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Biodiversity, Nitrogen Deposition, and CO_2 Affect Grassland Soil Carbon Cycling but not Storage

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Abstract

Grasslands are globally widespread and capable of storing large amounts of carbon (C) in soils, and are generally experiencing increasing atmospheric CO_2, nitrogen (N) deposition, and biodiversity losses. To better understand whether grasslands will act as C sources or sinks in the future we measured microbial respiration in long-term laboratory incubations of soils collected from a grassland field experiment after 9 years of factorial treatment of atmospheric CO_2, N deposition, and plant species richness on a deep and uniformly sandy soil. We fit microbial soil respiration rates to three-pool models of soil C cycling to separate treatment effects on decomposition and pool sizes of fast, slow, and resistant C pools. Elevated CO_2 decreased the mean residence time (MRT) of slow C pools without affecting their pool size. Decreasing diversity reduced the size and MRT of fast C pools (comparing monocultures to plots planted with 16 species), but increased the slow pool MRT. N additions increased the size of the resistant pool. These effects of CO_2, N, and species-richness treatments were largely due to plant biomass differences between the treatments. We found no significant interactions among treatments. These results suggest that C sequestration in sandy grassland soils may not be strongly influenced by elevated CO_2 or species losses. However, high N deposition may increase the amount of resistant C in these grasslands, which could contribute to increased C sequestration.

Key words: C sequestration; elevated CO_2; FACE experiment; soil C cycling; biodiversity; nitrogen deposition.

Introduction

Carbon (C) sequestration is an important ecosystem service that in combination with reduced fossil fuel CO_2 emissions and other measures could reduce already high atmospheric CO_2 levels (Hansen and others 2008). Globally, soils store approximately twice the amount of C as in the atmosphere and terrestrial biomass C pools combined (2,344 Pg, 0–3 m, Jobbagy and Jackson 2000), and that C has a mean residence time (MRT) of 50 years, greater than the MRTs of either atmospheric (5 years) or terrestrial biomass C pools (9 years, globally averaged, Schlesinger 1997; Scurlock and Hall 1998). Thus, increasing soil C sequestration is one key way to enhance long-term C sequestration in terrestrial

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ecosystems. In particular, grassland soil C has been found to be responsive to increases in atmospheric CO_2, nitrogen (N) deposition, and changes in species diversity (Fornara and Tilman 2008; Jastrow and others 2005; van Groenigen and others 2006). Here, we assess the effects of biodiversity, atmospheric CO_2 concentration, and N deposition on soil C pool sizes and turnover rates using a model grassland system to determine future C sequestration potential.

Elevated CO_2 increases plant biomass contributions to the soil (De Graaff and others 2006; Reich and others 2001a) which can result in modest increases in total soil C (Jastrow and others 2005). However, elevated CO_2 also increases soil microbial biomass and respiration (Craine and others 2001; Dijkstra and others 2005; Gill and others 2006; He and others 2010; Heath and others 2005; Rice and others 1994), reducing root-derived C sequestration (Heath and others 2005). C sequestration is further limited when new C inputs under elevated CO_2 are balanced by losses of old soil C (Adair and others 2009; Gill and others 2002), or contribute only to fast-cycling soil pools with little potential for long-term sequestration (Hungate and others 1997; Lichter and others 2005).

Although elevated CO_2 is expected to increase plant production, N availability limits primary productivity in many terrestrial ecosystems (LeBauer and Treseder 2008; Vitousek and Howarth 1991), and can reduce the response of plant productivity to elevated CO_2, constraining biomass inputs to soils and limiting C sequestration (Reich and others 2006a, b). N limitation also affects microbial decomposition, further affecting soil C sequestration. Under elevated CO_2, N-limited microbes may increase their access to soil N pools by increasing decomposition of soil organic matter to obtain N via the priming effect (Fontaine and others 2004, 2007). A CO_2-induced priming effect could reduce soil C sequestration by increasing the turnover rate of soil C, especially in low-nutrient soils (but see Dijkstra and others 2005; Fontaine and others 2004). In contrast, relieving N limitation, through N additions in elevated CO_2 environments, could increase plant biomass while maintaining decomposition rates, resulting in soil C sequestration (De Graaff and others 2006; Heath and others 2005; Reich and others 2006a; van Groenigen and others 2006). N additions may also increase C sequestration by stabilizing soil C in more resistant fractions (Neff and others 2002).

