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Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the Harvard Forest

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Abstract

At the Harvard Forest Long-term Ecological Research Site Chronic Nitrogen Amendment Study, a red pine and a mixed deciduous stand showed immediate changes in soil respiration following nitrogen additions (low N: 5 g N m^{-2} per year; high N: 15 g N m^{-2} per year) during the initial year (1988) of the study. In the hardwood stand, soil respiration rates increased after N additions in the first year (control: 482.0 g C m^{-2} per year; high N: 596.5 g C m^{-2} per year). This increase is attributed to increased productivity in the hardwood stand compared to the pine stand; N additions are hypothesized to have increased either root or microbial activity, or perhaps both. In the second year, however, respiration in the fertilized hardwood plots was not different from the control plot. In the pine stand, annual soil respiration was 21 and 25% lower, respectively, in low N and high N plots than the control (429.9 g C m^{-2} per year), with further reductions in the second year.

Weekly measures of soil respiration during summer 2001 showed that after 13 years of continuous nitrogen fertilization, soil respiration in the high N plots during growing season months was suppressed by 41% in both stands. To investigate the possibility that reduced microbial activity contributed to decreased total soil respiration, we incubated root-free soil and measured CO_2 fluxes. The pattern in average respiration for incubated soils was similar to that observed from total soil respiration measured in the field. Laboratory respiration rates from the hardwood high N and pine high N soils were 43 and 64%, respectively, lower than rates from control soils. This indicates that nitrogen additions have reduced microbial activity and thus CO_2 production in the field. Declines in forest productivity measured at both sites, as well as substantial tree mortality observed at the high N sites, may also lower root activity and rhizodeposition, and are also likely to reduce microbial decomposition by reducing organic matter available to soil microbes.

Keywords: Soil respiration; Carbon dioxide; N deposition; C sequestration; Forest soils

1. Introduction

Emissions of anthropogenic CO_2 (IPCC, 2001) and NO_x (Korner, 2000) into the atmosphere are of increasing importance to considerations of ecological economics and environmental policy (e.g. Wigley

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et al., 1996; Melillo and Cowling, 2002) because of potential impacts of altered atmospheric chemistry on forest soil health and services. Globally, soils contain 1500–1600 Pg C, second only to C storage in the deep oceans (Raich and Potter, 1995; Schimel, 1995). They also contain the largest stores of nitrogen in terrestrial ecosystems (Schlesinger, 1997). Carbon fluxes in forest soils of the northern hemisphere are of particular interest to environmental policy discussions because revegetation of abandoned agricultural areas is enabling these forests to serve as sinks for atmospheric C (Houghton, 1996; McGuire et al., 2001; Gurney et al., 2002), with sequestration rates up to 0.3 Pg C per year (Dixon et al., 1994; Nadelhoffer et al., 1999). Though there is considerable variability and uncertainty in the total sink, it is estimated that the C sink for eastern US forests ranges from 0.04 to 0.35 Pg per year (Houghton et al., 1999; Schimel et al., 2000; Goodale et al., 2002). Despite the importance of forest soil C and N storage, quantification of C and N stores, fluxes, and interactions within terrestrial ecosystems remain largely incomplete (McGuire et al., 2001). Nitrogen fertilization in northern temperate zones has been estimated to enhance C storage by 0.3–0.5 Pg C per year (Townsend et al., 1996; Nadelhoffer et al., 1999). However, other estimates suggest that stimulatory effects of N loading on ecosystems is not likely to account for significant C storage (Korner, 2000) and may actually reduce ecosystem productivity and C storage (Aber et al., 1989; Schulze, 1989; Cao and Woodward, 1998).

Soil respiration is one of the primary fluxes of C between soils and the atmosphere, with a global release of 75 Pg C per year (Schlesinger and Andrews, 2000). Understanding controls on soil respiration is critical because relatively small changes in respiration rates may dramatically alter atmospheric concentrations of CO_2 as well as rates of soil C sequestration. In temperate forest soils, respiration is influenced by a number of factors, including substrate quality (Waring and Running, 1998; Rout and Gupta, 1989; Fog, 1988), temperature (e.g. Rustad et al., 2001), soil moisture (e.g. Bowden et al., 1998; Savage and Davidson, 2001), root biomass (Pregitzer et al., 2000) and microbial activity and biomass (Fisk and Fahey, 2001). Nitrogen additions to forest soils have shown variable effects on soil CO_2 effluxes, including increases, decreases, or unchanged rates (Salonius and Mahendrappa, 1975; Brumme and Beese, 1992; Castro et al., 1994; Mattson, 1995; Haynes and Gower, 1995; Vose et al., 1995; Bowden et al., 2000; Burton et al., 2004). With increasing rates of anthropogenic N deposition (Vitousek et al., 1997), there is a strong need to understand links between N inputs and soil respiration.

Most studies reporting effects of N additions on soil respiration have been conducted over relatively short periods, often within the first few years of N additions. Importantly, however, there is a strong need to understand long-term effects of N deposition on soil respiration to better predict impacts on soil C fluxes and storage. The purpose of this study is to: (1) report initial and long-term effects of experimental N additions on soil respiration in a temperate hardwood stand and a red pine plantation at the Harvard Forest Long-term Ecological Research Site Chronic Nitrogen Amendment Study, and (2) to examine the role of N additions on microbial contributions to total soil respiration (Table 1).

