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Variable effects of nitrogen additions on the stability and turnover of soil carbon

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Soils contain the largest near-surface reservoir of terrestrial carbon¹ and so knowledge of the factors controlling soil carbon storage and turnover is essential for understanding the changing global carbon cycle. The influence of climate on decomposition of soil carbon has been well documented^{2,3}, but there remains considerable uncertainty in the potential response of soil carbon dynamics to the rapid global increase in reactive nitrogen (coming largely from agricultural fertilizers and fossil fuel combustion). Here, using ¹⁴C, ¹³C and compound-specific analyses of soil carbon from long-term nitrogen fertilization plots, we show that nitrogen additions significantly accelerate decomposition of light soil carbon fractions (with decadal turnover times) while further stabilizing soil carbon compounds in heavier, mineral-associated fractions (with multidecadal to century lifetimes). Despite these changes in the dynamics of different soil pools, we observed no significant changes in bulk soil carbon, highlighting a limitation inherent to the still widely used single-pool approach to investigating soil carbon responses to changing environmental conditions. It remains to be seen if the effects observed here—caused by relatively high, short-term fertilizer additions—are similar to those arising from lower, long-term additions of nitrogen to natural ecosystems from atmospheric deposition, but our results suggest nonetheless that current models of terrestrial carbon cycling do not contain the mechanisms needed to capture the complex relationship between nitrogen availability and soil carbon storage.

Human activity now fixes more atmospheric N₂ into biologically available forms each year than all natural processes combined⁴,

causing a wide range of cascading environmental responses, including a possible sink for excess atmospheric CO₂ through stimulation of plant growth in N-limited ecosystems⁵. However, on average, soils contain three times as much C as does terrestrial vegetation¹. Thus if changes in N availability alter soil C turnover, net C sinks from increased plant growth could be significantly enhanced or reduced, depending on the direction of the soil responses. Unfortunately, considerable uncertainty remains concerning the relationship between N availability and decomposition processes. Additions of N and/or natural variation in N concentrations have led to increases, decreases or no change in observed decomposition rates. This is true for field studies of litter and soil organic matter (SOM) decomposition, as well as for laboratory experiments^{6–10}.

In part, these varied results occur because decomposition studies are difficult to carry out and interpret. Soils are highly complex media with a diversity of substrates that vary in both the energy required for their breakdown and in their total N content. As a result, additions of N to soils could increase decomposition rates of some SOM fractions while simultaneously decreasing rates for other fractions. Past results suggesting widely varied—or no—responses of decomposition to N inputs may be due to varied and compensatory responses of SOM fractions that are difficult to detect with traditional measurements, such as mass loss or CO₂ efflux.

Data from long-term N-fertilized plots (defined as +N hereafter) at an alpine site on Niwot Ridge, Colorado, provide an example of the uncertainties associated with traditional measurements. Fertilized plots in dry meadow communities of alpine tundra at this site have received annual inputs of 10 g N m⁻² yr⁻¹ since 1990 (ref. 11) (see Methods section). The decade of N fertilization has greatly increased productivity; over the past ten years, an average of 72 g m⁻² yr⁻¹ of extra C has entered the fertilized plots (Table 1). Despite these increases in productivity, soil C concentrations are not significantly different between control and +N plots (Table 1). Against the large standing pools of SOM carbon on Niwot Ridge and elsewhere, large changes in C inputs or alteration of turnover times rarely result in statistically significant changes in soil C concentrations^{12,13}.

In contrast to bulk C values, the results of radiocarbon, ¹³C and compound-specific analyses of soil C on Niwot Ridge all point to an acceleration of intermediate-age SOM turnover, and a possible increase in the stabilization of some forms of C into mineral SOM pools. Atmospheric testing of atomic weapons in the early 1950s led to a rapid increase in the ¹⁴C content of atmospheric CO₂, followed by a gradual decline to the present¹⁴ (Fig. 1). The time-dependent change in ¹⁴CO₂ can be used to examine the turnover time of intermediate-age soil C formed in equilibrium with the atmosphere. For soil samples, we performed ¹⁴C measurements on 'light' and 'heavy' SOM fractions using a high-density solution. The heavy fraction is thought to be largely recalcitrant C associated with soil minerals, with turnover times of several decades to centuries, while the more labile light fraction reflects a mixture of compounds that includes microbial biomass, partially degraded plant material and older, more humified, by-products of decomposition^{15,16}. In the dry meadow communities on Niwot Ridge, the light fraction is not significantly altered by fertilization and contributes over half of total soil C to both control and +N plots (Table 1).

