days), and APE the atom percent excess over background (atom% ^{15}N–0.37 atom% ^{15}N).

Statistical analyses

In order to determine whether there were significant treatment effects, all data were analyzed with mixed-model (ring as random and treatments as fixed effects), split-plot (ring nested within atmospheric [CO_2] treatment) analyses of variance (ANOVAs) with SAS statistical analysis software (SAS Institute, Cary, NC, USA). Data were transformed to meet assumptions of ANOVA when necessary. Correlation analyses were also performed to determine relationships among parameters.

In addition to the tests of treatment effects with ANOVA, linear regressions were conducted as an additional test of whether the relationships between gross N mineralization and microbial respiration were altered

by the treatments. As no mineralization of N should occur in the absence of microbial respiration, these Type I linear regressions were forced through the origin. It is important to note that unconstrained Type I regressions were also compared with orthogonal regressions that do not assume zero variance in the *x*-axis, and the slopes and intercepts were not different between methods (results not presented).

Results

In partial contrast to our expectations, gross rates of N mineralization were strongly stimulated by increased plant species diversity as well as by N addition but not by elevated [CO_2] (Fig. 1, Table 1). Elevated N and diversity increased gross N mineralization by 11% and 38% on average, respectively. Also in contrast to our hypothesis, there were no significant interactions among any of the three treatments.

Microbial respiration generally responded positively to all three global change factors (Fig. 1, Table 1), with

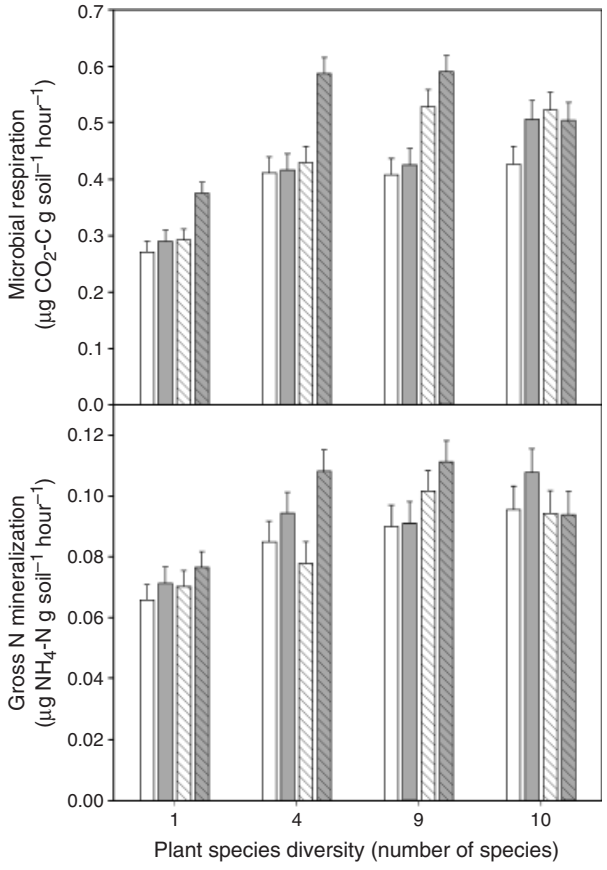

Fig. 1 Mean response to treatments (+1 SE) of gross N mineralization and microbial respiration. White bars are ambient nitrogen, gray bars are elevated nitrogen; unhatched bars are ambient [CO_2] and hatched bars are elevated [CO_2]. See Table 1 for results of statistical analyses.