Although plant species diversity has been widely recognized as an important determinant of ecosystem productivity, its role in determining soil C sequestration remains unclear. High species richness has been shown to increase soil C sequestration by as much as 600 % over monocultures (Fornara and Tilman 2008) or to increase sequestration to 2.7 Mg C ha^{-1} y^{-1} from no net sequestration in monocultures (Tilman and others 2006). However, much of that increase was attributed to the presence and abundance of specific species or functional groups (legumes, De Deyn and others 2009; legumes and C4 grasses, Fornara and Tilman 2008), rather than species richness per se. Despite examples of impressive increases in C sequestration, the absolute magnitude of the effect of grassland species richness on soil C sequestration (relative to monocultures) is small and highly variable (Fissore and others 2010). Increasing plant diversity (Tilman and others 2001, 1997), along with elevated CO_2 and N additions (Craine and Jackson 2010) have all been shown to increase plant biomass (Dijkstra and others 2006; Fornara and Tilman 2008), which should result in increased soil C inputs (Adair and others 2009) and thus changes in soil C dynamics.

The majority of research on the effects of elevated CO_2 and N deposition on soil C has considered soil C as a single pool (but see Dijkstra and others 2005; Neff and others 2002). However, because the soil C pool is so large, it is difficult to detect change over short periods of time. In addition, soil C varies in quality and accessibility to microbes from labile to recalcitrant. To represent this conceptually, models often divide soil C into two or more pools. The fastest cycling pools are typically the smallest and are referred to as active, labile, or fast pools. In two-pool models there is a second, slower pool, whereas in three-pool models there is a pool with intermediate turnover time that is referred to as the slow pool, and the slowest pool is referred to as resistant or recalcitrant. We will refer to three-pool soil C models using the fast, slow, and resistant terminology. Few studies have examined how diversity, CO_2 or N treatments influence the sizes and turnover rates of multiple soil pools. This distinction is important because long-term C sequestration depends largely on changes to slow and resistant pools.

Here, we describe a laboratory incubation study of field-collected soils from a large-scale experiment where CO_2, N, and plant diversity were manipulated for 9 years. We expand on previous study (Dijkstra and others 2005) by using a three-pool model that provides a more detailed representation of soil dynamics (Paustian and others 1992) and that allowed us to estimate the sizes and dynamics of the slow and resistant pools. We used estimated soil pool sizes and decomposition rates to

test several hypotheses about how soil C pools respond to multiple—and likely interacting—global change factors. Elevated CO_2 was hypothesized to increase the decay rates of the fast and slow pools by increasing labile C inputs and priming microbial decomposition (Fontaine and others 2004). We expected N additions to increase the size of the resistant pool and decrease the decay rate of the slow pool by decreasing lignin decomposition (Dijkstra and others 2004) and by reducing priming (Fontaine and others 2004; Pregitzer and others 2008; Zak and others 2008). N additions were expected to interact with elevated CO_2 to increase total soil C by reducing nutrient limitation of primary production (increasing soil inputs) and microbial respiration (decreasing priming losses, Fontaine and others 2004). High plant diversity was hypothesized to increase the decay rate of the fast pool by increasing microbial metabolism through increased plant biomass and C inputs to soils (Zak and others 2003). Finally, we hypothesized that elevated CO_2, N, and plant diversity treatments would increase biomass and therefore increase total soil organic C.

Methods

BioCON

This research was conducted within the biodiversity, CO_2, and N experiment (BioCON, Reich and others 2001a, b), which was established in 1997 in a nearly level old field in the Cedar Creek Ecosystem Science Reserve (CCESR), Minnesota, USA (Lat. 45°N, Long. 93°W). Soils in this area are very homogeneous, sandy, and nutrient poor (Typic Udipsamments on the Anoka sand plain, Grigal and others 1974). Mean annual precipitation is 660 mm with mean monthly temperatures of −11°C in January and 22°C in July.

In 1997, the vegetation from six 20-m-diameter circular areas was removed. Soil was tilled uniformly to a depth of 25 cm and fumigated with methyl bromide to eliminate the soil seed bank. Soils were reinoculated with microbes from surrounding old field soils. By 2000, arbuscular mycorrhizal fungal communities and soil respiration had recovered to levels similar to surrounding old field areas (Wolf and others 2003, unpublished soil C flux data). In June 1997, 296 2 × 2-m plots were seeded with 1, 4, 9, or 16 grassland species, randomly chosen from 16 species in four functional groups (C3 and C4 perennial grasses, non-legume forbs, and legumes) at a rate of 12 g m^{-2}, with seed mass divided evenly among the species in a plot. The 16 species used were all native or naturalized to the CCESR: the C4 grasses *Andropogon gerardii* Vitman, *Bouteloua gracilis*, *Schizachyrium scoparium* (Michaux) Nash, and *Sorghastrum nutans* (L.) Nash; the C3 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 irrigated during the 1997 growing season, but not in subsequent years.