2. Site description

The study site is at the Harvard Forest, Petersham, Massachusetts, USA (42°30′N, 72°20′W). Two adjacent 2.3 ha stands with well-documented histories were used (Bowden et al., 1990; Aber et al., 1993): an approximately 55-year-old mixed hardwood stand and a 75-year-old red pine (*Pinus resinosa* Ait.) plantation (planted 1926). Soils are of glacial origin, and are stony- to sandy-loams classified as Typic Dystrochrepts. Soils in both stands are mors, although the hardwood stand has a thicker organic horizon (6.5 ± 1.3 cm (S.E., $n = 9$)) than the pine stand (4.6 ± 0.3 cm (S.E., $n = 9$)). Organic horizons in both stands are acidic, with mean pH values of 3.3 in the hardwood stand and 3.2 in the pine stand.

Mean monthly temperatures range from 19 °C in July to −12 °C in January; average annual precipitation is 112 cm (Magill et al., 2000). Total nitrogen deposition to the forest is approximately 0.8 g m^{-2} per year (wet: 0.6 g m^{-2} per year; dry: 0.2 g m^{-2} per year; Ollinger et al., 1993).

Current forests developed following abandonment from pastures in the early 1900s, and have a well-

Table 1
Annual rates (g C m^{-2} per year) of soil respiration at control and fertilized hardwood and red pine stands, and deviation of fertilized plots from control plots, at the Harvard Forest Long-term Ecological Research Site Chronic Nitrogen Amendment Study

Sampling year	Hardwood					Pine				
	Control	Low N		High N		Control N	Low N		High N	
	Rate	Rate	% change from Control	Rate	% change from Control	Rate	Rate	% change from Control	Rate	% change from Control
1988–1989										
X	482.0	606.5	+25.8	596.5	+23.8	429.9	428.7	−11.1	358.7	−25.6
S.E.	40.9	25.6		27.5		41.9	47.1		32.0	
1989–1990										
X	533.6	605.7	+13.5	475.6	−10.9	480.5	417.7	−21.7	372.9	−30.1
S.E.	53.2	29.2		30.7		21.2	15.7		26.3	

described history (Bowden et al., 1990). The hardwood stand is dominated by black oak (*Quercus velutina* Lam.) and red oak (*Quercus rubra* L.) that together comprised 76% of total basal area of 21.8 m^2 ha^{-1}. The remaining vegetation consists mostly of black birch (*Betula lenta* L.), paper birch (*Betula papyrifera* Marsh.), red maple (*Acer rubrum* L.), and American beech (*Fagus grandifolia* Ehrh.). The pine stand is nearly pure red pine (98% of total basal area of 51.9 m^2 ha^{-1}).

3. Methods

3.1. Plot establishment

In May 1988, three 30 m × 30 m plots were established in each stand. One plot serves as a control, one has received 5 g N m^{-2} per year (low N plots), and one has received 15 g N m^{-2} per year (high N plots). In the first year only, the low N plot received 3.7 g N m^{-2} per year and the high N plot received 12 g N m^{-2} per year. Nitrogen, added as NH_4NO_3, was applied in liquid form with a backpack sprayer in six equal additions from April through September. Untreated corridors at least 50 m wide separate plots. Each plot was divided into 5 m × 5 m subplots, and the outermost subplots in each plot served as buffer strips and were not sampled. Three interior subplots from each plot were randomly selected for measurement of gaseous fluxes. Soil sampling was restricted to the remaining subplots—no soil was removed from subplots used to measure gas fluxes.

3.2. Field soil respiration measurements, years 1 and 2

Carbon dioxide flux measurements began in May 1988 and were conducted routinely for 2 years (year 1 = 1988−1989, year 2 = 1989−1990). Measurements were made at least once per month from March through December, and twice per month during most spring, summer and fall months; fluxes were measured at least 2 weeks after each fertilizer application. Soil respiration measurements were suspended when soils became snow-covered (approximately December), and resumed in the spring (April or May) when the upper 5 cm of soil began to thaw. Soil fluxes during winter were assumed to be the average of the final December estimate and the first measurement conducted in spring. Mean spring soil temperatures (0–2.5 cm depth) in the pine stand were 4.5 °C in year 1 and −1.5 °C in year 2. In the hardwood stand, spring soil temperatures were 6.5 and 0 °C in years 1 and 2, respectively. Soil below 5 cm was still frozen in the pine stand, and the upper 5 cm of soil in the hardwood stand had thawed within the previous week.

Soil carbon dioxide fluxes were measured using an in situ chamber incubation technique (Bowden et al., 1990; Raich et al., 1991). Chambers, constructed of 1.8 cm thick polyvinyl chloride (PVC) plastic, consisted of a 28.7 cm diameter × 4.0 cm tall chamber top and a 5.2 cm tall lower portion (anchor) attached to the organic horizon with stainless steel stakes. Anchors were pushed <1 cm into the organic horizon to minimize disturbance, and the volume within each anchor was measured. Anchors were removed during

the winter of 1988–1989, but were left in place throughout the year in 1989–1990. Incubations were conducted by placing chamber tops, colored white to minimize solar heating, on the anchors and holding them in place with two 4.5 kg weights. Total chamber volume, measured when anchors were implanted 1 cm, was approximately 5.4 L. Gas samples were withdrawn from the headspace via a stainless steel luer-lock needle that extended 2.5 cm into the headspace.

Fluxes were measured six times (every 4 h) in a 24 h period and were timed to provide sampling at approximate minimum and maximum daily soil temperatures. During each 30 min flux measurement, headspace air samples (20 cm^3) were collected at the beginning of the incubation and at 10 min intervals thereafter. Samples were withdrawn with 20 mL nylon syringes. Chamber tops were removed from anchors upon completion of each flux measurement.

