

# **Chronic nitrogen additions suppress decomposition and sequester soil carbon in temperate forests**

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## SUPPLEMENATRY TABLES

**Supplementary Table S1.** Soil carbon content (%) and stocks (g C m<sup>-2</sup>) for soil samples collected from plots amended with 0, 50, or 150 kg N ha<sup>-1</sup> yr<sup>-1</sup>. Samples were collected from both the hardwood and pine stands at the Chronic Nitrogen Amendment Experiment at the Harvard Forest Long-Term Ecological Research (LTER) site. Organic horizon samples were collected by removing a 20 x 20 cm square of the O horizon (Oe and Oa layers) to the depth of the mineral soil. Mineral soil samples were collected in 10-cm increments to a depth of 40 cm (bottom of soil profile) using a gas-powered soil auger with a 9-cm diameter core. Samples were sieved (<2 mm) and roots, rocks and other debris >2 mm removed. Bulk density, determined for each soil depth increment within a N addition treatment, was used to calculate the volumetric C stocks (g C m<sup>-2</sup>). Values are means of 4-6 replicates with one standard error in parentheses.

	Nitrogen addition (kg N ha <sup>-1</sup> yr <sup>-1</sup> )					
	0		50		150	
	%C	g C m <sup>-2</sup>	%C	g C m <sup>-2</sup>	%C	g C m <sup>-2</sup>
<b>Hardwood stand</b>						
Organic horizon						
Oe	39 (2.3)	1792 (430)	44 (0.8)	2189 (292)	50 (4.3)	3645 (433)
Oa	17 (2.0)	1222 (129)	24 (2.5)	1813 (199)	28 (3.5)	1775 (510)
Mineral Soil						
0-10 cm	5.3 (0.4)	4023 (193)	5.2 (0.2)	4021 (153)	6.6 (0.5)	4768 (211)
10-20 cm	2.7 (0.2)	2674 (114)	2.7 (0.8)	2302 (298)	4.3 (0.7)	3560 (425)
20-30 cm	1.6 (0.1)	1587 (142)	1.4 (0.2)	2112 (346)	3.2 (0.3)	3905 (299)
30-40 cm	1.2 (0.0)	1261 (141)	1.5 (0.6)	1590 (456)	2.4 (0.3)	2579 (0.0)
<b>Pine stand</b>						
Organic horizon						
Oe	39 (2.6)	1660 (272)	46 (0.4)	3811 (167)	42 (3.6)	3574 (816)
Oa	16 (2.0)	1470 (171)	24 (1.3)	953 (111)	25 (4.0)	1090 (259)
Mineral Soil						
0-10 cm	5.5 (0.5)	4682 (360)	4.4 (0.1)	4430 (172)	4.2 (0.3)	4305 (283)
10-20 cm	3.0 (0.3)	3240 (198)	2.9 (0.4)	3378 (519)	2.5 (0.2)	2961 (263)
20-30 cm	2.0 (0.3)	2368 (280)	1.9 (0.4)	1957 (275)	1.7 (0.1)	2323 (92)
30-40 cm	1.4 (0.2)	1602 (258)	1.4 (0.4)	2069 (682)	1.1 (0.1)	1591 (192)

**Supplementary Table S2.** Light fraction (free particulate) proportion (%) for mineral soil samples collected from hardwood and pine plots amended with 0, 50, or 150 kg N ha<sup>-1</sup> yr<sup>-1</sup>.

Values are means with one standard error in parentheses for samples with 3-6 replicates. After samples were sieved (<2 mm) and roots, rocks and other debris >2 mm removed, soils were dispersed in a 1.85 g cm<sup>-3</sup> density solution of sodium polytungstate by reciprocal shaking for several hours. After aspirating off the light fraction, samples were washed with distilled water, dried, and weighed. ND = not determined.

	Hardwood			Pine		
	Nitrogen addition (kg N ha <sup>-1</sup> yr <sup>-1</sup> )			Nitrogen addition (kg N ha <sup>-1</sup> yr <sup>-1</sup> )		
	0	50	150	0	50	150
0-10 cm	4.5 (0.7)	5.4 (0.5)	ND	4.8 (0.1)	5.2 (0.3)	ND
10-20 cm	5.9 (1.3)	2.4 (0.3)	6.7 (2.2)	2.9 (1.2)	3.9 (----)	ND
20-30 cm	3.5 (0.4)	3.7 (1.7)	2.5 (0.3)	1.5 (1.1)	5.3 (3.3)	ND
30-40 cm	4.2 (1.7)	3.4 (0.8)	1.9 (0.2)	1.3 (0.8)	5.1 (----)	ND

**Supplementary Table S3.** Specific respiration rates and N concentrations for fine roots (< 1 mm) from the organic horizon and surface mineral soil of the hardwood and pine stands. Values represent the mean and one standard error of four replicate root samples collected both in Oct 2008 and Aug 2009. The four samples per treatment each year were each composites of three 5 cm-diameter by 10 cm-deep soil cores. These cores were then divided into organic horizon and mineral soil, with samples of fine roots hand sorted from each soil horizon and brushed free of adhering soil and organic matter, with approximately 2 g fresh weight placed in a respiration cuvette attached to an infrared gas analyzer (Burton et al. 2003). All root respiration samples were subsequently analyzed for N concentration using an elemental analyzer. Differences among N addition treatments, soil horizons, and years were examined using a three-factor analysis of variance for each forest type. Nitrogen additions significantly increased fine root N concentration in the organic horizon and mineral soil of both forests ( $P < 0.0001$ ), but corresponding increases in root respiration rates were not observed ( $P = 0.107$ ). In the organic horizon of the pine stand, respiration rates were significantly reduced by N addition ( $P = 0.022$ ). As a result of these responses, respiration per unit N was lower in organic horizon roots from both forests and in mineral soil roots from the pine stand (Supplementary Figure S1).