The Δ¹⁴C of incoming plant C determined from summer plant harvests from 1990 to the present is similar to the Δ¹⁴C of

Table 1 Productivity and soil C and N concentrations on Niwot Ridge

	Above ground productivity (g m ⁻² yr ⁻¹)	%C	Soil C in the light fraction (%)	%N	C:N ratio
Control	151 (11)*	8.61 (1.12)*	55.12 (2.14)*	0.67 (0.07)*	12.65 (0.74)*
+N	223 (16)†	10.40 (1.24)*	60.44 (1.91)*	0.83 (0.08)*	12.37 (0.33)*

Values shown are means with standard errors in parentheses. Statistically significant differences below the $P < 0.05$ level are shown by contrasting symbols within columns. Average annual productivity in the control and fertilized plots is significantly different ($F = 54.54$, $P < 0.001$). +N, long term N-fertilized plots.

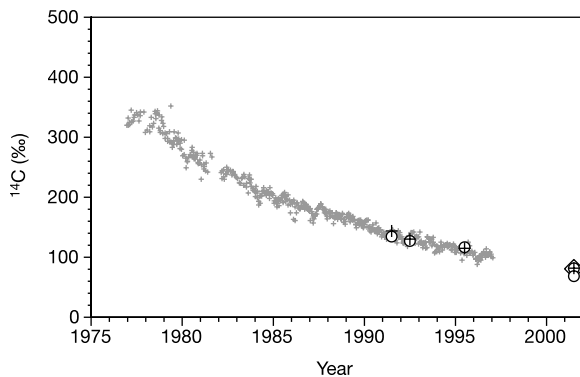


Figure 1 ^{14}C activity of Niwot Ridge plant material compared to atmospheric trends¹³. $\Delta^{14}\text{C}$ of atmospheric CO_2 from ref. 14 is shown by small grey crosses. $\Delta^{14}\text{C}$ of plant biomass for 1992, 1993, 1996 and 2001 is shown for control (open circles) and N-fertilized plots (crosses and diamond symbols).

atmospheric CO_2 , and exhibits a decline to a contemporary $\Delta^{14}\text{C}$ value of approximately 80‰ (Fig. 1). The light fraction of SOM from control plots has a $\Delta^{14}\text{C}$ of 126‰, indicative of a pool of soil C with a turnover of approximately a decade. Heavy-fraction $\Delta^{14}\text{C}$ of control plots is -13‰, reflecting C deposited mainly before atmospheric testing of nuclear weapons¹⁴. Fertilization leads to a significant reduction in light-fraction SOM $\Delta^{14}\text{C}$, to an average of 54.6‰; this value is below the 2001 input signal of approximately 80‰ (Table 2).

The most likely cause for the decline in +N light-fraction $\Delta^{14}\text{C}$ is the accelerated decomposition of highly labelled organic matter deposited roughly 10–30 years ago when atmospheric $\Delta^{14}\text{C}$ values were higher, combined with increased productivity (and inputs of less-enriched carbon) over the past decade. Changes in the structure of soil carbon after N addition suggest that relatively unaltered plant carbon resides in these soils for years to decades and then disappears as a direct or indirect result of fertilization.

Three biomarkers for a range of plant compounds that are consistent across a number of species, and reflect different plant biochemical constituents, also indicate an acceleration of the decomposition of residual plant material in fertilized soils¹⁷. Both 2-methoxy-4-vinylphenol (a biomarker for relatively undegraded plant lignin¹⁷) and two plant polysaccharide markers¹⁷ (5-methyl-2-furanone and 2-hydroxy-3-methyl-2-cyclopentenone) are substantially lower in the light fraction of fertilized plots. The concentration of the lignin biomarker is 93% lower in fertilized plots than in control plots, while the two polysaccharide markers decline by an average of 91% (Table 2). In addition, increasing lignin biomarker content in the heavy fraction of fertilized soils suggests that some plant material may be moving directly into stabilized, mineral-associated SOM pools, which may explain the small increase in the $\Delta^{14}\text{C}$ of the fertilized heavy-fraction soils.

The combination of lower light-fraction $\Delta^{14}\text{C}$ and the significant reduction in both polysaccharide and lignin plant biomarkers in fertilized soils suggests that N additions trigger an increase in the decomposition of plant-related compounds that had been resident in the soil for the past several decades. This acceleration could be mechanistically controlled by direct N effects on decomposer organisms, or indirectly by enhanced decomposition of SOM as the result of increased labile C input to soils (a 'priming' effect), or by other associated changes in plant or microbial community composition. N additions on Niwot Ridge caused a change in the dominant plant community from sedges to grasses¹¹. Previous studies of the lignin biomarkers we examined suggest that increasing grass cover should lead to greater concentration of lignin biomarkers in soil rather than to the declines observed here. The decline in both lignin and polysaccharide biomarkers provides unambiguous evidence for the acceleration of the turnover of a broad range of plant compounds in these soils^{18,19}.

