

REPORT

Nitrogen deposition and plant species interact to influence soil carbon stabilization

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Abstract

Anthropogenic nitrogen (N) deposition effects on soil organic carbon (C) decomposition remain controversial, while the role of plant species composition in mediating effects of N deposition on soil organic C decomposition and long-term soil C sequestration is virtually unknown. Here we provide evidence from a 5-year grassland field experiment in Minnesota that under elevated atmospheric CO₂ concentration (560 ppm), plant species determine whether N deposition inhibits the decomposition of soil organic matter via inter-specific variation in root lignin concentration. Plant species producing lignin-rich litter increased stabilization of soil C older than 5 years, but only in combination with elevated N inputs (4 g m⁻² year⁻¹). Our results suggest that N deposition will increase soil C sequestration in those ecosystems where vegetation composition and/or elevated atmospheric CO₂ cause high litter lignin inputs to soils.

Keywords

Carbon isotopes, elevated CO₂, grassland species, humification, lignin, nitrogen isotopes, root litter, soil carbon sequestration, soil organic matter decomposition.

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INTRODUCTION

Increased nitrogen (N) deposition, largely caused by agricultural fertilizer use and fossil fuel combustion, potentially affects the global carbon (C) cycle by directly influencing terrestrial ecosystem processes such as productivity and decomposition (Magill *et al.* 1997; Vitousek *et al.* 1997). Additionally, N effects on community attributes such as biodiversity and exotic species invasions (Vitousek *et al.* 1997; Dukes & Mooney 1999; Sala *et al.* 2000; Zavaleta *et al.* 2003; Stevens *et al.* 2004) can influence the quantity and chemistry of C fixed by plants and ultimately decomposed in soils. However, the interactive effects of increased N deposition and plant species composition on long-term soil C sequestration are unknown.

The amount of organic C that is stored in the soil (1.5 × 10¹⁸ g C) is globally about twice that of the total C in the atmosphere (Schlesinger 1997). Most of the organic C in soils occurs as humic substances produced by the transformation of plant litter into more persistent organic compounds (humification). Because of the long turnover times of humified soil organic matter, even small changes in the humification rate can result in substantial changes in soil C storage over the long-term.

Preliminary evidence suggests that N plays an important role in humification and the formation of stable organic compounds in soils. Additions of N as chronically high N deposition, N fertilization, or N fixation have led to reduced decomposition of humus (Berg & Matzner 1997; Matzner 2002) and increased formation of refractory soil C (Neff *et al.* 2002; Resh *et al.* 2002; Hagedorn *et al.* 2003). Additionally, N enrichment can reduce the competitive ability of fungi that are responsible for lignin decomposition (Fog 1988), suppress the formation of enzymes that break down lignin (Carreiro *et al.* 2000; Saiya-Cork *et al.* 2002), and N can react with lignin residues (phenolic compounds) to form complexes highly resistant to microbial degradation (Nömmik & Vahtras 1982; Stevenson 1994; Zech & Kögel-Knabner 1994). Consequently, plant litter high in lignin content could in theory enhance the formation and stability of refractory soil C in response to N addition. However, to our knowledge, this has never been demonstrated.

We used ¹³C and ¹⁵N isotopes to study the influence of N addition on the stability of refractory soil C for 12 grassland species planted as monoculture plots under elevated CO₂ (560 ppm) in Minnesota, USA. We provide evidence that plant species with higher root lignin litter inputs increase the stability of refractory soil C relative to

species with lower root lignin litter inputs, but only when inorganic N ($4 \text{ g m}^{-2} \text{ year}^{-1}$) is added.