Stand	Date	Response variable	Nitrogen addition (kg N ha <sup>-1</sup> yr <sup>-1</sup> )		
			0	50	150
<b>Hardwood</b>					
O horizon	Oct-08	Respiration (nmol CO <sub>2</sub> g <sup>-1</sup> s <sup>-1</sup> )	5.43 (0.45)	4.67 (0.66)	4.01 (0.42)
		Nitrogen (g kg <sup>-1</sup> )	13.4 (0.6)	14.6 (0.7)	17.6 (0.6)
	Aug-09	Respiration (nmol CO <sub>2</sub> g <sup>-1</sup> s <sup>-1</sup> )	4.37 (0.56)	4.43 (0.49)	6.81 (0.85)
		Nitrogen (g kg <sup>-1</sup> )	10.6 (0.6)	13.8 (0.9)	14.8 (2.5)
Mineral soil	Oct-08	Respiration (nmol CO <sub>2</sub> g <sup>-1</sup> s <sup>-1</sup> )	2.15 (0.25)	2.94 (1.07)	3.25 (0.61)
		Nitrogen (g kg <sup>-1</sup> )	8.4 (0.7)	10.6 (1.7)	13.6 (2.0)
	Aug-09	Respiration (nmol CO <sub>2</sub> g <sup>-1</sup> s <sup>-1</sup> )	2.55 (0.23)	3.32 (0.64)	3.77 (0.33)
		Nitrogen (g kg <sup>-1</sup> )	8.0 (0.1)	8.7 (0.6)	10.7 (0.7)

Pine					
O horizon	Oct-08	Respiration (nmol CO <sub>2</sub> g <sup>-1</sup> s <sup>-1</sup> )	5.92 (0.86)	4.07 (0.33)	5.02 (0.28)
		Nitrogen (g kg <sup>-1</sup> )	15.2 (1.6)	19.5 (0.7)	18.3 (0.4)
	Aug-09	Respiration (nmol CO <sub>2</sub> g <sup>-1</sup> s <sup>-1</sup> )	8.39 (1.33)	3.88 (0.47)	5.54 (1.08)
		Nitrogen (g kg <sup>-1</sup> )	15.7 (0.6)	16.8 (0.7)	13.3 (1.9)
Mineral soil	Oct-08	Respiration (nmol CO <sub>2</sub> g <sup>-1</sup> s <sup>-1</sup> )	3.69 (0.44)	4.17 (0.36)	3.83 (0.16)
		Nitrogen (g kg <sup>-1</sup> )	11.6 (0.5)	17.7(1.0)	16.0 (1.0)
	Aug-09	Respiration (nmol CO <sub>2</sub> g <sup>-1</sup> s <sup>-1</sup> )	4.57 (0.53)	5.22 (0.63)	4.12 (0.54)
		Nitrogen (g kg <sup>-1</sup> )	11.5 (0.5)	15.0 (0.3)	14.0 (0.5)

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**Supplementary Table S4.** Fine-root production and specific root length in the hardwood stand.

Root production and specific root length, indicators of nutrient foraging and stress of roots (Robinson 1986; Ostonen et al. 2007), were assessed using *in situ* soil cores of sieved, root-free soil (Vogt and Persson 1991; Fahey and Hughes 1994; Hendricks et al. 2006) that were installed in June 2011. Soil from the inner 6.4-cm diameter  $\times$  20-cm depth of root in-growth cores was collected after approximately four months. Roots were separated from soil with forceps, rinsed of soil particles, photographed on a flatbed scanner (Epson 10000XL), and dried for 48 hr at 65°C. Root length was measured with ImageJ software (<http://rsb.info.nih.gov/ij>). Values are means with one standard error in parentheses (n = 4 except as noted). Root parameters did not differ among N addition treatments within soil-depth increments ( $P \geq 0.05$  in all cases).

Parameter	Depth	Nitrogen addition (kg N ha <sup>-1</sup> yr <sup>-1</sup> )		
		0 <sup>†</sup>	50	150
Root production (g m <sup>-2</sup> y <sup>-1</sup> )	0-10 cm <sup>‡</sup>	152 (70)	186 (71)	172 (57)
	10-20 cm	38 (18)	52 (16)	41 (12)
Specific root length (m g <sup>-1</sup> )	0-10 cm	46 (12)	47 (6)	47 (3)
	10-20 cm	62 (32)	48 (10)	35 (9)

<sup>†</sup>n = 3.

<sup>‡</sup>Includes top 10 cm of soil, including organic horizon. The deeper depth (10-20 cm) was mineral soil only.

**Supplementary Table S5.** Oxidative enzyme activity ( $\mu\text{mol g}^{-1} \text{ soil hr}^{-1}$ ) for the organic horizon and the top 10 cm of mineral soil in the hardwood stand. Samples were collected and analyzed on five dates between May and Dec 2012 using the substrate 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) as described by Floch et al. (2007), but modified for 96-well microplates (Saiya-Cork et al. 2002). Field-moist soil was sieved to  $< 2 \text{ mm}$ , homogenized 30 s in modified universal buffer (pH 4.5, 1:100 soil:buffer [w/v]), and dispensed as a suspension in 200- $\mu\text{L}$  aliquots. There were sixteen analytical replicates that received 50  $\mu\text{L}$  3.35 mM ABTS and eight buffer, substrate, and sample blanks. Incubations were standardized at  $22^\circ\text{C}$  and 1.5-2-hr after a preliminary study that optimized sensitivity and confirmed linearly increasing absorbance through time. The upper 100  $\mu\text{L}$  of reaction product was transferred to a 96-well plate and absorbance was measured at 420 nm on a Synergy HT microplate reader (Biotek, USA). The concentration of ABTS converted during the incubation was estimated by an extinction coefficient of  $18,460 \text{ M}^{-1} \text{ cm}^{-1}$  (Floch et al. 2007). Values shown are means of N treatments across dates with standard errors ( $n = 5$ ) in parentheses. Letters indicate significant differences among treatments within soil horizons ( $P \leq 0.05$ ).