Samples were usually analyzed within 4 days of collection. Laboratory tests showed that syringes were inert to CO_2, showed no ''memory effect'' after exposure to high concentrations (1970 ppm CO_2-C) and could store samples for up to 9 days with less than a 4% change in the original concentration. Carbon dioxide was analyzed on a Shimadzu Mini-2 gas chromatograph (GC) equipped with a 3 m × 3.2 mm diameter stainless steel column filled with Poropak Q (80/100 mesh), and a ^{63}Ni electron capture detector. Carrier gas (95% Ar, 5% CH_4) was maintained at 20 mL min^{-1}. Injection and detector temperatures were 275 °C and column temperature was 50 °C. Samples and standards were passed through a silica gel (80/100 mesh) trap to remove water vapor and were then carried into the GC via a stainless steel sampling valve equipped with a 1 mL sampling loop. Certified CO_2 standards (299.7, 1010, and 1970 ppm CO_2-C in N_2) were obtained from Scott Specialty Gases, Plumsteadville, PA, USA.

Carbon dioxide concentrations were corrected to STP conditions and fluxes were calculated using the initial linear portion of the CO_2 concentration increase within the chamber (usually the first two samples over the 30 min incubation). The minimum detection limit for individual flux measurements was approximately 2 mg CO_2-C m^{-2} h^{-1}, depending upon combined field and analytical precision on each sampling date. Coefficients of variation of triplicate samples taken from the same chamber at the same time ranged from 2.4 to 6.1%.

Annual CO_2 fluxes for each treatment were calculated as the mean of the annual fluxes of the three chambers. Each sampling date was considered the midpoint of a sampling period, and the annual flux was the sum of C respired during all sampling periods.

Ambient air (at 1 m), chamber air, and soil temperatures (0–2.5 and 2.5–5.0 cm) were measured during each flux measurement. Air temperature was measured at one location in each stand, and chamber air and soil temperatures were measured at one chamber in each plot. Organic horizon samples (humic material, exclusive of litter overlying mineral soil) ($n = 3-9$) were gravimetrically analyzed for moisture during each sampling (Bowden et al., 1990).

3.3. Field soil respiration measurements, summer, year 13

During the summer of 2001, soil respiration was measured approximately weekly from June until September using a manual soil chamber system with a portable infrared gas analyzer (Savage and Davidson, 2003) (Table 2). Fluxes were measured between 09:00 and 12:00, the time of day when the average flux of the diel cycle occurs (Davidson et al., 1998).

Six 10.2 cm diameter × 10 cm tall white polyvinyl chloride collars were placed randomly in each of the treatment plots within each stand. Collars were inserted approximately 2–4 cm into the soil, and stood approximately 6–8 cm above the soil surface. A vented, 10 cm tall acrylonitrile–butadiene–styrene plastic chamber top was attached to the IRGA and placed over the soil collars during each soil respiration measurement, and a pump circulated air from the chamber to the IRGA at 0.5 L min^{-1}. A backpack-mounted Licor 6252™ portable IRGA system was used to record CO_2 concentrations every 12 s over a flux measurement period of 5 min. The IRGA was calibrated before each measurement using a zero CO_2 standard and a known certified standard (523 ppmv CO_2). Linear regressions (concentration versus time) on concentrations determined rates of CO_2 flux.

3.4. Laboratory soil incubations, year 13

To assess the possibility that N fertilization has depressed microbial respiration in the mineral soil horizon, we collected soils from each of the plots

Table 2
Cumulative soil respiration (g C m^{-2}) during growing season (June 6–September 6, 2001) from control and fertilized hardwood and red pine forest plots, and deviation of fertilized plots from control plots, at the Harvard Forest Long-term Ecological Research Site Chronic Nitrogen Amendment Study

Sampling year	Hardwood					Pine				
	Control	Low N		High N		Control N	Low N		High N	
	Rate	Rate	% change from control	Rate	% change from control	Rate	Rate	% change from control	Rate	% change from control
1988										
X	232.5	325.1	+39.8	310.9	+33.7	204.9	192.3	−17.3	163.7	−29.6
S.E.	15.2	19.1		10.1		23.9	23.0		13.8	
1989										
X	323.6	370.0	+14.3	274.5	−15.2	249.4	210.7	−34.9	185.7	−42.6
S.E.	37.9	18.1		7.1		7.8	13.7		10.8	
2001										
X	276.9	236.7	−14.5	163.3	−41.0	263.1	182.7	−34.0	163.8	−40.9
S.E.	16.2	21.4		16.5		15.7	12.6		7.3	

within each stand, and measured the rate of soil respiration by root-free soils incubated in the laboratory. A-horizon soils were collected with an oakfield corer in early July near each of the collars used for soil respiration sampling. Roots were removed, soils were passed through a 2 mm sieve, and soils were maintained for 4 days in the laboratory at approximately 5 °C prior to incubation. A subsample of each of the soil samples was air-dried at 35 °C to determine field soil moisture content. Prior to incubation, 20 g of fresh weight soil from each sample was adjusted to field moisture content and placed into a 0.46 L (1 pt) canning jar. Six replicates from each of the treatment plots for each stand (36 total) were incubated and allowed to reach ambient temperature of approximately 20 °C; the jars were open just prior to the incubation so that headspaces would equilibrate with ambient air. Jars were then sealed, maintained in the laboratory at ~20 °C, and 5 mL of headspace were collected at times 0, 60, 120 and 180 min. Each gas sample was injected immediately into a Licor 6252™ Infrared Gas Analyzer. The flux rate of CO_2 was calculated using linear regression on initial linear portion of the concentration curve. After incubations were complete, incubated soils were dried at 105 °C, and respiration rates are reported on a dry weight basis.