Plots in three of the six rings have been treated with ambient +180 ppm CO_2 during each growing season since 1998 (using FACE technology). Beginning in 1998, half of the plots were fertilized with 4 g N m^{-2} y^{-1} applied in three doses during the year (May, June, and July) as slow release NH_4NO_3. All plots were burned two of every 3 years (2000, 2002, 2003, and 2005), a common management practice that mimics natural fire frequencies in tall grass prairies.

The BioCON main experiment is a split-plot arrangement of treatments in a completely randomized design. The CO_2 treatment is the whole-plot factor. The subplot treatments of species richness and N addition were randomly distributed and replicated in individual plots among the six rings. For this research, we utilized all of the 16 species plots and 8 of the 16 monoculture treatments, 2 from each functional group: C4 grasses *Andropogon gerardii* Vitman and *Sorghastrum nutans* (L.) Nash; the C3 grasses *Agropyron repens* (L.) Beauv.and *Bromus inermis* Leysser; the forbs *Asclepias tuberosa* L., and *Solidago rigida* L.; and the legumes *Amorpha canescens* Pursh and *Lespedeza capitata* Michaux. We limited this experiment to just the monocultures and 16 species plots to capture the largest possible differences in belowground biomass and to keep the experiment a manageable size.

In August 2006, we sampled soils from the 48 16 species plots and 64 monoculture plots (total 112 plots) by taking three 2.5-cm-diameter cores (0–20 cm) per plot. Soils were composited by plot and immediately sieved (2 mm). Visible roots were removed by hand. We took immediate subsamples for soil C respiration incubations and gravimetric soil water content. The remaining soil was air-dried, ground, and subsampled for total C and N and nonhydrolyzable C and N analyses.

Soil Analyses

Resistant soil C was estimated using an acid digest procedure that hydrolyzes polysaccharides and

nitrogenous material, leaving a residue consisting primarily of lignin and polyaromatic humics (Martel and Paul 1974; Sollins and others 1999). Identifiable plant materials were removed from air-dried, ground soil. One-gram soil samples were refluxed for 16 h in digestion tubes with 10 ml of 6 M hydrochloric acid solution. The remaining residue was filtered, washed with 100 ml of nanopure water, dried for 24 h in a 60°C oven, weighed, and analyzed for total C by combustion (Model ECS 4010, COSTECH Analytical, Valencia, California). The remaining nonhydrolyzable, or chemically resistant, C represents resistant soil C, which ^{14}C-dating indicates is much older than bulk soil (Paul and others 2006). A subsample of the dried and ground whole soil was also analyzed for total organic C by dry combustion (as above).

To quantify organic soil C pools and decomposition rates we placed 20 g of moist soil into 120 ml polyethylene specimen cups and brought soils to a common moisture content [70 % field capacity to prevent rapid drying in these sandy soils (Dijkstra and others 2006)] using nanopure water to ensure that no additional nutrients were added. Specimen cups were placed in 1-l glass jars and were incubated aerobically in the dark at a constant temperature (21°C) for 391 days. On each sampling day (1, 4, 7, 13, 27, 46, 74, 152, 168, 222, 273, 324, and 391 days), we sampled CO_2 production over 24 h. On each date, jars were capped and the headspace was sampled immediately through a septum in the lid. Headspace was sampled again after 24 h. Headspace samples were immediately analyzed for CO_2 on a gas chromatograph (Shimadzu GC14, Shimadzu Scientific Instruments, Wood Dale, Illinois) using a thermal conductivity detector and a Poropak N column. Daily soil C respiration rates were calculated by determining the difference between CO_2 concentrations in the initial (time = 0) and final (time = 24 h) samples. Between sampling periods, jars were covered with a polyethylene film to allow O_2 exchange and minimize soil water loss.

Fitting one, two, or three-pool models to soil incubation respiration data allows for the analytical estimation of soil C pools and fluxes. Either cumulative respiration or daily respiration rate data may be used for model fitting. Several authors have suggested that using cumulative respiration data accumulates errors while dampening noise and providing a false sense of security in the form of high R^2 values (Alvarez and Alvarez 2000; Ellert and Bettany 1988; Hess and Schmidt 1995). Thus, to avoid autocorrelation in residuals and dependence among data points (Hess and Schmidt 1995), we fit all models to daily respiration rates.