Table 1 Results of the statistical analyses of microbial activity variables

Variable	Source of variation	*F* (nDF, dDF)	*P*
Gross N mineralization (μg NH_4-N g $soil^{-1}$ h^{-1})			
	Diversity	27.92 (3,263)	**<0.0001**
	[CO_2]	0.66 (1,4)	0.4611
	N	7.26 (1,263)	**0.0075**
	Diversity × [CO_2]	1.79 (3,263)	0.1496
	Diversity × N	1.39 (3,263)	0.2476
	[CO_2] × N	0.42 (1,263)	0.5194
	Diversity × [CO_2] × N	1.01 (3,263)	0.3876
Microbial respiration (μg CO_2-C g $soil^{-1}$ h^{-1})			
	Diversity	58.80 (3,272)	**<0.0001**
	[CO_2]	37.12 (1,4)	**0.0037**
	N	13.53 (1,272)	**0.0003**
	Diversity × [CO_2]	2.61 (3,272)	**0.0516**
	Diversity × N	0.57 (3,272)	0.6370
	[CO_2] × N	2.16 (1,272)	0.1429
	Diversity × [CO_2] × N	2.86 (3,272)	**0.0373**
Gross N mineralization/respiration (μg N μg C^{-1})			
	Diversity	9.05 (3,259)	**<0.0001**
	[CO_2]	9.22 (1,4)	**0.0385**
	N	0.37 (1,259)	0.5447
	Diversity × [CO_2]	1.44 (3,259)	0.2304
	Diversity × N	0.86 (3,259)	0.4628
	[CO_2] × N	0.46 (1,259)	0.4987
	Diversity × [CO_2] × N	0.50 (3,259)	0.6841

All analyses were conducted as mixed-model split-plot (plot as random effect and nested within the [CO_2] treatment) ANOVAs and variables were transformed as necessary to meet assumptions of ANOVA. See text for methodological details. Bold indicates $P \leq 0.05$.

average stimulations of 21%, 12%, and 60% due to CO_2, N, and diversity, respectively. There were, however, significant higher order interactions for microbial respiration. For example, at ambient [CO_2] the effect of elevated N on microbial respiration was substantial only at the highest diversity. At elevated [CO_2], however, elevated N stimulated microbial respiration at all except the highest diversity.

As expected (but see Schimel, 1986), gross N mineralization rate and microbial respiration were positively correlated ($R = 0.66$). The slopes of regression lines for the elevated CO_2 treatments in both ambient and elevated N were significantly lower (both = 0.19) than were the slopes for ambient CO_2 (both = 0.22; $P < 0.0001$; Fig. 2), consistent with a lower mean ratio of N mineralized to microbial CO_2 respired that was also observed (Fig. 3, Table 1). Species number did not significantly affect the slopes of these regressions, although the mean ratio of N mineralized to CO_2 respired decreased significantly with increasing diversity (Fig. 3, Table 1).

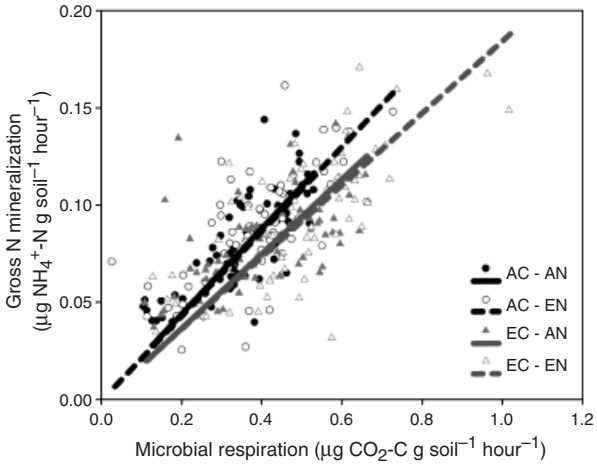

Fig. 2 Relationship between gross N mineralization and microbial respiration representing 'yield' of inorganic N from C respired for the four CO_2 and N treatments. The fitted lines were constrained to a zero intercept and were significantly different by CO_2 treatment but not by N treatment. Fits for diversity did not yield significant differences (see text for details). The legend indicates ambient (A) or elevated (E) CO_2 (C) and nitrogen (N) treatments.