MATERIALS AND METHODS

Field experiment

The BioCON (Biodiversity, CO_2 and N) grassland field experiment is located at the Long-Term-Ecological Research site at the Cedar Creek Natural History Area in Minnesota, USA, on a nutrient-poor sandy outwash plain. In 1997, 354 plots ($2 \text{ m} \times 2 \text{ m}$) evenly distributed among six circular areas or rings (diameter 20 m) were sown with either 1, 4, 9, or 16 species of a pool of 16 grassland species equally divided over four functional groups (C4 grasses, C3 grasses, C3 legumes, C3 forbs). An additional 12 plots (two in each ring) were kept free of vegetation (bare plots) during the experiment. Three rings were treated with 560 ppm atmospheric CO_2 concentration using FACE technology (starting in April 1998), a concentration expected to occur globally before the end of this century (Intergovernmental Panel on Climate Change 2001). The CO_2 received by the elevated CO_2 plots is strongly depleted in ^{13}C ($\delta^{13}\text{C} = -41.2$). Since 1998, half of the plots received $4 \text{ g N m}^{-2} \text{ year}^{-1}$ (NH_4NO_3), which is comparable with N deposition rates in highly industrialized areas (Vitousek 1994). The N fertilizer applied was spiked with ^{15}N (atom% ^{15}N of fertilizer = 0.38498%). The experiment was set up in a fully blocked design (for more details see Reich *et al.* 2001a,b). For this study, we focused on the 12 bare plots and the 48 monoculture plots planted with one of the 12 C3 plant species (the grasses *Agropyron repens*, *Bromus inermis*, *Koeleria cristata*, and *Poa pratensis*; the forbs *Achillea millefolium*, *Anemone cylindrica*, *Asclepias tuberosa*, and *Solidago rigida*; and the legumes *Amorpha canescens*, *Lespedeza capitata*, *Lupinus perennis*, and *Petalostemum villosum*) in the elevated CO_2 rings. We excluded the monoculture plots with C4 grasses in the elevated CO_2 rings from our study because we were unable to separate soil C pools in these plots using ^{13}C isotopes.

Sampling and analyses

In July 2002, we sampled three soil cores (diameter 2.5 cm) to 10-cm depth from each plot. We sieved soils (2 mm) to remove roots and then separated soils into light and heavy soil fractions in a sodium iodide (NaI) solution (density 1.8 g cm^{-3} , Gregorich & Ellert 1993). We placed 15 g of air-dry soil into 50 mL plastic centrifuge tubes and added 30 mL NaI solution. After 16 h of shaking and 1 day of settling we removed the light soil fraction floating on top of the solution. Both light and heavy fraction were filtered (through Magna 0.45 μm nylon and Whatman G/F glass fiber filter, respectively) and thoroughly rinsed with

nanopure water. Light and heavy fractions were dried and ground with mortar and pestle. We calculated light and heavy soil fractions per m^2 using soil bulk densities that were measured from a soil core (diameter 5 cm) to 10-cm depth taken in each plot in August 2003. The heavy soil fraction consists of clay-associated soil organic matter that is more recalcitrant while the light soil fraction is thought to contain partially degraded organic compounds that undergo further relatively rapid decomposition (Gregorich & Janzen 1995).