	Nitrogen addition ( $\text{kg N ha}^{-1} \text{ y}^{-1}$ )		
	0	50	150
O horizon	3.31 (1.14) <sup>a</sup>	1.93 (0.48) <sup>b</sup>	0.43 (0.12) <sup>c</sup>
0-10 cm mineral soil	1.37 (0.22) <sup>a</sup>	1.39 (0.22) <sup>a</sup>	0.20 (0.04) <sup>b</sup>

**Supplementary Table S6.** Relative percentages and ratios of chemical classes in the organic horizon collected from the hardwood stand as determined using pyrolysis-GC/MS (Wickings et al. 2011). Samples were pyrolyzed at 600°C and products transferred to a GC where compounds were separated on a 60 m capillary column with a starting temperature of 40°C followed by a temperature ramp of 5°C min<sup>-1</sup> to 270°C followed by a final ramp (30°C min<sup>-1</sup>) to 300°C. Compounds were transferred to an ion trap mass spectrometer where they were ionized, detected via electron multiplier, and identified using a compound library from the National Institute of Standards and Technology (NIST). Numbers in parentheses are standard errors. Values followed by a different lowercase letter are significantly different at  $P \leq 0.05$ .

Chemical class	Nitrogen addition (kg N ha <sup>-1</sup> yr <sup>-1</sup> )		
	0	50	150
Lignin	25.76 (1.58) <sup>b</sup>	36.86 (2.54) <sup>a</sup>	37.97 (6.04) <sup>a</sup>
Lipids	17.28 (1.99) <sup>a</sup>	7.41 (0.90) <sup>b</sup>	8.14 (1.21) <sup>b</sup>
Phenols	8.42 (0.73)	6.59 (0.77)	6.22 (0.75)
N-bearing	4.13 (0.33) <sup>a</sup>	2.76 (0.27) <sup>b</sup>	3.33 (0.09) <sup>b</sup>
Polysaccharides	16.62 (1.63)	15.75 (1.13)	13.13 (1.11)
Proteins	3.96 (0.23)	3.72 (0.28)	3.86 (0.24)
Unknown origin	23.83 (2.38)	26.90 (2.46)	27.36 (5.68)
Lignin: phenols	3.13 (0.32) <sup>b</sup>	5.90 (0.91) <sup>a</sup>	6.09 (0.43) <sup>a</sup>
Lignin: N <sup>†</sup>	3.22 (0.26)	5.75 (0.50)	5.35 (0.98)

<sup>†</sup>Represents the sum of N-bearing compounds and proteins



**Supplementary Table S7.** Dissolved organic carbon ( $\mu\text{g DOC g}^{-1}\text{ TOC}$ ) extracted from samples collected from the organic horizon (Oe and Oa layers) and 0-10 cm mineral soil depth. The DOC was extracted with water (10:1 water:soil) and the extracts were analyzed using a TOC analyzer (Shimadzu TOC-VCPH that measures organic carbon by high temperature catalytic oxidation). Numbers in parentheses are standard errors. Values followed by a different lowercase letter are significantly different at  $P \leq 0.05$ .

	Nitrogen addition ( $\text{kg N ha}^{-1}\text{ yr}^{-1}$ )		
	0	50	150
Hardwood stand			
Oe	12.2 (2.8) <sup>a</sup>	7.1 (0.3) <sup>b</sup>	3.3 (0.7) <sup>c</sup>
Oa	5.1 (0.2)	5.8 (0.9)	4.3 (0.7)
0-10 cm	6.4 (0.2)	6.3 (0.9)	6.7 (0.5)
Pine stand			
Oe	6.5 (2.3) <sup>a</sup>	3.6 (0.2) <sup>b</sup>	3.9 (1.1) <sup>b</sup>
Oa	4.7 (0.4)	4.6 (0.5)	3.9 (0.3)
0-10 cm	6.8 (0.9)	7.8 (0.9)	6.6 (0.8)

**Supplementary Table S8.** Mean residence time (MRT) of soil C pools separated by density fractionation. Mineral soil (0-10 cm depth) was separated into pools by sequential fractionation in sodium polytungstate solution at densities of 1.85, 2.4, and 2.8 g mL<sup>-1</sup> by centrifugation and floatation (Sollins et al. 2006). This method is laborious and costly; therefore, the 150 kg N ha<sup>-1</sup> yr<sup>-1</sup> treatment was not included in the analysis. The MRT was estimated by matching radiocarbon data (Supplementary Fig. S7) to modeled values using a four-pool, time-dependent, steady state model (Trumbore et al. 1995; Gaudinski et al. 2000). Assumptions of the model were that the inputs equaled the outputs for each pool and that the radiocarbon values of the inputs were equal to the atmospheric value on a three year time lag to account for time spent as live tissue. Values are means  $\pm$  one standard error. The MRT among treatments for a given forest stand and density size class was not significantly different ( $P \leq 0.05$ ).

Stand	N addition rate (kg N ha <sup>-1</sup> yr <sup>-1</sup> )	Mean residence time (yr)		
		<1.85 g mL <sup>-1</sup>	1.85-2.4 g mL <sup>-1</sup>	2.4-2.8 g mL <sup>-1</sup>
Hardwood	0	161 $\pm$ 37	200 $\pm$ 33	378 $\pm$ 86
	50	169 $\pm$ 11	205 $\pm$ 28	403 $\pm$ 40
Pine	0	195 $\pm$ 8	284 $\pm$ 25	374 $\pm$ 11
	50	193 $\pm$ 9	281 $\pm$ 6	390 $\pm$ 19