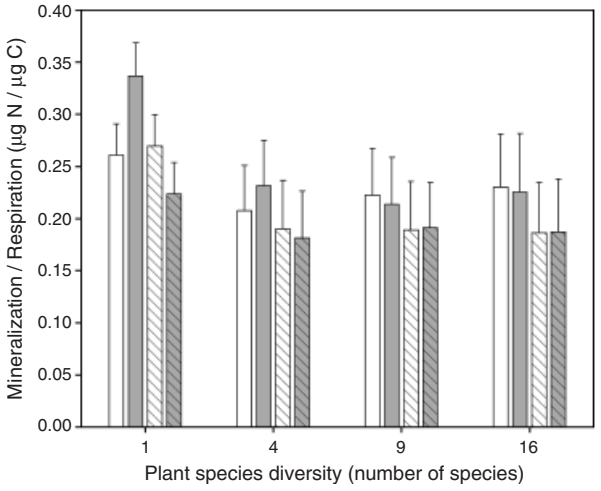

Fig. 3 Mean response to treatments (+1 SE) of the ratio of gross N mineralization to microbial respiration (inorganic N 'yield'). White bars are ambient nitrogen, grey bars are elevated nitrogen; unhatched bars are ambient [CO_2] and hatched bars are elevated [CO_2]. See Table 1 for results of statistical analyses.

Discussion

Gross N mineralization showed strong, positive responses to both increasing diversity and N addition. These responses were consistent with our expectations and suggest that previous observations in BioCON of divergent responses of net N mineralization in response to N addition (positive) and diversity (negative), as seen both *in situ* (Reich *et al*., 2001), and in lab incubations (Dijkstra *et al*., 2005), were due to differences in gross N immobilization between treatments. In contrast to our expectations, however, there was no significant response of gross N mineralization to elevated [CO_2], and there were no significant interactions among treatments. The lack of response of gross N mineralization to elevated [CO_2] is consistent with the majority of published reports (Zak *et al*., 2003a, but see Hungate *et al*., 1999), suggesting that, although there are often large changes in other ecosystem components and fluxes, the rate of inorganic N liberation from organic matter does not change. This does not necessarily imply that plant N supply does not change with elevated atmospheric [CO_2], but that, if it does, it is most likely linked to changes in microbial immobilization or loss of inorganic N. With the exception of microbial respiration, interactions between treatments were not observed in these results, increasing our confidence in the information gained from single-factor experiments.

The lack of gross N mineralization response to elevated [CO_2] was likely a result of the counteracting effects of increased total organic matter supply and reduced N concentrations in plant organic inputs. Elevated [CO_2] has increased plant productivity and labile C inputs in this experiment (Reich *et al*., 2001, 2006; Dijkstra *et al*., 2005), but there has been a strong temporal trend in plant productivity and total plant N (Reich *et al*., 2006). This temporal trend in plant N complicates the interpretation of the microbial response to these plant inputs, as it is likely a response to some integration of recent plant inputs and the soil organic matter present before treatment application. The simultaneous measurement of gross N mineralization and microbial respiration allowed us to evaluate the ratio of NH_4^+-N mineralized per CO_2-C respired by microbes. This ratio represents the yield of inorganic N from microbial activity and should reflect the relative limitation of microbial activity by C vs. N (Hart *et al*., 1994a; Booth *et al*., 2005).

Although gross N mineralization rates alone did not respond to elevated [CO_2], there was a decline in the N yield parameter in response to elevated [CO_2]. This response is consistent with our suggestion that elevated [CO_2] induced N limitation of microbes by decreasing N concentrations in plant inputs to the soil organic matter pool while increasing labile C inputs to soils via plant production and root exudation (Dijkstra *et al*., 2005). Other work in this experiment suggests that a CO_2-induced N limitation to plant production has developed over time in the ambient N treatment (Reich *et al*., 2006), and is consistent with reports from other systems (e.g. Hu *et al*., 2005; Janus *et al*., 2005). Reich *et al*., (2006) reported that over time, N fertilization

increasingly enhanced the positive response of biomass production and total plant N pools to elevated [CO_2]. If this plant productivity response can be explained in part by declining soil N supply at elevated [CO_2] and ambient N, our results suggest that this decline resulted from increasing microbial demand for N in the elevated [CO_2] treatment. Clearly more work needs to be done to improve our understanding of the coupling between plants and soil microbes, and to help resolve ongoing debates about these relationships (e.g. Knops *et al*., 2002; Chapman *et al*., 2005).