Aboveground biomass sampled in June 2002 from $10 \times 100 \text{ cm}$ strips clipped just above the soil surface (Reich *et al.* 2001b) and the light and heavy soil fractions were analysed for total C and $\delta^{13}\text{C}$ on a mass spectrometer (ThermoFinnigan Delta Plus, Bremen, Germany). Plant biomass of the 12 C3 grassland species grown under elevated CO_2 showed ^{13}C signatures that were significantly lower ($\delta^{13}\text{C}$ between $-41.8 \pm 0.6\text{‰}$ and $-39.8 \pm 0.4\text{‰}$, mean \pm SE, among species) than the ^{13}C signature of soil C in the bare plots ($\delta^{13}\text{C} = -23.6 \pm 0.1\text{‰}$). Because of the absence of litter inputs in the bare plots, the soil ^{13}C signature of these plots represents that of 'old' soil C, i.e. C formed before the CO_2 treatment started in 1998. During the experiment, incorporation of 'new' C (i.e. C formed after the initiation of the CO_2 treatment) into the soil in the vegetated plots treated with elevated CO_2 decreased the soil ^{13}C signature over time. The fraction of new C (f_{new}) in each soil fraction was calculated with $f_{\text{new}} = (\delta^{13}\text{C}_{\text{soil}} - \delta^{13}\text{C}_{\text{bare}}) / (\delta^{13}\text{C}_{\text{biomass}} - \delta^{13}\text{C}_{\text{bare}})$ where $\delta^{13}\text{C}_{\text{soil}}$ is the $\delta^{13}\text{C}$ of the light or heavy soil fraction of each planted plot, $\delta^{13}\text{C}_{\text{bare}}$ is the average $\delta^{13}\text{C}$ of the light or heavy soil fraction from the six bare plots in the ambient CO_2 rings (pooled across N treatment), and $\delta^{13}\text{C}_{\text{biomass}}$ is the $\delta^{13}\text{C}$ of the plant biomass of each planted plot. We used aboveground biomass for the ^{13}C isotope measurements instead of belowground biomass to avoid potential C contamination from soil organic matter. Although above- and belowground biomass may differ slightly in $\delta^{13}\text{C}$ (e.g. less than 1‰, Nadelhoffer & Fry 1988; Lin *et al.* 1999), these differences fall within intra-specific variation among individual plants and are small compared with the difference in $\delta^{13}\text{C}$ between new and old C (c. 17‰). For the N fertilized and bare plots we analysed the light and heavy soil fraction for total N and $\delta^{15}\text{N}$, simultaneous with $\delta^{13}\text{C}$. The fraction of N from the N fertilizer stored in each soil fraction after 5 years of application ($f_{\text{fertilizer}}$) was calculated with $f_{\text{fertilizer}} = (\delta^{15}\text{N}_{\text{soil}} - \delta^{15}\text{N}_{\text{bare}}) / (\delta^{15}\text{N}_{\text{fertilizer}} - \delta^{15}\text{N}_{\text{bare}})$ where $\delta^{15}\text{N}_{\text{soil}}$ is the $\delta^{15}\text{N}$ of the light or heavy soil fraction of each planted plot, $\delta^{15}\text{N}_{\text{bare}}$ is the average $\delta^{15}\text{N}$ of the light or heavy soil fraction from the six unfertilized bare plots (pooled across CO_2 treatment, $\delta^{15}\text{N} = 1.6$), and $\delta^{15}\text{N}_{\text{fertilizer}}$ the $\delta^{15}\text{N}$ of the spiked N fertilizer ($\delta^{15}\text{N} = 51.2$).

In June 1999 and 2002, we sampled roots to 20-cm soil depth for chemical analysis by taking three soil cores (diameter 2.5 cm) from each plot. Roots were collected by sieving soils (2 mm), and then dried and ground. A 0.5 g sub-sample was analysed for acid insoluble compounds (lignin and other recalcitrant compounds such as suberin, henceforth collectively called lignin) on a Fiber Analyser (Ankom, Macedon, NY, USA; Van Soest 1994). We focused on roots because they represent roughly three-fourths of plant biomass in these grassland ecosystems (Reich *et al.* 2001b) and are a critical source of litter inputs. We used root ingrowth cores to measure root growth during multiple incubation periods. At each plot one soil core (diameter 3.8 cm) was taken to 20-cm depth. Roots were separated from soil by sieving (2 mm) and handpicking. A core made out of chicken wire was placed into the hole that was then filled back up with the root-free soil. At the end of each incubation period the core was pulled and soils were sieved and handpicked for roots that grew into the core. Roots were dried and weighed. There were three incubation periods during each growing season (May to September) and one during the remainder of the year (September to May) from 1998 to 2001. The last incubation ended in August 2002. We calculated total root death in the top 10 cm of the soil by adding the root mass grown into the cores from all incubation periods minus the net change in standing root biomass in the soil between May 1998 and August 2002, divided by two. We calculated lignin input through root litter during this period by multiplying root lignin concentrations with total root death.

We used analysis of variance (ANOVA) to test for significant N fertilization and plant species identity effects and interactions on new and old C pools in the light and heavy soil fraction. We used analysis of covariance (ANCOVA) with lignin input through root litter or root lignin

concentration as a covariate to test for significant covariate effects and N fertilization \times covariate interactions on new and old C pools in the light and heavy soil fraction. We used ANOVA to test for species identity effects on N fertilizer stored in the light and heavy soil fraction and linear regressions to test for significant relationships between N fertilizer retained in the soil and the amount of old or new soil C. Some variables data were transformed (natural log) to meet assumptions of normality and homoscedasticity (JMP 4.0.4).