Because incubation data alone are not sufficient to analytically estimate the size and flux of resistant C, we used the nonhydrolyzable C fraction as an estimate of the resistant pool, and fit a three-pool model to the daily respiration rates (Paul and others 2006, 1999; Pendall and King 2007):

$$C_{\text{rate}}(t) = k_f\left(C_f e^{-k_f t}\right) + k_s\left[(C_t - C_f - C_{\text{NHC}})e^{-k_s t}\right] + k_r\left(C_{\text{NHC}} e^{-k_r t}\right),$$

where C_{rate} is the daily respiration rate (mg C g soil^{-1} day^{-1}), C_f is the labile C pool (mg C g soil^{-1}), C_{NHC} is nonhydrolyzable or resistant C (NHC; mg C g soil^{-1}), C_t is total C (mg C g soil^{-1}), k_f, k_s, and k_r are the decomposition rates of the labile (fast), slow, and resistant pools (respectively; day^{-1}), and t is time in days. The slow C pool (C_s) is defined in the above equation as C_t minus the sum of C_f and C_{NHC}. The MRT of the resistant C pool was constrained to be more than 1,000 years (Paul and others 2006; Pendall and King 2007 $k_r = 2.7 \times 10^{-6}$ day^{-1}). As was found by Paul and others (2001a, b) and Pendall and King (2007), the choice of a k_r (100–1,000 years) did not influence the parameter estimates of the faster soil C pools or fluxes (Appendix A).

Although we fit one-, two-, and three-pool models to the daily respiration data from each soil incubation jar (Appendix A), we chose to focus on the parameter estimates from the three-pool model for several reasons: (1) the two- and three-pool models better accounted for the long-term dynamics of soil respiration in our incubations than did the single pool model; (2) the two and three-pool models fit the data equally well (Appendix A); (3) obtaining separate estimates for slow and resistant pools and fluxes allowed us to parse out the effects of BioCON treatments on each pool (vs. a two-pool model which lumps resistant and slow C into the second, slow pool) and expand upon previous study in BioCON that analyzed long-term incubations using a two-pool model (2005); and (4) Paul and others (2001a, b) found that using a two-pool model (constrained by total C) substantially underestimated both the size and decomposition rate of the slow C pool. We also found that the two-pool model consistently resulted in a slower k_s, but a larger C_s (Appendix A). Estimates of k_f and C_f were unaffected by model choice (linear regressions of parameter estimates from both models had intercepts ~0 and slopes ~1; Appendix A). The three-pool model explained greater than 90 % of the variation in daily respiration rates in 66/112 cases; between 70 and 90 % of the variation in the data in 42/112 cases; and between 60 and 70 % of the variation in the data in 4 cases (Appendix A).

Data Analysis

We performed several different analyses to investigate the effects of N, CO_2, and species richness on soil C pools and fluxes. Total soil C and nonhydrolyzable C were analyzed using an ANOVA with ring nested within CO_2 treatment as a random effect. All treatments were considered fixed effects. The same ANOVA was performed on the soil C pool and flux parameter estimates from the three-pool model: k_f, k_s, C_f, and C_s. We used an ANCOVA with total plant biomass in each plot averaged over the experiment duration (1998–2006) as a covariate in our mixed-effects model to determine the degree to which plant biomass was responsible for treatment effects (JMP 9.0.1, SAS Institute, Cary, North Carolina). We also used a similar ANCOVA with below-ground plant biomass in each plot averaged over the experiment duration, but the results were nearly identical to the total plant biomass results (Appendix B), thus we focused on the results for total plant biomass. Dependent variables with non-normal residuals (Shapiro-Wilk test for normality) were natural log transformed (k_f, k_s, C_f, and C_s) to meet normality assumptions.

Results

Averaged across all treatments, total soil C was 6.03 mg C g soil^{-1}, fast or labile C was 0.14 mg C g soil^{-1} or 2 % of total soil C, slow C was 4.02 mg C g soil^{-1} or 67 % of total soil C, and resistant C (NHC) was 1.87 mg C g soil^{-1} or 31 % of total soil C. The MRT of fast and slow C averaged 19 days (k_f = 18.6 y^{-1} or 0.051 day^{-1}) and 9 years (k_s = 0.10 y^{-1} or 0.00027 day^{-1}), respectively.

Contrary to our expectations, total soil C did not change significantly in response to CO_2, diversity or N or their interactions (Figure 1). Although total C storage did not change with treatments, the distribution of C among fast, slow, and resistant pools did.