$\delta^{13}\text{C}$ values in plant tissue and in lignin biomarkers provide another line of evidence for increased decomposition of the light fraction. The $\delta^{13}\text{C}$ signature of 2-methoxy-4-vinylphenol in the N-fertilized light fraction closely parallels that of unmodified plant lignin, reflecting the flush of new plant material into fertilized soils combined with the loss of older biomarker pools. In contrast, the $\delta^{13}\text{C}$ value of the same marker in control soils is enriched by 2.1‰ ($P < 0.001$, Table 2), in response to isotopic fractionation that can occur in the first stage of plant material decomposition in soils^{17,20}.

While we show several lines of evidence for N-induced acceleration of light-fraction turnover, such data cannot fully distinguish the mechanisms behind this change. However, regardless of the specific mechanism, the clear result of this study is that N additions increase the decomposition of decadal-age SOM, despite the fact that the dry meadow alpine plots analysed here are cold and dry terrestrial environments. In such environments, it is thought that decomposition is controlled primarily by temperature and perhaps by moisture²¹. Indeed, the presence of relatively unmodified plant biomarkers in alpine soils would be predicted from the standard conceptual model for decomposition. The substantial reduction in the concentration of plant biomarkers with fertilization, however, indicates that N availability exerts a major control over the decomposition of these materials in this setting. However, one important issue to resolve is whether the changes in soil C cycling resulting from high rates of fertilizer addition over short time periods are similar to the impacts caused by relatively low, but chronic, additions of N to ecosystems from atmospheric deposition.

Finally, despite clear evidence for significant changes in soil C processing, the net effects of increased N on soil C storage are not certain. Our data suggest that the responses of alpine ecosystems to fertilization include both increased productivity and increased decomposition of the light fraction of SOM, resulting in no statistically detectable change in total SOM carbon. There is a well developed literature on plant responses to increasing N inputs, the bulk of which suggests that initial increases in productivity will eventually plateau, and perhaps even decline at high levels of N

Table 2 Isotope and compound-specific data from Niwot Ridge plots

	$\Delta^{14}\text{C}$	$\delta^{13}\text{C}$	Lignin marker area (Vs)	Lignin marker $\delta^{13}\text{C}$	Polysaccharide marker area (Vs)	Polysaccharide marker $\delta^{13}\text{C}$
Control light fraction	126.12 (13.29)*	-25.60 (0.08)*	37.54 (7.37)*	-27.34 (0.41)*,†	34.11 (6.28)*	-19.44 (0.44)*
+N light fraction	54.60 (15.31)†	-26.01 (0.17)†	0.68 (0.12)†	-29.40 (0.42)*	1.08 (0.07)†	-19.76 (0.55)*,†
Control heavy fraction	-13.31 (7.91)†	-24.78 (0.07)†	2.10 (1.01)†	-25.90 (0.73)†	0.95 (0.33)†	-21.08 (0.18)†
+N heavy fraction	-1.05 (7.37)†	-24.90 (0.07)†	2.56 (0.29)†	-25.35 (0.21)†	2.80 (0.41)†	-20.42 (0.17)*,†

Values shown are means with standard errors in parentheses (see Supplementary Information for individual $\Delta^{14}\text{C}$ values). Contrasting symbols within each column indicate significant differences determined from Tukey post-hoc analyses following two-way ANOVA. Statistical results for $\Delta^{14}\text{C}$ illustrate significant fertilization ($F = 76.45$, $P < 0.001$), SOM density ($F = 3.23$, $P < 0.01$) and density by fertilization interaction ($F = 13.59$, $P < 0.001$) effects. Soil $\delta^{13}\text{C}$ is affected by SOM density ($F = 82.49$, $P < 0.001$) and fertilization ($F = 6.50$, $P < 0.015$). The concentrations of 2-methoxy-4-vinylphenol, a plant lignin biomarker, are shown as lignin marker area (Vs is normalized abundance). Significant effects are shown for fertilization ($F = 18.19$, $P < 0.001$), SOM density ($F = 18.19$, $P < 0.001$) and density by fertilization interactions ($F = 21.40$, $P < 0.001$). The $\delta^{13}\text{C}$ of the marker for lignin shows significant density ($F = 30.70$, $P < 0.001$) and density \times treatment interactions ($F = 2.32$, $P < 0.022$). Variations in plant polysaccharides were analysed by combining normalized abundance (to 1 mg sample) values for 5-methyl-2-furanone and 2-hydroxy-3-methyl-2-cyclopentenone and these values show significant fertilization ($F = 24.40$, $P < 0.001$), SOM density ($F = 30.04$, $P < 0.001$) and density by fertilization interactions ($F = 24.00$, $P < 0.001$). The $\delta^{13}\text{C}$ of the polysaccharide markers is influenced by SOM density ($F = 8.758$, $P < 0.001$). Vs, volt second.