RESULTS

After 5 years of treatment, both the old and new C pools in the light soil fraction were 25–30% greater in the N enriched than in the unamended plots (Table 1). The old and new C pools in the heavy soil fraction were also higher in the N fertilized plots, although not significantly so. The 12 grassland species differed significantly in the amount of old and new C in the light soil fraction and responded differently to N fertilization in terms of the old C pool (Fig. 1, Table 1). *Poa pratensis* and *Solidago rigida* showed especially large increases in old and new C in the light soil fraction with N fertilization. The old C pool in the heavy soil fraction did not show any significant treatment effects, likely in part because this pool is comparatively large and its dynamics are slow relative to the 5-year duration of the study.

Both root lignin concentration and cumulative input of lignin through dead roots (i.e. root litter lignin) differed significantly among plant species ($P = 0.04$ and $P = 0.0002$, respectively, Fig. 2). Species differences in cumulative input of lignin through dead roots were driven mainly by species differences in root biomass production, as these two parameters were tightly correlated ($r = 0.91$). None of

	Light fraction		Heavy fraction	
	Old C (g m ⁻²)	New C (g m ⁻²)	Old C (g m ⁻²)	New C (g m ⁻²)
Control	66.9(4.2)	42.1 (7.1)	454 (25)	43.9 (8.8)
N fertilized	84.8 (5.0)	55.3(7.6)	506 (21)	56.5 (7.4)
<i>F</i> ratios				
N fertilization	12.60**	3.09 [†]	2.33	1.47
Species identity	2.31*	4.75***	0.92	1.90 [†]
N \times species identity	2.57*	1.04	0.28	0.98

Table 1 Old and new carbon pools (mean \pm SE) in the light and heavy soil fraction

Old carbon was present in soils before treatments were initiated.

F ratios are derived from ANOVA using all 48 planted plots with N fertilization and species identity as main effects. Means of control and N-fertilized treatments are shown averaged across species treatments. We found no significant differences among functional groups or interactions with N fertilization.

Symbols used for level of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; [†] $P < 0.1$.

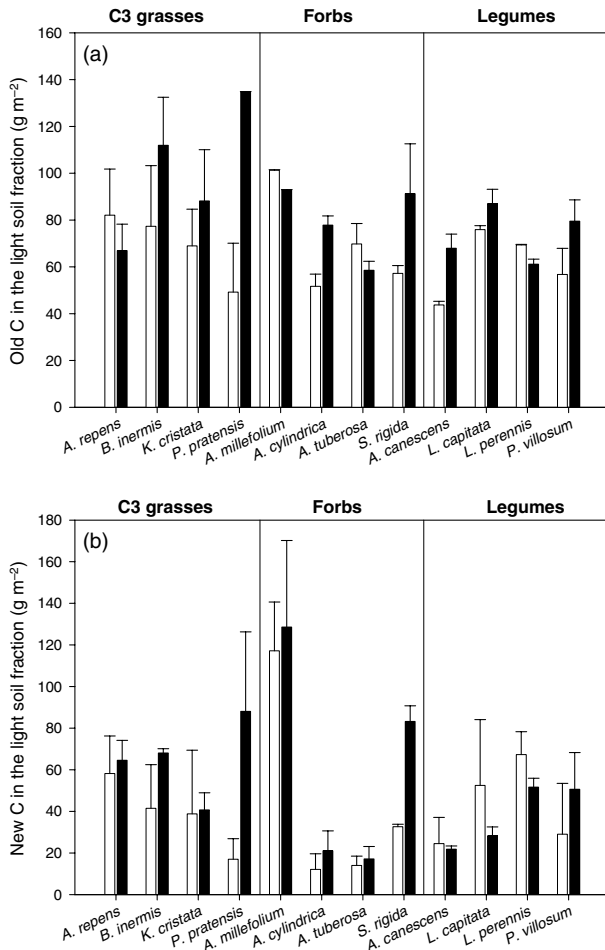


Figure 1 (a) Old and (b) new carbon in the light soil fraction separated by species identity and N fertilization treatment (open bars: unfertilized, solid bars: fertilized). After 5 years of treatment species identity showed significant effects on old carbon ($P = 0.04$) and new carbon ($P = 0.0007$) in the light soil fraction, while old carbon showed a significant interaction with the N treatment ($P = 0.02$). No significant differences were found among functional groups.

these factors (root lignin concentration, root biomass production, or cumulative root lignin inputs) were significantly affected by N fertilization and none showed significant plant species by N fertilization interactions; hence they represent intrinsic species traits.