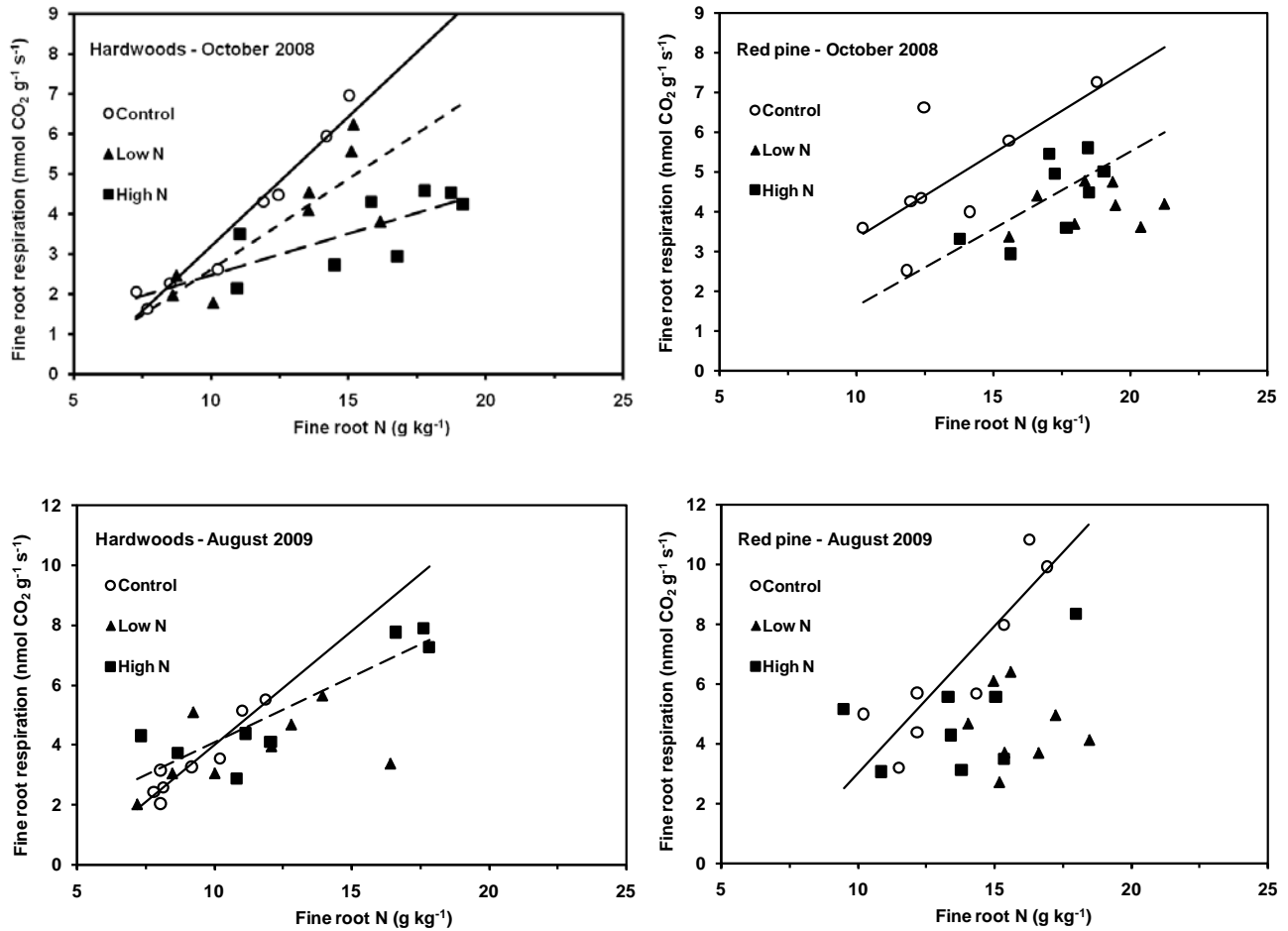
**Supplementary Table S9.** The quantity (nmol g<sup>-1</sup> soil) of microbial biomarkers representing bacterial and fungal groups extracted from the Oe horizon of the forest floor and determined by phospholipid fatty acid (PLFA) analysis (Guckert et al. 1985). Numbers in parentheses are standard errors. ND = not detectable. These data were used as an index of bacterial and fungal biomass (Table 2 in main text) and to examine N treatment differences in microbial community composition (Fig. 5 in main text).

Biomarker	Hardwood			Pine		
	Nitrogen addition (kg ha <sup>-1</sup> yr <sup>-1</sup> )			Nitrogen addition (kg ha <sup>-1</sup> yr <sup>-1</sup> )		
	0	50	150	0	50	150
<i>Bacteria</i>						
i15:0	63.8 (5.7)	74.9 (12.4)	43.6 (9.6)	79.7 (23.7)	42.0 (3.7)	28.0 (3.2)
a15:0	25.8 (1.4)	30.7 (5.3)	11.7 (2.7)	25.0 (7.4)	12.6 (1.1)	9.5 (1.3)
15:0	10.5 (2.0)	8.5 (1.7)	4.1 (0.5)	5.8 (1.2)	1.5 (1.5)	0.0 (0.0)
i16:0	7.4 (0.7)	7.4 (1.5)	0.0 (0.0)	56.2 (18.7)	18.4 (2.4)	14.7 (1.2)
16:1 $\omega$ 7 $c$	58.6 (2.8)	57.7 (9.1)	17.2 (3.9)	6.8 (1.6)	1.4 (1.4)	ND
16:1 $\omega$ 7 $t$	7.0 (2.2)	7.7 (0.4)	ND	26.4 (12.5)	6.8 (0.8)	8.2 (1.2)
i17:0	7.1 (0.8)	6.9 (1.6)	0.9 (0.9)	10.7 (4.6)	1.9 (1.9)	1.1 (1.1)
a17:0	12.3 (2.1)	10.7 (2.8)	6.9 (1.2)	10.2 (2.7)	7.0 (0.1)	5.5 (0.9)
cy17:0	21.4 (1.1)	22.8 (4.0)	15.1 (3.0)	17.8 (4.3)	12.4 (1.2)	10.0 (1.0)
18:1 $\omega$ 7 $c$	9.1 (2.7)	12.8 (1.8)	10.9 (2.0)	11.7 (3.6)	4.8 (2.8)	4.3 (2.7)
cy19:0	54.7 (0.5)	66.8 (17.4)	38.0 (3.7)	79.0 (25.6)	44.2 (4.2)	27.6 (3.6)
<i>Actinomycetes</i>						
10me16:0	4.0 (0.7)	3.9 (0.1)	4.0 (0.3)	2.6 (0.1)	2.0 (0.3)	1.5 (0.2)
<i>Fungi</i>						
18:2 $\omega$ 9,12 $c$	151.9 (13.7)	14.9 (4.5)	12.3 (9.7)	41.6 (3.9)	19.8 (3.9)	21.5 (4.0)
18:1 $\omega$ 9 $c$	66.1 (1.6)	56.0 (10.1)	18.7 (3.7)	72.9 (28.3)	24.1 (4.3)	17.9 (2.0)

**Supplementary Table S10.** The quantity (nmol g<sup>-1</sup> soil) of microbial biomarkers representing bacterial and fungal groups extracted from the Oa horizon of the forest floor and determined by phospholipid fatty acid (PLFA) analysis (Guckert et al. 1985). Numbers in parentheses are standard errors. ND = not detectable. These data were used as an index of bacterial and fungal biomass (Table 2 in main text).