loading²². Despite the fact that soil C pools greatly exceed vegetation storage, there is no comparable conceptual or quantitative model for soil C responses to changing N availability. An improved understanding of the mechanisms of soil C response to changing N availability, and more accurate SOM carbon measurement techniques will be needed for accurate modelling^{5,21} of ecosystem C storage in response to a changing N cycle. □

Methods

Site description

The experimental plots were located at the National Science Foundation, Long-Term Ecological Research site on Niwot Ridge, an 8-km ridge that extends east from the continental divide in the Front Range of the Colorado Rocky Mountains (<http://culter.colorado.edu>). The soils of the dry meadow community are Inceptisols with texture of 39% sand, 28% silt and 23% clay²³. The N-fertilization experiment was carried out in a dry meadow community dominated by *Kobresia myosuroides* and including other species in the Cyperaceae. Forbs constitute the other major growth form. Experimental fertilization plots were established in dry meadow sites in May 1990, and consist of five replicate 2 × 2 m plots including control and fertilizer addition plots where N has been applied every year since 1990 at an average rate of 25 g N m⁻² yr⁻¹ as urea-N for the first two years and at 10 g N m⁻² yr⁻¹ thereafter. For reference, atmospheric deposition rates at Niwot Ridge are 0.5 g N m⁻² yr⁻¹ (ref. 24). Full details on the plot design, fertilizer additions and plant measurements can be found in ref. 11.

Soil measurements

We collected soils from the top 10 cm of the experimental plots. For most analyses, we sieved soils (2 mm) and then separated the soils into light and heavy fractions by floating soils in sodium polytungstate (density ~1.6 g cm⁻³)¹⁵. For C concentrations, ¹⁴C/¹²C and ¹³C/¹²C determinations, we took two samples from each plot and averaged these for a single plot-value used in statistical tests. Targets for ¹⁴C measurement were prepared at the Laboratory for Radiocarbon Preparation and Research at the Institute for Arctic and Alpine Research and analysed at the National Ocean Sciences Accelerator Mass Spectrometer Facility at the Woods Hole Oceanographic Institution. Δ¹⁴C values were decay corrected to the date of collection²⁵ and presented as per mil deviations from fraction modern (F_m) = 1, where F_m = (¹⁴C/¹²C)_{snl}/(¹⁴C/¹²C)_{on} and the subscript sn denotes the sample value normalized to a δ¹³C value of -25‰ and the subscript on indicates the radiocarbon standard value normalized to a δ¹³C value of -25‰ (ref. 24) (see Supplementary Information for individual radiocarbon results).

Compound-specific analyses

Compound-specific analyses were performed at the Max Planck Institute for Biogeochemistry in Jena, Germany, using pyrolysis-gas chromatography/mass spectrometry-combustion interface-isotope ratio-mass spectrometry (py-GC/MS-C-IRMS). Briefly, samples were pyrolysed for 9.9 s in a 0316 Fisher pyrolyser using a ferromagnetic tube with a Curie temperature of 590 °C. Pyrolysis products were separated on a BPX 5 column (60 m × 0.32 mm, film thickness 1.0 μm) and detected on a Thermoquest GCQ operated at 70 eV. Compound-specific ¹³C measurements were made on a Finnigan MAT Delta^{plus}XL. Additional analytical details are available in ref. 17.

Statistical calculations

Our statistical calculations were carried out with a two-way factorial analysis of variance (ANOVA) combined with Tukey post-hoc pair-wise comparisons to examine statistical differences between soil densities (light versus heavy) and control versus fertilization treatments.

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Quantifying nitrogen-fixation in feather moss carpets of boreal forests

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Biological nitrogen (N) fixation is the primary source of N within natural ecosystems¹, yet the origin of boreal forest N has remained elusive. The boreal forests of Eurasia and North America lack any significant, widespread symbiotic N-fixing plants^{1–6}. With the exception of scattered stands of alder in early primary successional forests⁷, N-fixation in boreal forests is considered to be extremely limited. Nitrogen-fixation in northern European boreal forests has been estimated² at only 0.5 kg N ha⁻¹ yr⁻¹; however, organic N is accumulated in these ecosystems at a rate of 3 kg N ha⁻¹ yr⁻¹ (ref. 8). Our limited understanding of the origin of boreal N is unacceptable given the extent of the boreal forest region, but predictable given our imperfect knowledge of N-fixation^{1,9}. Herein we report on a N-fixing symbiosis between a cyanobacterium (*Nostoc* sp.) and the