The higher amounts of new and old soil C in the light fraction with N fertilization was particularly pronounced for plants that produced relatively more root litter lignin (Fig. 3). When we included cumulative lignin input through root litter as a covariate in ANCOVA models for old and new C in the light soil fraction, we found significant lignin input ($P = 0.004$ for old C and $P = 0.03$ for new C) and N \times lignin input interaction effects ($P = 0.009$ for old C

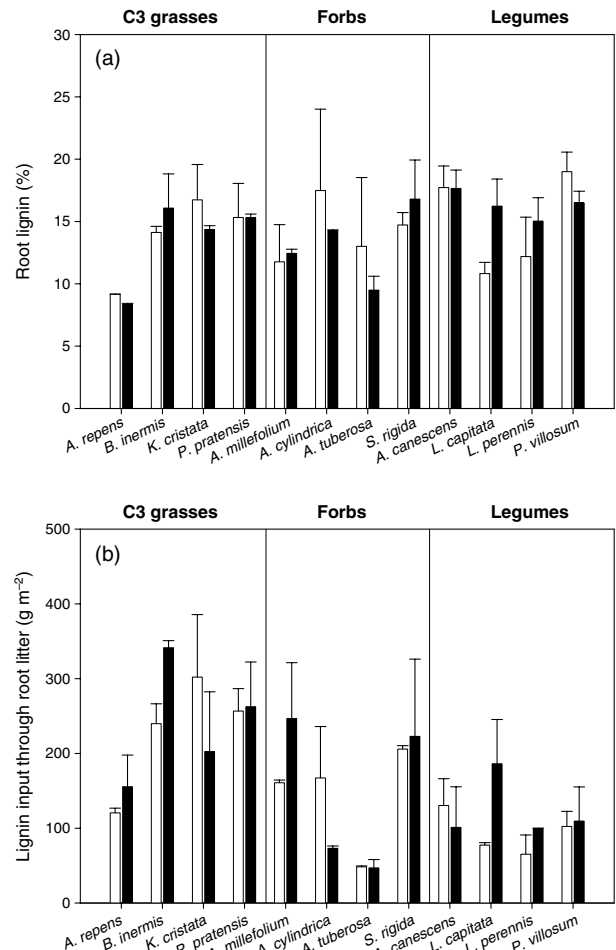


Figure 2 (a) Root lignin concentration and (b) cumulative lignin input through root litter (total input from spring 1998 to autumn 2002) separated by species identity and N fertilization treatment (open bars: unfertilized, solid bars: fertilized). Species identity showed significant effects on root lignin concentration ($P = 0.04$) and cumulative lignin input through root litter ($P = 0.0002$), while the N treatment or N \times species identity interactions were not significant ($P > 0.1$). Cumulative lignin input through root litter differed significantly among functional groups ($P = 0.0003$).

and $P = 0.03$ for new C). The ANCOVA models could explain more of the variance in old and new C with cumulative lignin input through root litter ($R^2 = 0.41$ and 0.26 , respectively) than with total root biomass production as a covariate ($R^2 = 0.28$ and 0.19 , respectively), despite the tight relation between the two. Further, ANCOVA models with total root biomass production as a covariate showed no significant N \times total root biomass production interactions. In contrast, when we used root lignin concentration as a covariate, we found significant root lignin concentration ($P = 0.02$) and N \times root lignin concentration effects ($P = 0.003$) for the old C in the light fraction. These results