All statistical tests were conducted using SigmaStat Statistical Software Ver. 2.0 (SPSS Software Products, 1997).

4. Results

Field soil respiration, years 1 and 2 soil temperatures (Fig. 1) were not significantly different among the plots within each stand. Organic horizon moisture content, however, did differ among treatments. In the hardwood stand, the control plot ($x = 1.054$ g H_2O g $soil^{-1}$) was significantly drier (one-way RMANOVA on mean treatment values by date, $F = 5.322$, $P < 0.009$ over the year) than the high N plot ($x = 1.336$ g H_2O g $soil^{-1}$). In the pine stand, the control (1.170 g H_2O g $soil^{-1}$) and high N (1.051 g H_2O g $soil^{-1}$) were significantly wetter (Friedman one-way RMANOVA on mean treatment values by date, chi-square 11.7, $P < 0.003$) than the low N (0.930 g H_2O g $soil^{-1}$).

Carbon dioxide effluxes showed a strong seasonal pattern (Fig. 2), with highest rates observed in July–August in both stands, and lowest rates in early winter. Rates ranged from approximately 20 mg C m^{-2} h^{-1} during winter months, to over 150 mg C m^{-2} h^{-1} in the pine stand and over 200 mg C m^{-2} h^{-1} in hardwood stands in mid-summer. Soil respiration was nearly always higher in the hardwood stand than the pine stand.

Fluxes exhibited positive exponential relationships with temperature and negative relationships with moisture content of the organic horizon (Fig. 3). Exponential relationships between soil temperature

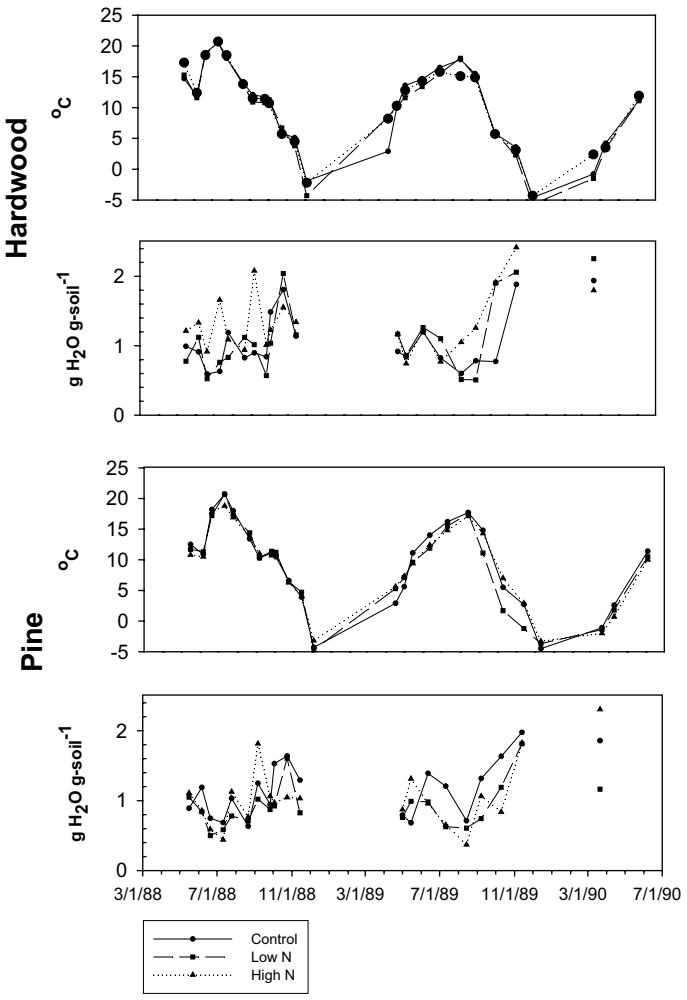

Fig. 1. Soil temperature (0–2.5 cm depth) and moisture (organic horizon) in control and fertilized hardwood and red pine forest stands (1988–1990) at the Harvard Forest Long-term Ecological Research Site Chronic Nitrogen Amendment Study.

and soil respiration were very highly significant ($P < 0.0001$) in all plots, with r^2 values ranging from 0.72 to 0.86. An overall pattern of decreased respiration with increased organic horizon moisture content is apparent, but individual relationships among fluxes and moisture within each treatment during the study period were less clear. Exponential decay relationships between respiration and soil moisture over the 1988–1990 sampling period were significant in two of the six plots, with r^2 values ranging from 0.13 to 0.35.

Nitrogen fertilization showed different effects in the two stands during the first year of fertilization. In the hardwood stand (Fig. 4), N fertilization increased annual CO_2 effluxes by 26 and 24% (one-way RMANOVA, $F = 9.348$, $P < 0.001$, Tukey's test) in the low N and high N plots (Table 1), respectively, over the control plots (482.01 ± 40.94 g C m^{-2} per year). In contrast, pine stand control plot soil respiration (429.87 ± 41.89 g C m^{-2} per year) was 20% higher (Friedman one-way RMANOVA, chi-square 21.571, $P < 0.001$) than the high N plot, though not significantly different than the low N plot.