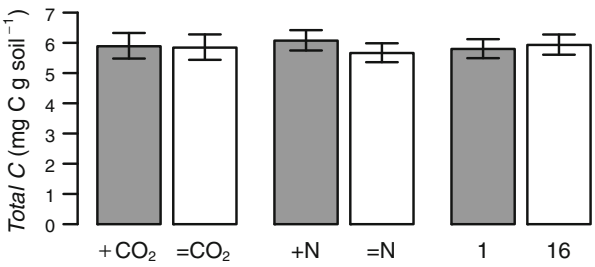

Figure 1. Least squares mean ± standard error of total C for all treatments. In the ANOVAs there were no significant treatment effects.

High species richness nearly doubled the fast pool size (C_f) and decreased the decay rate of the fast pool by 39 % compared to monocultures (Figure 2). High species richness also increased the decay rate of the slow pool by 27 % compared to monocultures. There was no concurrent change in slow pool size between species-richness treatments, indicating that monoculture plots had lower inputs to the slow pool to match the lower observed decay rates.

In response to elevated CO_2 we observed a marginally significant 22 % increase in the slow pool decay rate. There was no concurrent change in slow pool size in elevated CO_2 plots, indicating that inputs to the slow pool under elevated CO_2 were increased at a rate that roughly matched the observed decay rate (Figure 3).

Although N additions had no significant effect on fast and slow pool sizes or decomposition rates, we observed a 10 % increase in the size of the resistant C pool (NHC) in the N addition treatment compared to the ambient treatment, although this effect was only marginally significant (Figure 4).

In contrast to our hypothesis that elevated CO_2 would increase soil C in the presence of sufficient N (N additions), there were no significant interactions among CO_2, N, and species richness (Table 1).

Because higher levels of all three treatments increased plant biomass (Reich and others 2006b), we further analyzed our results with the total plant biomass in each plot averaged over the experiment duration (1998–2006) as a covariate in our mixed-effects model. Total plant biomass accounted for all of the effects of CO_2, N, and diversity, except on the slow pool decay rate (ANCOVAs, Table 2). Accounting for total plant biomass reversed the effect of diversity on k_s. The slow pool decay rate increased by 43 % in single species treatments (k_s = 0.116 y^{-1} or 0.00032 day^{-1}, MRT 8.6 years) compared to 16 species treatments (k_s = 0.081 y^{-1} or 0.00022 day^{-1}, MRT 12.3 years; ANCOVA, *diversity*: P = 0.0244).

Discussion

Our results suggest that grasslands on coarse-textured soils subjected to increasing atmospheric CO_2, N deposition and species losses may not become strong C sinks. Although we found no significant change in total soil organic C after 9 years of treatments, we found significant effects of species diversity on fast pool size and decay rate; of diversity and CO_2 on slow pool cycling; and of N on resistant pool size. Changes to the fast pool size and decay rate caused by loss of species richness are