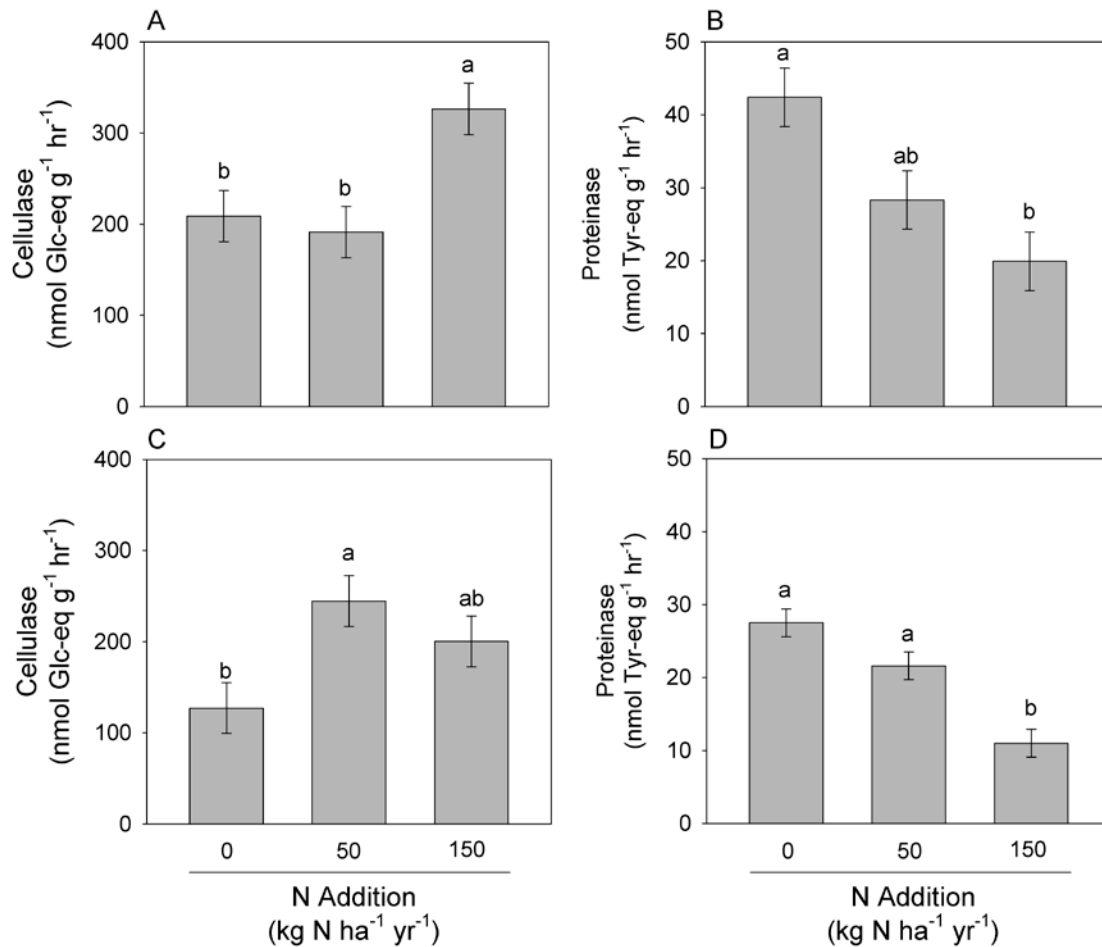
Biomarker	Hardwood			Pine		
	Nitrogen addition (kg ha <sup>-1</sup> yr <sup>-1</sup> )			Nitrogen addition (kg ha <sup>-1</sup> yr <sup>-1</sup> )		
	0	50	150	0	50	150
<i>Bacteria</i>						
i15:0	24.9 (9.5)	15.0 (3.0)	12.7 (4.8)	24.5 (4.9)	22.7 (7.1)	6.6 (1.0)
a15:0	10.0 (2.9)	6.0 (1.2)	3.6 (2.1)	6.9 (1.2)	6.5 (2.2)	ND
15:0	1.3 (1.3)	ND	ND	ND	ND	ND
i16:0	4.6 (1.5)	2.1 (1.1)	ND	2.2 (1.2)	1.7 (1.7)	ND
16:1 $\omega$ 7 $c$	19.3 (6.3)	10.5 (2.4)	3.9 (2.6)	14.8 (4.4)	9.1 (6.8)	ND
16:1 $\omega$ 7 $t$	2.5 (1.4)	1.1 (1.1)	ND	0.8 (0.8)	0.9 (0.9)	ND
i17:0	4.2 (0.7)	1.7 (0.8)	ND	4.4 (0.5)	7.2 (2.0)	ND
a17:0	4.6 (0.8)	1.7 (0.9)	1.1 (1.1)	2.1 (1.1)	1.4 (1.4)	0.9 (0.9)
cy17:0	6.0 (1.7)	3.5 (0.9)	2.3 (1.3)	4.3 (0.4)	2.2 (2.2)	ND
18:1 $\omega$ 7 $c$	5.8 (1.2)	2.2 (1.1)	ND	2.3 (1.2)	1.5 (1.5)	ND
cy19:0	35.3 (6.7)	31.7 (5.0)	18.6 (8.2)	38.7 (3.4)	25.9 (5.5)	7.8 (0.8)
<i>Actinomycetes</i>						
10me16:0	1.9 (0.4)	1.6 (0.2)	1.8 (0.5)	2.1 (0.5)	2.4 (0.9)	0.8 (0.2)
<i>Fungi</i>						
18:2 $\omega$ 9,12 $c$	11.4 (3.1)	4.2 (2.7)	2.7 (2.7)	2.9 (0.5)	3.1 (3.1)	ND
18:1 $\omega$ 9 $c$	33.0 (9.5)	15.6 (0.8)	7.1 (2.4)	28.9 (10.3)	12.4 (8.7)	0.8 (0.8)