In the second year of fertilization, the hardwood stand began to show a depression in soil respiration due to N fertilization; low N and high N rates did not differ significantly (one-way RMANOVA, $F = 7.503$,

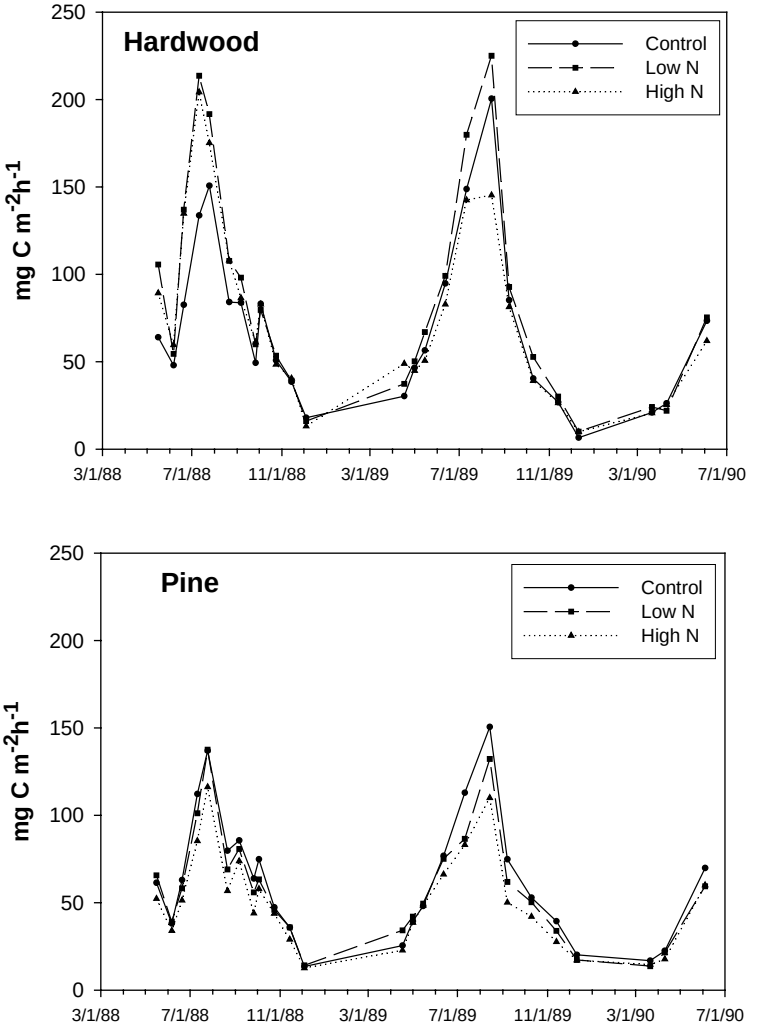

Fig. 2. Soil respiration (1988–1990) in control and fertilized hardwood and red pine forest stands at the Harvard Forest Long-term Ecological Research Site Chronic Nitrogen Amendment Study.

$P < 0.004$, Tukey's test) from control rates, although respiration in the high N plot was significantly lower (27%) than the low N plot. In the pine stand, respiration in both fertilized plots were significantly lower than the control (one-way RMANOVA, $F = 15.567$, $P < 0.001$, Tukey's test) (Fig. 5).

4.1. Field soil respiration, year 13

Mean growing season soil respiration (Table 2, Fig. 5) in the N amended plots was further reduced in 2001 in the hardwood stand compared to results observed in the 1989–1990 sampling year. The high N plot exhibited significantly lower respiration rates than the control plot (one-way ANOVA, $F = 9.471$, $P < 0.002$, Tukey's test) (Fig. 6). The soil respiration pattern during 2001 in the Pine Stand was nearly identical to the pattern observed in the 1989–1990 sampling season, with both fertilized plots exhibiting significantly lower rates than those observed in the control (one-way ANOVA, $F = 17.850$, $P < 0.001$, Tukey's test).