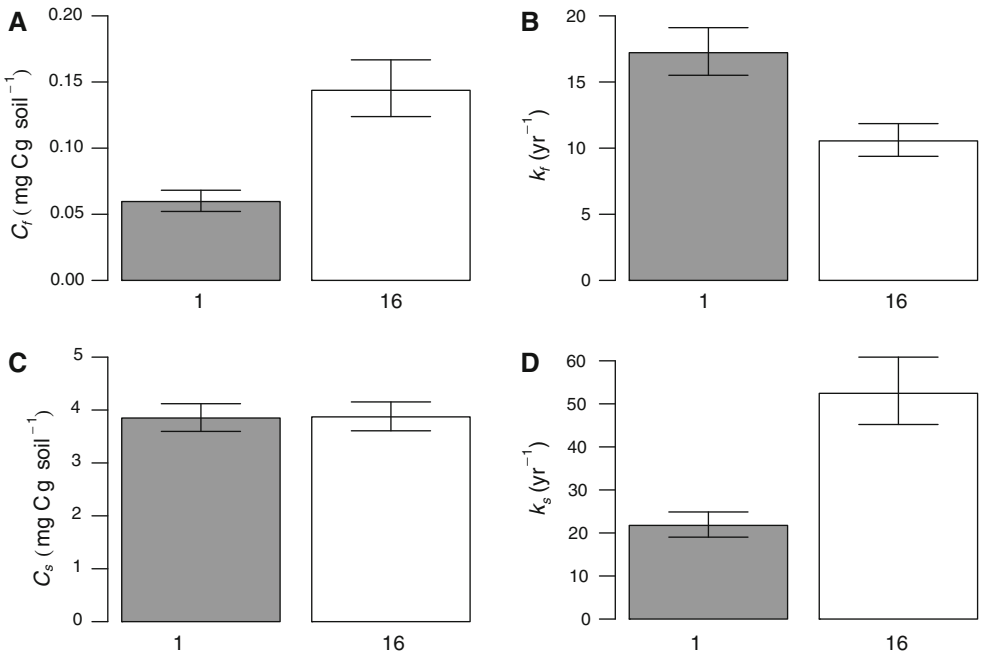

Figure 2. Least squares mean ± standard errors of the estimated **A** fast C pool size (mg C g soil^{-1}), **B** fast pool decomposition rate (y^{-1}), **C** slow C pool size (mg C g soil^{-1}), and **D** slow pool decomposition rate (y^{-1}) in monocultures and 16 species plots. In the ANOVAs, species richness significantly increased fast pool size, but decreased the fast decomposition rate. Species richness had no significant effect on the size of the slow pool.

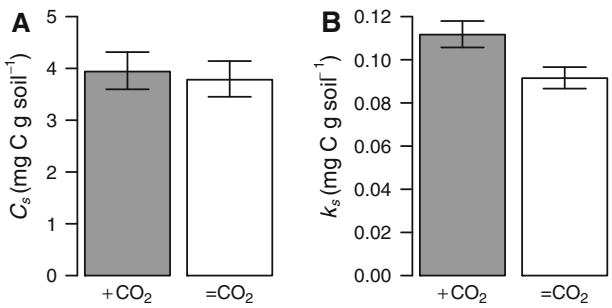

Figure 3. Least squares mean ± standard errors of the estimated **A** slow C size (mg C g soil^{-1}) and **B** decomposition rate (y^{-1}) in soils from the elevated and ambient CO_2 treatments. In the ANOVA, elevated CO_2 tended to increase the slow decomposition rate.

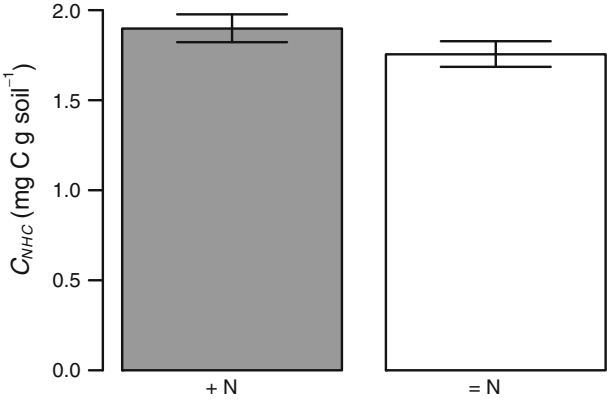

Figure 4. Least squares mean ± standard errors of the resistant C size (mg C g soil^{-1}) in soils from the elevated and ambient N treatments. In the ANOVA, elevated N tended to increase the resistant C pool size.

unlikely to result in substantial loss of C sequestration potential because of the small size of the fast pool (2 % of total soil organic C) and its short MRT (19 days). Elevated CO_2 and increasing diversity both increased the rate of C cycling in the slow pool without affecting its size. Finally, although the effect of N additions on the resistant pool was small (10 % increase in resistant C which is 31 % of total soil organic C) our results suggest that N additions could slowly increase long-term C storage.

Biodiversity

Although the effect of biodiversity on productivity is relatively well understood (Cardinale and others 2006; Reich and others 2001b), few studies have examined the effects of species richness on soil C (but see Fornara and Tilman 2008; Tilman and others 2006), and even fewer have investigated its effects on different soil C pools and fluxes (but see Dijkstra and others 2005). In our study, high species richness resulted in a larger fast-cycling pool (consistent with results reported for fast-cycling pools in four species versus monoculture treatments, Dijkstra and others 2005) that decomposed at a slower rate. The results of the ANCOVA suggest that these species-richness effects were due to greater plant biomass in the high richness treatments (as also concluded by Dijkstra and others 2005). Despite the increase in total plant biomass, there was no stimulation of old or resistant C decomposition as previously reported at this site

Table 1. ANOVA Results for Three-Pool Model

	N	C_f	k_f	C_s	k_s	C_{NHC}	Total C
CO_2							
Ambient	56	0.089	0.033	3.781	0.00025†	1.9	5.8
Elevated	56	0.096	0.041	3.939	0.00031†	1.8	5.9
N							
Ambient	56	0.086	0.035	3.761	0.00027	1.8†	5.7
Elevated	56	0.099	0.039	3.961	0.00028	1.9†	6.1
Species number							
1	64	0.060**	0.047**	3.849	0.00025**	1.8	5.8
16	48	0.144**	0.029**	3.87	0.00031**	1.8	5.9
R^2		0.25	0.17	0.23	0.1	0.15	0.21