## SUPPLEMENTARY FIGURES



**Supplementary Fig. S1** Relationships between fine root (<1 mm) respiration rates and fine root N concentration for roots from the forest floor and surface soil of the hardwood and pine stands in October 2008 and August 2009. Relationships between root N and respiration rate for each forest and year were examined using linear regression, with differences between control and N addition treatments in root respiration:tissue N relationships examined using analysis of covariance (ANCOVA), with N as the covariate. Nitrogen additions increased fine root N concentrations in both forests during both years ( $P \leq 0.0001$ ). Despite lower N concentrations, specific respiration rates of roots collected from the forest floor were significantly greater in the

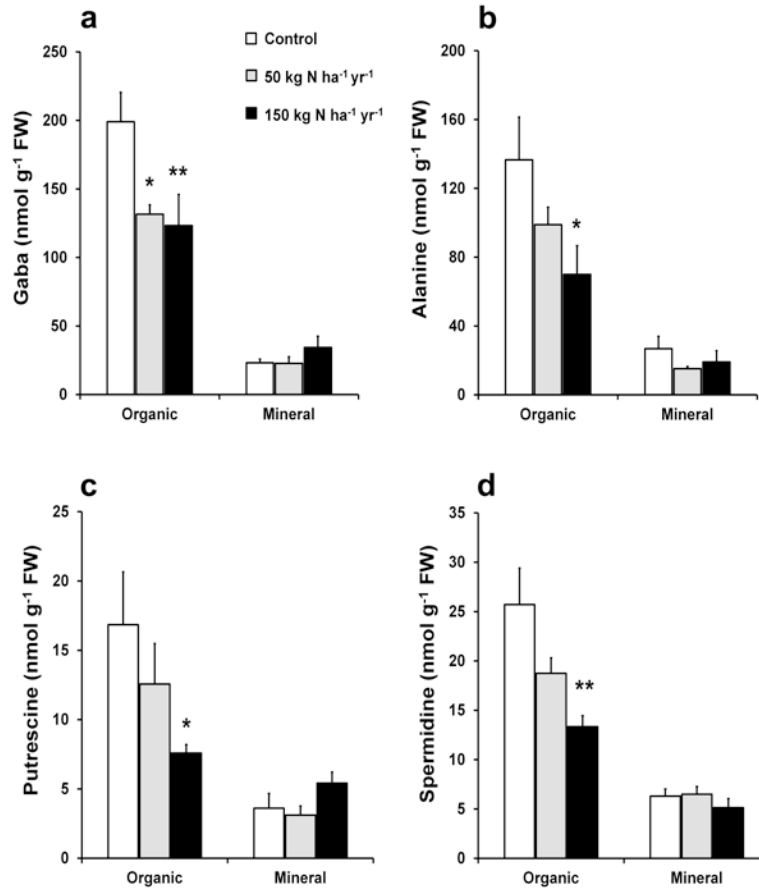
control plots for the pine stand ( $P = 0.022$ ). Respiration per unit N was significantly greater for the control treatment in both years for pine and in 2008 for the hardwoods. Significant linear relationships ( $P \leq 0.05$ ) between fine root N and specific respiration rate always occurred for the control treatment (solid lines) but only occasionally existed for the low N (short-dashed line) and high N (long-dashed line) treatments.



**Supplementary Fig. S2** Cellulase and proteinase activity in the 0-10 cm mineral soil collected from the hardwood stands (A and B) and the pine stands (C and D). Potential cellulase activity was measured as glucose release from carboxymethyl-cellulose over 24 hr at 30°C. Potential proteinase activity was measured as aromatic amino acid release from casein following the method of Ladd and Butler (1972). Values are means  $\pm$  one standard error. Significant differences across N addition treatments at the  $P \leq 0.05$  level are indicated by different letters. The proteolytic enzyme activity data shown in this figure were collected from a different set of subsamples and analyzed in a different lab than those shown in Fig. 4 in the main text. The two

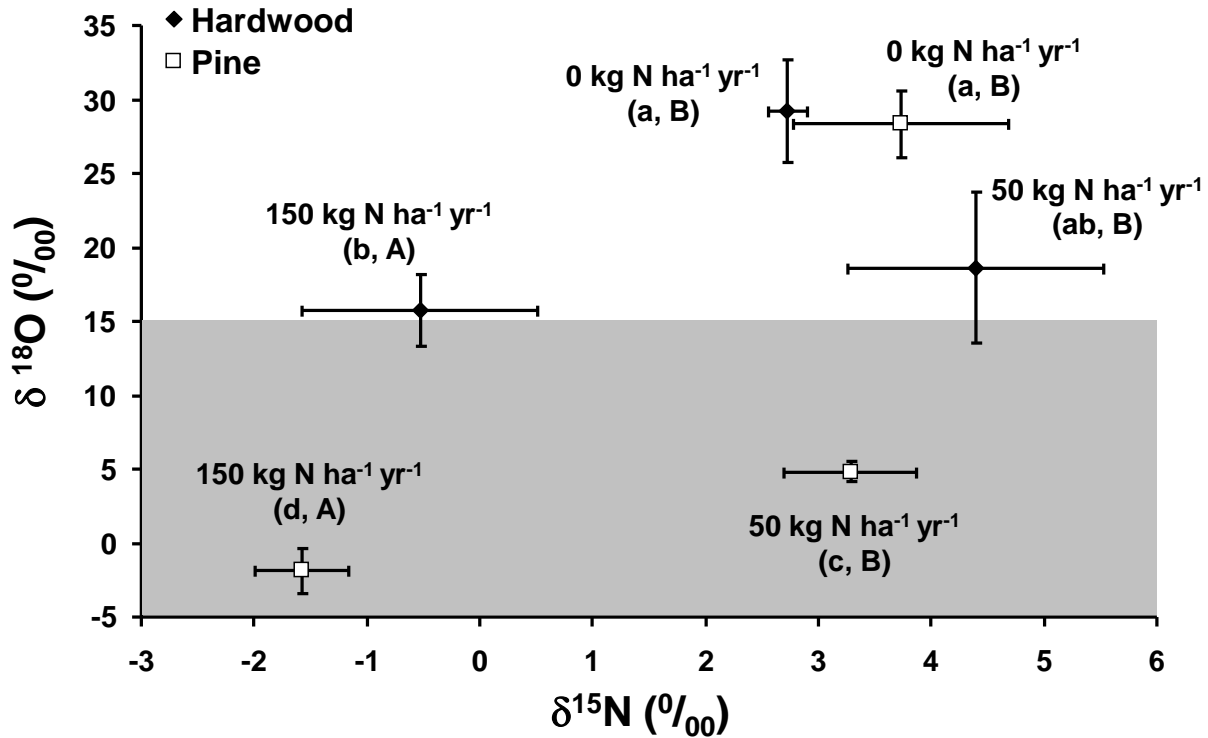
datasets show consistent results, with proteolytic activity declining across the N addition gradient.