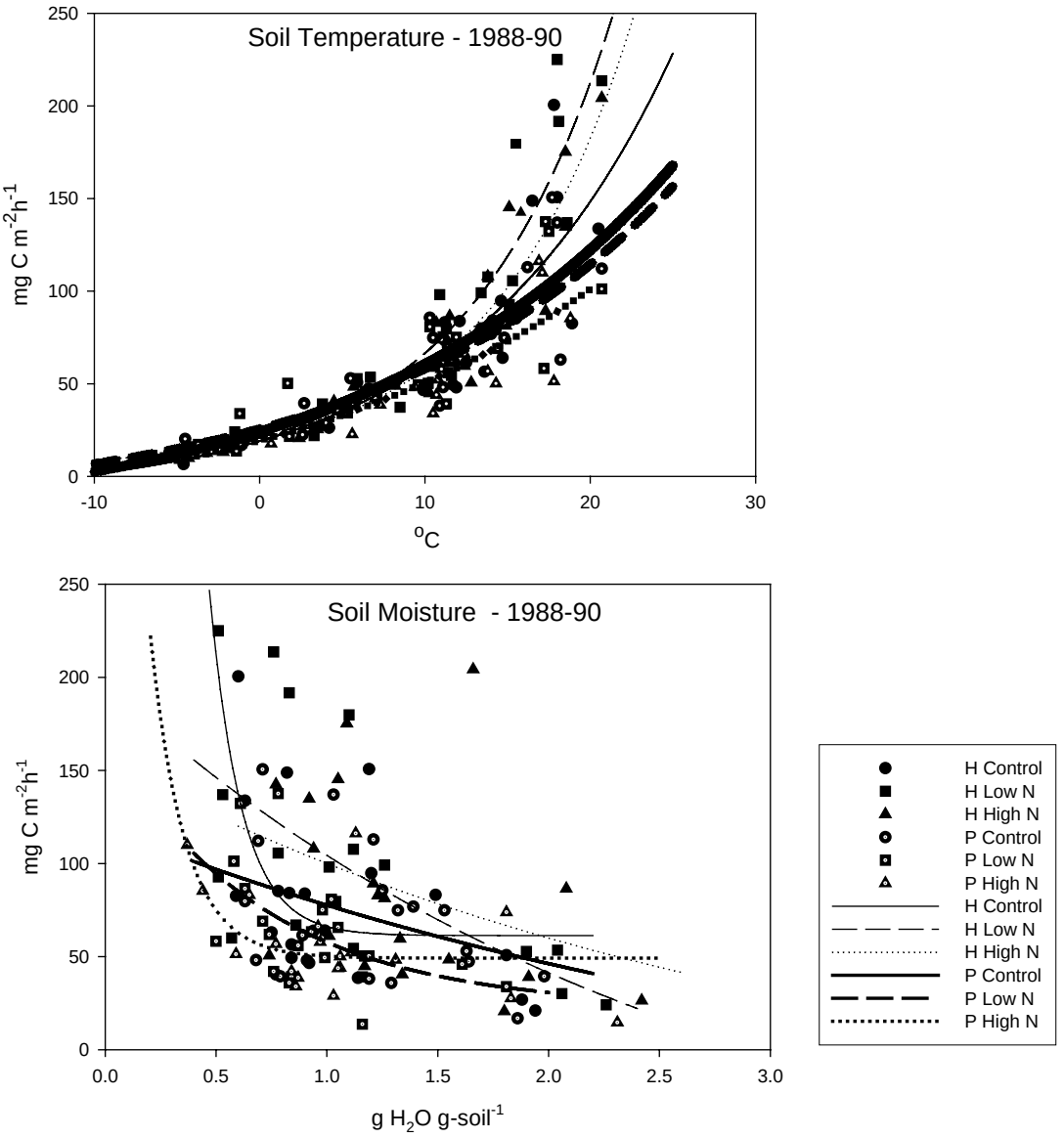

Fig. 3. Relationships between soil respiration and soil temperature (0–2.5 cm depth) and soil moisture (organic horizon) in control and fertilized hardwood and red pine forest stands at the Harvard Forest Long-term Ecological Research Site Chronic Nitrogen Amendment Study. H: hardwood, P: pine. Temperature relationships ($P < 0.0001$ for all plots)—hardwood control: flux $= -13.2 + 32.25^{(0.0806\times\text{temperature})}$, $r^2 = 0.72$; hardwood low N: flux $= 0.1703 + 20.69^{(0.1164\times\text{temperature})}$, $r^2 = 0.86$; hardwood high N: flux $= 3.8 + 16.28^{(0.1198\times\text{temperature})}$, $r^2 = 0.86$; pine control: flux $= -28.6 + 52.66^{(0.0527\times\text{temperature})}$, $r^2 = 0.75$; pine low N: flux $= -18.7 + 44.35^{(0.0550\times\text{temperature})}$, $r^2 = 0.73$; pine high N: flux $= -31.7 + 51.11^{(0.0477\times\text{temperature})}$, $r^2 = 0.73$. Moisture relationships—hardwood control: flux $= 61.3 + (4703\,e^{-6.8747\times\text{moisture}})$, $r^2 = 0.29$, $P = 0.058$; hardwood low N: flux $= -93.4 + (290.3\,e^{-0.3844\times\text{moisture}})$, $r^2 = 0.35$, $P = 0.025$; hardwood high N: flux $= -44.5 + (199.9\,e^{-0.3248\times\text{moisture}})$, $r^2 = 0.13$, $P = 0.318$; pine control: flux $= -51.9 + (170.9\,e^{-0.2775\times\text{moisture}})$, $r^2 = 0.18$, $P = 0.184$; pine low N: flux $= 22.0 + (147.3\,e^{-1.4179\times\text{moisture}})$, $r^2 = 0.26$, $P = 0.078$; pine high N: flux $= 49.2 + (645.8\,e^{-6.4395\times\text{moisture}})$, $r^2 = 0.33$, $P = 0.033$.

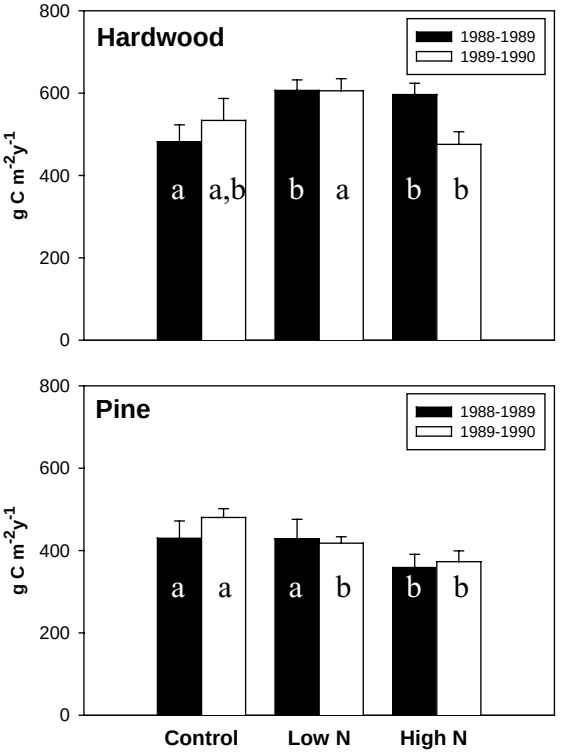

Fig. 4. Annual soil respiration (1998–1989, 1989–1990) in control and fertilized hardwood and red pine forest stands at the Harvard Forest Long-term Ecological Research Site Chronic Nitrogen Amendment Study.