Mixed-effects model parameter estimates of fast and slow C pool sizes (C_f, C_s, C_{NHC}, and total C: mg C g soil^{-1}), and decomposition rates (k_f and k_s: day^{-1}).
*$^{\dagger}P \leq 0.1$, *$P \leq 0.05$, **$P \leq 0.01$ (ANOVA). No significant interactions were found $P \leq 0.1$.*

Table 2. ANCOVA Results for Three-Pool Model

	N	C_f	k_f	C_s	k_s	C_{NHC}	Total C
CO_2							
Ambient	56	0.09	0.033	3.78	0.00025	0.19	5.864
Elevated	56	0.082	0.043	3.95	0.00028	0.17	5.844
N							
Ambient	56	0.094	0.034	3.75	0.00029	0.18	5.676
Elevated	56	0.079	0.042	3.98	0.00025	0.19	6.037
Species number							
1	64	0.093	0.04	3.81	0.00032*	0.19	5.868
16	48	0.079	0.036	3.92	0.00022*	0.18	5.84
Total biomass	112	0.00153**	−0.000545	−0.000036	0.00087**	0.00011	0.0000403
R^2		0.34	0.18	0.23	0.25	0.15	0.21

Mixed-effects model parameter estimates of fast and slow C pool sizes (C_f, C_s, C_{NHC}, and total C: mg C g soil^{-1}), and decomposition rates (k_f and k_s: day^{-1}).
*$^{\dagger}P \leq 0.1$, *$P \leq 0.05$, **$P \leq 0.01$ (ANCOVA). No significant interactions were found $P \leq 0.1$.*

(Dijkstra and others 2006), and also no offset of soil respiration by increased litter inputs associated with N additions in high species-richness plots (Dijkstra and others 2005).

Increasing species richness decreased the fast pool decay rate, a change associated with higher total plant biomass in more diverse plots. Increasing species richness also increased the slow pool decay rate without affecting its size—suggesting an associated increase in slow pool inputs and accelerated cycling of slow soil C. Increased slow pool C cycling could be due to an increase in slow C inputs under non-limiting N conditions for microbes (Kaye and Hart 1997; Kuzyakov 2002), changes in the quality of slow C inputs (for example, the ratio of root litter to exudates or decreased C/N of root litter and exudates), or the rhizosphere priming effect (Fontaine and others 2004; Pregitzer and others 2008; Zak and others 2008). High root C inputs should increase slow C decomposition when there is sufficient N available for microbes or if the inputs are of higher quality (for example, lower C/N). The rhizosphere priming effect would cause an increase in decomposition of older, N-rich soil C when N supply to microbes is insufficient. Unfortunately, our results do not allow us to reject any of the potential causes of increased slow pool C cycling. However, there is evidence that the increase in total belowground C allocation in diverse plots is due to root biomass—suggesting a higher ratio of root tissues to exudates in diverse plots relative to monocultures (Adair and others 2009)—hinting that changes in slow pool inputs may be driving the increased slow pool cycling.

Our results contrast those of similar research at Cedar Creek Ecosystem Science Reserve (Fornara and Tilman 2008; Tilman and others 2006) that found significantly higher soil C accumulation in 16 species plots compared to monocultures at the same depths that we sampled. In that study, the

topsoil was removed from the plots before the start of their experiment. Thus, initial soil C concentrations were lower, which may have contributed to the higher rates of total C accumulation in diverse plots in that study compared to in our study.

Nitrogen

Soil C dynamics have been shown to depend heavily on the available N in soils (DeForest and others 2004; Fontaine and others 2003, 2004, 2011; Pregitzer and others 2008), with old soil C acting as a nutrient bank (sensu Fontaine and others 2011). Consistent with this mechanism, N additions in this low-nutrient grassland have stimulated cellulose decomposition (Keeler and others 2009) and increased the decomposition rate of labile C in the soil (Dijkstra and others 2005, 2006) and litter (Hobbie and others in revision) probably due to the alleviation of N limitation of C decomposition. N additions increased resistant C by 10 % in our three-pool model of soil C, with no changes to the slow pool size or rate. As hypothesized, this increase was associated with higher total plant biomass. Our results are consistent with the nutrient bank hypothesis (sensu Fontaine and others 2011), although we can not differentiate between the possible mechanisms of physio-chemical stabilization (for example, stabilization of lignin-rich litter inputs by N additions, Dijkstra and others 2004; von Lützow and others 2008) that were responsible for the marginally significant increase in resistant C that we observed in the presence of N additions.