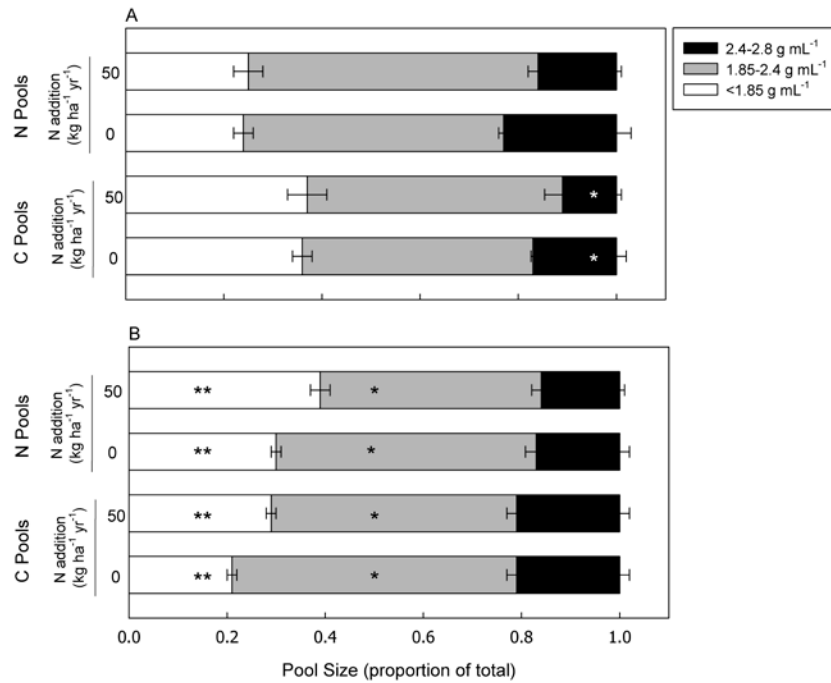
**Supplementary Fig. S3** Effects of chronic N additions on the concentrations of common amino acids,  $\gamma$ -aminobutyric acid (GABA) (a) and alanine (b) and soluble polyamines, putrescine (c) and spermidine (d) in soil extracts of samples collected from the hardwood stand. Soils were collected in 2009 and processed according to the procedure described in Turlapati et al. (2013). Amino acids and polyamines were extracted in 5% perchloric acid and analyzed according to the procedure described in Minocha and Long (2004). Symbols \* and \*\* above the bars indicate significant differences at  $P \leq 0.05$  and 0.01, respectively. The data presented represent the means  $\pm$  one standard error of five field replicates, with two analytical replicates per field sample. Polyamines are single chained organic cations that are rich in N and are present in all living organisms and essential for growth and development. Cellular contents of polyamines,

specifically putrescine, are indicative of the physiological response of foliage of forest trees to an array of environmental stress conditions including a shortage of soil available Ca, excess Al, and chronic N accumulation (Minocha et al. 1997; Minocha et al. 2000; Minocha et al. 2010; Wargo et al. 2002).