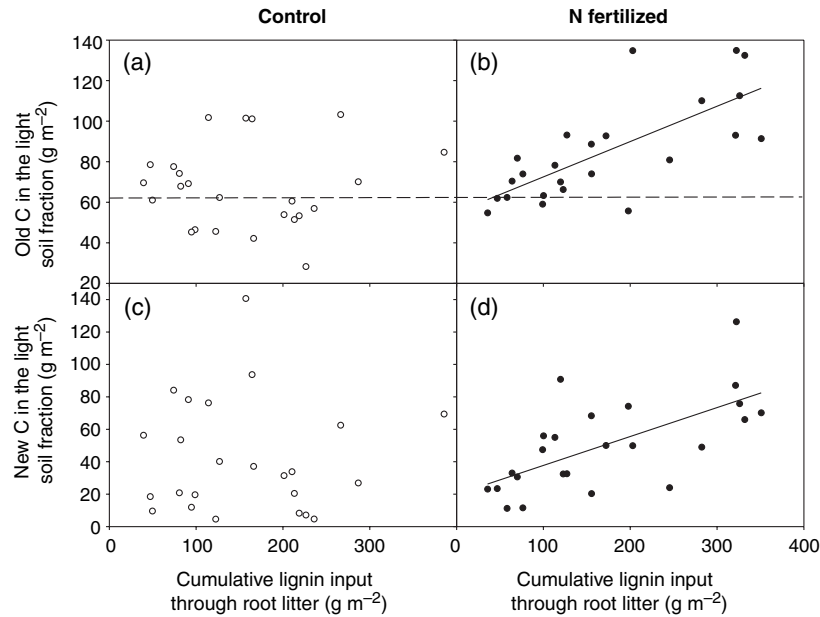


Figure 3 (a and b) Old and (c and d) new carbon in the light soil fraction as a function of cumulative root lignin input for all 48 planted plots. After 5 years of treatment, there was a significant interaction between lignin input and N treatment in an analysis of their effects on old and new carbon in the light soil fraction (see text). Therefore, we did separate regressions of light fraction soil carbon against root lignin for control (a, c) and N fertilized (b, d) treatments. Old and new carbon in the light soil fraction were significantly positively related to root lignin input (total input from spring 1998 to autumn 2002) in the nitrogen fertilized plots (old C: $R^2 = 0.54$, $P < 0.0001$, new C: $R^2 = 0.25$, $P = 0.01$), but not in the control plots ($P > 0.1$). Dashed line in (a) and (b) shows the average pool size of the old carbon in the light soil fraction in the bare plots under ambient atmospheric CO₂ ($n = 6$) for comparison.

indicate that root lignin and N \times lignin interactions (particularly for the old C pool) are not entirely a result of effects of variation in the quantity of plant litter inputs.

The amount of N fertilizer retained in the light soil fraction was significantly positively related (Fig. 4) to both the old ($R^2 = 0.31$, $P = 0.002$) and new ($R^2 = 0.64$, $P < 0.0001$) C pool in the light soil fraction. Much of the variation in the amount of N fertilizer retained in the light soil fraction was explained by plant species composition (ANOVA: $R^2 = 0.73$, $P = 0.03$), likely because of plant species differences in litter inputs.

DISCUSSION

Our results indicate that N availability in the soil interacts with root lignin to stabilize old and new C in the light soil fraction, with the greatest stabilization occurring under high N and high root lignin inputs. Because old soil C pools did not receive any litter inputs since the start of the experiment, treatment effects on the old soil C pool size in the light soil fraction must have been caused by differences in decomposition. Both the unfertilized and fertilized planted plots had on average higher old C pools in the light soil fraction than the bare plots (average increase of 6 and 24 g m⁻², respectively), suggesting that the presence of plants reduced

the decomposition of old C in the light soil fraction, especially when plots were fertilized with N. Higher new C pools under a combination of N fertilization and higher root lignin inputs were likely caused by increased litter inputs, although reduced decomposition may have contributed to the increase in new C. Increased decomposition of fresh litter with N addition has also been observed, but then especially for litter types with low lignin concentration (Hobbie 2000).

Further evidence for the effects of N fertilization on old soil C stabilization comes from the positive relationship between the amount of N fertilizer retained and the old C pool in the light soil fraction. This relationship suggests that some of the N fertilizer was incorporated (perhaps abiotically) into the old C pool causing an increase in the stability of that pool. The positive relationship between the amount of N fertilizer retained and the new C pool in the light fraction likely resulted from incorporation of N fertilizer through plant uptake of N fertilizer and subsequent death and decomposition of plant litter.

One possible mechanism for the interaction between soil N availability and plant litter lignin input is that N reacts abiotically with lignin breakdown products to form compounds that are resistant to further microbial decomposition (Nömmik & Vahtras 1982; Zech & Kögel-Knabner 1994).