The decline in microbial N yield with increasing diversity suggests increasing N limitation to microbial activity across this gradient at the same time that the rate of NH_4^+ release from organic matter is increasing. These results suggest that although microbial activity is becoming increasingly N limited, the total N flux through the microbial pool is greater. Although previous reports have suggested a positive relationship between plant diversity and inorganic N supply to plants (e.g. Zak *et al*., 2003b), whether this truly represents a positive feedback between plant diversity and productivity (Hobbie, 1992) remains to be determined.

Interestingly, in contrast to the results for CO_2 and diversity, there was no change in microbial N yield in response to N fertilization, nor did the slopes relating gross N mineralization to microbial respiration change in response to N fertilization. This lack of response in N yield contrasts with the strong, positive responses to N fertilization observed for both gross N mineralization and microbial respiration individually. Added N shifted microbial activity along the linear gross N mineralization–respiration relationship, tending to increase both without fundamentally changing the relationship, a response that contrasts with the response to elevated [CO_2].

Although very few studies have quantified both simultaneously, prior work has suggested a link between gross N fluxes and microbial respiration (Schimel, 1986; Hart *et al*., 1994a). Hart *et al*. (1994a) concluded, based on results of a long-term incubation of forest soils, that the strong correlation between gross N fluxes and microbial respiration they observed indicated significant controls on N mineralization by microbial C availability. These authors further suggested that this relationship may be common in soils, and that rates of microbial respiration may generally indicate rates of N cycling. As a caveat to this extension of their results, Hart *et al*. (1994a) suggested that the slope of the relationship between gross N mineralization and microbial respiration may vary in response to different soil conditions. Our results are consistent with the observations of Hart *et al*. (1994a). With linear regressions not constrained to a zero intercept, Hart *et al*. (1994a) reported a slope of 0.20 for forest soils, and Schimel (1986) reported a shallower slope (0.05) for grassland soil gross N immobilization. Across our dataset, we find a slope of 0.13 when not constrained to a zero intercept (results not presented). It should be noted that our incubations were relatively short (<24 h) and those of Hart *et al*. (1994a) were conducted for over a year. Our observations are, therefore, relevant to the rapid-cycling soil organic matter pool, and any changes to other soil pools would not have been observed in this study. Given this, it is interesting that we observe similar correlations between C and N dynamics as Hart *et al*. (1994a), suggesting that there may be important, fundamental controls on microbial activity across very different organic matter sources.

Our results aid in the interpretation of net N mineralization results presented previously (Reich *et al*., 2001, 2006). Although short-term (24 h) laboratory incubations are not directly comparable with month-long field incubations because of potential changes in the organic matter pools accessed by microbes as decomposition proceeds (Hart *et al*., 1994a), qualitative comparison suggests that atmospheric [CO_2] effects on rates of net N mineralization resulted from effects on immobilization rather than mineralization. In other words, the lack of a [CO_2] effect on gross N mineralization observed here suggests that reduced net N mineralization under elevated [CO_2] and ambient N (Reich *et al*., 2006) resulted from enhanced immobilization. Analogously, then, under elevated N, increased net N mineralization with elevated [CO_2] likely arose from reduced, or at least unchanged, immobilization.

In conclusion, our results demonstrate that gross NH_4^+ production in this system is sensitive to changes in plant species diversity, increases in atmospheric [CO_2], and the addition of reactive N to ecosystems, but in different ways. The linkages reported here between plant productivity and microbial biomass and respiratory activity should improve efforts to model terrestrial ecosystem response to future global change (e.g. Rastetter *et al*., 1997; Van Oene *et al*., 1999), and ongoing work should help to resolve many of the remaining unanswered questions.

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