4.2. Laboratory soil respiration

Root-free soil respiration rates in the laboratory-incubated soils during summer of year 13 (Fig. 7) were greater in the control soil than the high N plot soil for both the hardwood and pine stands. In the hardwood soil, respiration in the high N plot was significantly lower than either the control or low N plots (one-way ANOVA, $F = 4.093$, $P < 0.038$, Duncan's multiple range). In the pine soil, respiration in the control plot was significantly greater than either the low N or high N plots (one-way ANOVA, $F = 14.492$, $P < 0.001$, Tukey's test).

5. Discussion

Lower respiration rates in the pine stand (429.9 (year 1) − 480.5 (year 2) g C m^{-2} per year) compared to the hardwood stand (482.0 (year 1) − 533.6 (year 2) g C m^{-2} per year) may reflect generally lower rates of gross primary productivity by the pine stand. The first 2 years of our results fit within the wide range of results reported in the literature for soil respiration responses to N additions. For example, increased respiration in the hardwood stand agrees with results for young aspen cuttings (Pregitzer et al., 2000) as well as for those studies that report a positive correlation between root N concentrations and soil respiration (e.g. Zogg et al., 1996; Burton et al., 2002). Decreased respiration is consistent with a number of studies, including those in mixed hardwoods (Bowden et al., 2000; Fisk and Fahey, 2001; Burton et al., 2004), as well as in coniferous forests (Mattson, 1995; Maier and Kress, 2000) Looking at growing season months only, respiration was reduced 14% in the low N plot and 41% in the high N plot in 2001, which is substantially greater than the reductions observed in year 2 of the study. Suppression of soil respiration was also pronounced in the pine stand after 13 years, but rates of suppression (34–41%) were essentially identical to those rates measured in year 2.

We cannot explain completely the pattern that we observed in the first 2 years, but we suspect that increased soil respiration was related to the increases in aboveground biomass woody increments and litterfall, as well as to standing root biomass in fertilized plots observed by Magill et al. (1997). It is likely that added C from aboveground litter and from decomposing roots stimulated heterotrophic respiration, and the greater mass of roots and thus root exudation enhanced autotrophic respiration in the fertilized plots. Recent work (Hogberg et al., 2001) has shown a rapid link between photosynthetic production and belowground C allocation. The reversal of the N addition response in the hardwood stand during the second year may reflect changes in root activity associated with nutrient uptake. A large fraction of root respiration is allocated to N assimilation (Bloom et al., 1992), but with larger doses of N readily available for uptake, energetic costs of N assimilation may have been reduced. The early response in the pine stand may have been driven by direct impacts of N additions on the soil microbial community, as well as by changes in overall forest growth. Whereas aboveground litterfall and standing root biomass were greater in the fertilized plots than in the control during