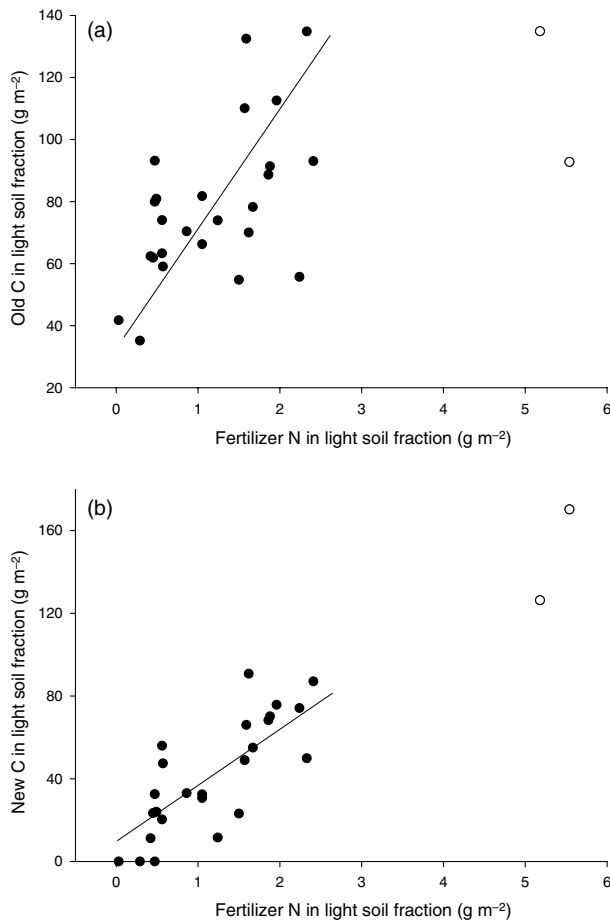


Figure 4 (a) Old and (b) new C in the light soil fraction plotted against fertilizer nitrogen in the light soil fraction in the nitrogen fertilized plots after 5 years of treatment (bare plots included). Linear regressions showed that both old ($R^2 = 0.31$, $P = 0.002$) and new carbon ($R^2 = 0.64$, $P < 0.0001$) were positively related to the nitrogen fertilizer in the light soil fraction. Outliers (open symbols) were excluded from the analysis.

Alternatively, N enrichment has been shown to inhibit the formation of enzymes that break down lignin (Carreiro *et al.* 2000; Saiya-Cork *et al.* 2002) possibly leading to accumulation of lignin. This mechanism could have retarded decomposition of new C, and could have altered old C pools if the same oxidative enzymes involved in lignin degradation are also involved in humus decomposition. However, it would not explain the relationship between retention of fertilizer N with light fraction C pool sizes. A third possibility is that N fertilization reduced the competitive ability of microorganisms that decompose lignin (Fog 1988) and perhaps other recalcitrant compounds. Regardless of the mechanism, our results are the first to show that plant species with higher lignin concentrations in their tissues increase the stabilization and storage of soil C in the presence of added N through reductions in decomposition.

Our estimates of the stabilizing effect of lignin on old C in the light soil fraction are conservative for the following reason: lignin is depleted in ^{13}C relative to most other organic compounds (Benner *et al.* 1987; Wedin *et al.* 1995), and thus increased inputs of lignin to soil may have decreased the soil ^{13}C in the soil even more than the decrease in ^{13}C caused by the incorporation of new C from the ^{13}C depleted CO_2 fumigation treatment alone. Therefore, we may have underestimated the size of the old C pool, especially in plots receiving high inputs of lignin.

Our results suggest that increased anthropogenic N deposition has the potential to increase stable soil organic matter pools, but that this potential strongly depends on plant species composition and associated lignin inputs. Any systematic trends in lignin inputs that result from plant species shifts caused by global change such as land use change, exotic invasions, and woody plant encroachment, as well as potential increases in plant tissue lignin concentrations caused by elevated atmospheric CO_2 (Norby *et al.* 2001), could lead to corresponding changes in soil C stabilization with potential impacts on the global carbon cycle.

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