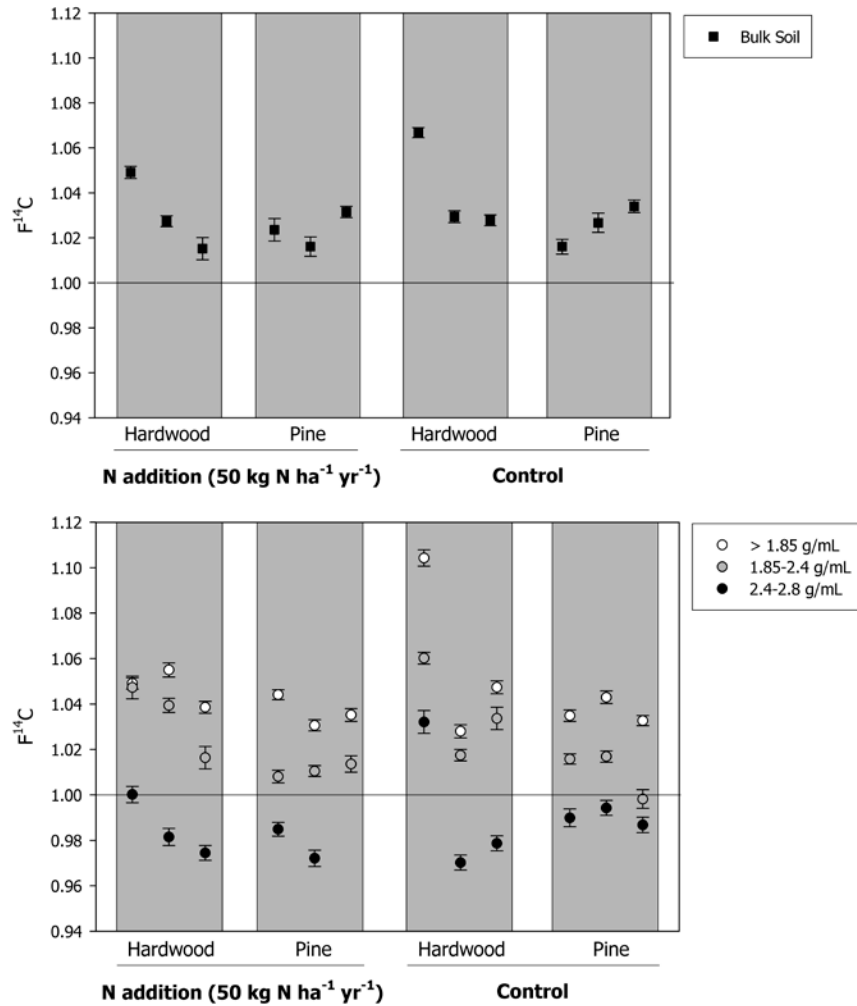


**Supplementary Fig. S4** Natural abundance <sup>15</sup>N and <sup>18</sup>O in soil solution NO<sub>3</sub><sup>-</sup> collected from lysimeters installed in each treatment plot. Samples were analyzed according to Templer and Weathers 2011). Data are means ± one standard error. Different upper-case letters represent statistically significant differences in δ<sup>15</sup>N values and different lower-case letters represent statistically significant differences in δ<sup>18</sup>O values (*P* ≤ 0.05). The shaded region indicates NO<sub>3</sub><sup>-</sup> microbially produced from nitrification only. There was wide separation in mean δ<sup>15</sup>N and δ<sup>18</sup>O values between soil solution NO<sub>3</sub><sup>-</sup> in the ambient compared to treatment plots. There was no significant difference in δ<sup>15</sup>N among forest types (*P* = 0.76), but δ<sup>15</sup>N in soil solution was significantly lower in the high N plots compared to the ambient and low N plots for both forest types (*P* = 0.0002). δ<sup>18</sup>O values of the ambient plots in the hardwood and pine stands were both significantly greater than the high N plots in the hardwood stand, as well as the low N and high N plots of the pine stand (*P* < 0.0001). Overall, δ<sup>18</sup>O values were significantly greater in the

hardwood compared to the pine stand ( $P < 0.0001$ ) and was significantly greater in the ambient plots compared to the low N and high N plots ( $P < 0.0001$ ). These results suggest that a greater proportion of  $\text{NO}_3^-$  in soil solution came from nitrification rather than atmospheric deposition or fertilizer N inputs in the higher N plots. If  $\text{NO}_3^-$  came directly from atmospheric deposition or fertilizer N inputs, one would expect the  $\delta^{18}\text{O}$  values of soil solution  $\text{NO}_3^-$  to be significantly higher in the fertilized plots compared to the ambient plots since  $\text{NO}_3^-$  inputs from both fertilizer ( $\delta^{18}\text{O} = 28.1\text{‰}$ ) and atmospheric deposition (71‰) are both equal to or greater than the  $\delta^{18}\text{O}$  values of soil solution  $\text{NO}_3^-$  in the ambient plots (29.2‰ in hardwood and 28.3‰ in pine plots). Instead, the  $\delta^{18}\text{O}$  values in soil solution  $\text{NO}_3^-$  of the fertilized plots is significantly lower than the unfertilized plots, suggesting a greater proportion of  $\text{NO}_3^-$  coming from microbial production of  $\text{NO}_3^-$ . However, the  $\delta^{15}\text{N}$  values of soil solution  $\text{NO}_3^-$  in the high N plots are significantly lower than the low N plots for both forest types, suggesting slower N cycling rates relative to  $\text{NO}_3^-$  losses with higher rates of N addition.



**Supplemental Figure S5.** Carbon and nitrogen pool sizes of mineral soil (0-10 cm) density fractions as a proportion of total soil stock for the control and N addition (50 kg N ha<sup>-1</sup> yr<sup>-1</sup> treatment only) hardwood (A) and pine (B) plots. Mineral soil (0-10 cm depth) was separated into pools by sequential fractionation in sodium polytungstate solution at densities of 1.85, 2.4, and 2.8 g mL<sup>-1</sup> by centrifugation and floatation (Sollins et al., 2006). This method is laborious and costly; therefore, the 150 kg N ha<sup>-1</sup> yr<sup>-1</sup> treatment was not included in the analysis. Data presented are means  $\pm$  one standard error. An \* indicates a significant difference between N treatments at the  $P \leq 0.1$  level and \*\* indicates significant differences at the  $P \leq 0.05$  level. Twenty years of N addition in both stands did not significantly alter the C and N pool sizes in the mineral-dominated soil fractions (>1.85 g mL<sup>-1</sup>) of the 0-10 cm depth increment ( $P < 0.05$ ). In contrast, there was a significant increase in both C and N pools associated with the lightest density fraction (<1.85 g mL<sup>-1</sup>) isolated from samples collected in the pine stand.



**Supplemental Figure S6.** Radiocarbon data, expressed as F<sup>14</sup>C (fraction modern) values for bulk soil and density fractions isolated from mineral soil (0-10 cm) for the control and N addition plots (50 kg N ha<sup>-1</sup> yr<sup>-1</sup> treatment only). These data were used to estimate the mean residence time (MRT) of the three density fractions described in Supplementary Figure S7 above. The <sup>14</sup>C/<sup>12</sup>C and <sup>13</sup>C/<sup>12</sup>C ratios of the samples were measured by accelerator mass spectrometry at the <sup>14</sup>CHRONO Centre for Climate, the Environment, and Chronology at Queen's University Belfast. The <sup>14</sup>C/<sup>12</sup>C ratios were corrected for decay since 1950 and for isotope fractionation.

Error bars are analytical error. The MRT values estimated from these data are given in Supplementary Table S10.

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