Because the effect of N additions on the resistant pool was small and the resistant pool is less than one-third of total SOC, there was no detectable change in total SOC. However, the resistant pool has an assumed MRT of 1,000 years, so even a modest increase of 10 % could result in a long-term increase in C sequestration. The projected increase in total SOC in N addition treatments compared to ambient N treatments would be 5 % after 13 years and 20 % after 49 years.

Elevated CO_2

As expected, elevated CO_2 alone did not increase C storage, and actually led to increased turnover of slow C which taken alone would result in decreased C storage over the long-term. However, the slow pool size remained constant between ambient and elevated CO_2, indicating a concurrent increase in inputs to the slow pool in the elevated CO_2 treatments. Our results therefore contrast with the decline in sequestration associated with elevated CO_2 reported by Heath and others (2005), but provide further support for increased rates of belowground C cycling under elevated CO_2 (Adair and others 2009; Hagedorn and others 2003; Hungate and others 1997; van Kessel and others 2000). The implied increase in C inputs is consistent with increased plant biomass observed in response to elevated CO_2 in grasslands (Adair and others 2009; Dijkstra and others 2005, 2006; Reich and others 2006a, b). The higher decay rate of the slow pool in elevated CO_2 was associated with increases in plant biomass at elevated CO_2. Consistent with the soil nutrient bank hypothesis (sensu Fontaine and others 2011), and the progressive N limitation hypothesis, our results suggest that additional plant production in elevated CO_2 may increase soil C:N, causing N-limited microbes to increase decomposition of slow C for access to N (Gill and others 2002). Using a three-pool model of soil C turnover we were able to detect faster C cycling and higher C inputs in the slow pool that others were not able to detect with a simpler two-pool model (Dijkstra and others 2005).

Interactions

Contrary to our expectations, we found no significant interactions between CO_2 and N on any soil C pools or turnover rates. Interestingly, our results suggest that CO_2 and N affect belowground C cycling in different ways: CO_2 increased the inputs to the slow pool and its decomposition rate, whereas N increased the resistant pool size. The differences in effects are likely due to differences in total belowground carbon allocation (TBCA). Adair and others (2009) found that both CO_2 and N increased TBCA at BioCON. Although the effect of N was entirely due to concurrent increases in root biomass, the effects of CO_2 on TBCA could not be explained by root biomass alone (Adair and others 2009), implying an increase in allocation to root exudates or arbuscular mycorrhizae (AM), consistent with studies elsewhere (Pendall and others 2004; Treseder and Allen 2000; Treseder 2004). Increased allocation to mycorrhizae at BioCON is supported by increased AM spore volume in elevated CO_2 plots (Antoninka and others 2011; Wolf and others 2003). An increase in either root exudates or allocation to AM caused by elevated CO_2 is likely to prime decomposition of older, N-rich soil C to alleviate N limitation (Fontaine and others 2011; the microbial activation hypothesis, Kuzyakov 2002). In contrast, the N additions resulted in allocation of TBCA to root biomass (Adair and others 2009). The lack of additional C inputs to root exudates or AM in elevated N treatments limits

mycorrhizal exploration and the decomposition of older, N-rich soil C, possibly preserving slow and resistant soil C. Thus, we found CO_2 and N treatment effects to be additive and not interactive.

Conclusions

Our results suggest that although elevated CO_2, added N and changes in diversity alter belowground C cycling, these changes are unlikely to result in rapid, substantial C sequestration in course textured soils such as studied here. Elevated CO_2 only increased the cycling rate of slow C, suggesting that a portion of the previously observed increases in rates of belowground cycling at elevated CO_2 (Adair and others 2009; Hungate and others 1997) is likely associated with slowly cycling soil pools of C (in the absence of concurrent labile plant C inputs). Increasing species richness also increased belowground cycling of slow C, but also resulted in larger, more slowly decaying fast C pools; changes that are unlikely to increase total C storage. We believe that the removal of all topsoil in previous studies of the effects of species diversity on soil C storage (Fornara and Tilman 2008; Tilman and others 2006) may explain the large soil C increases that others have observed that we were unable to replicate. Although CO_2 and species-richness treatments are unlikely to increase soil C storage, our results suggest that N additions to N-limited grasslands on coarse-textured soils may result in a small, long-term sink for soil C.

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