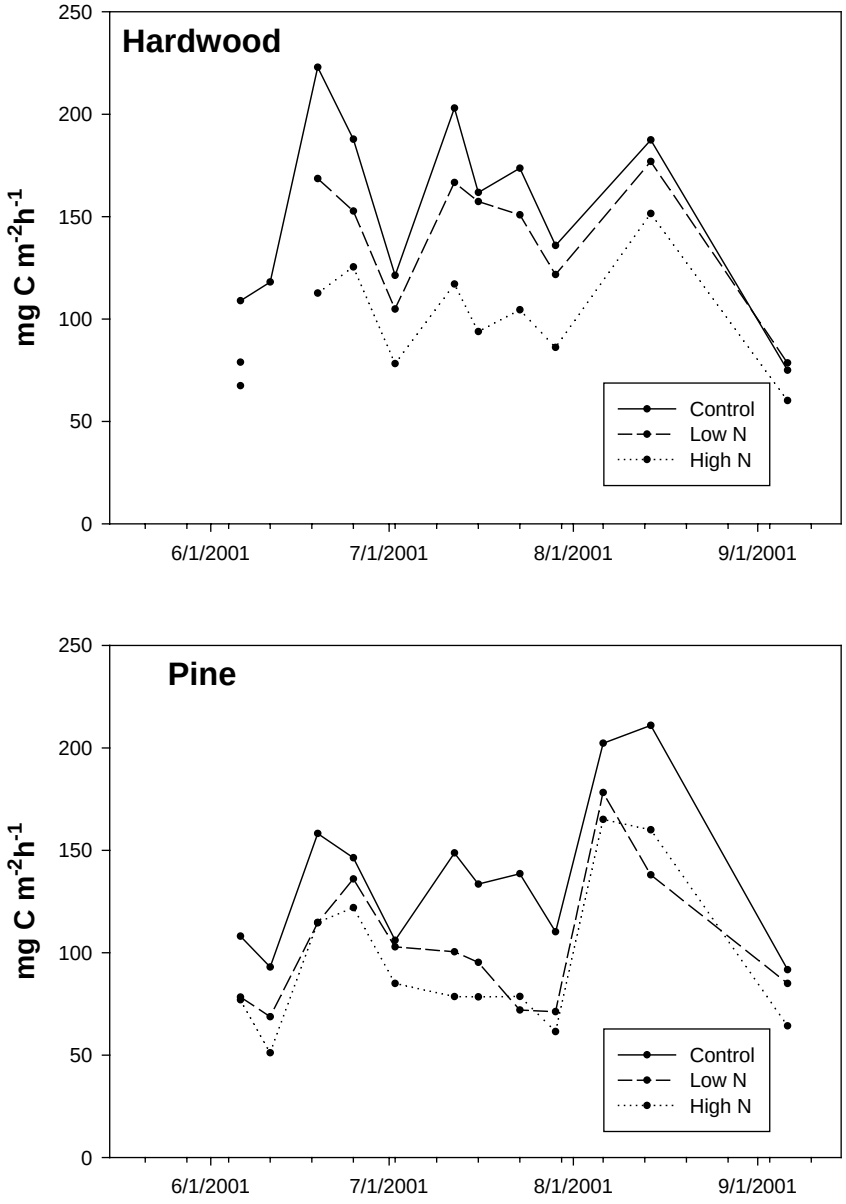

Fig. 5. Soil respiration in control and fertilized hardwood and red pine forest stands during the 2001 growing season at the Harvard Forest Long-term Ecological Research Site Chronic Nitrogen Amendment Study.

the period from 1989 to 1993, the woody aboveground increment was lower in the high N plot than either the control or low N plots. An additional factor, especially in the pine stand, may be the response of the ectomycorrhizal fungal community, which has been shown to be quite sensitive to N inputs (Harley and Smith, 1983).

Laboratory incubation of root-free soil shows that heterotrophic respiration from the microbial community in fertilized plots is reduced. Although Micks et al. (2004) did not find a microbial respiration response immediately (within days) following N fertilizer addition, Compton et al. (2004) found that chronic N additions decreased microbial biomass and diversity,

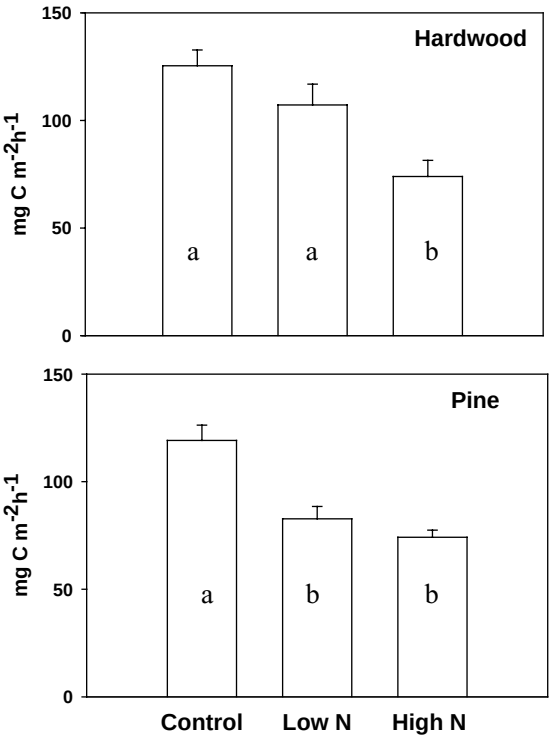

Fig. 6. Mean rates of soil respiration in control and fertilized hardwood and red pine forest stands at the Harvard Forest Long-term Ecological Research Site Chronic Nitrogen Amendment Study over the growing season, 2001. Rates (within each stand) with the same letter are not significantly different.

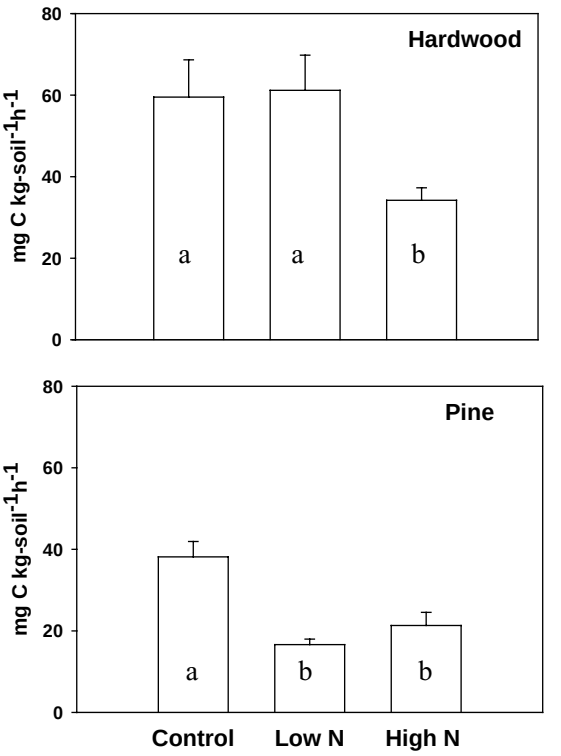

Fig. 7. Respiration rates (per gram of soil dry weight) of laboratory-incubated, root-free soils from control and fertilized hardwood and red pine forest stands at the Harvard Forest Long-term Ecological Research Site Chronic Nitrogen Amendment Study. Rates (within each stand) with the same letter are not significantly different.

and Frey et al. (2004) observed that active fungal biomass was lower in fertilized plots than in control. Frey et al. (2004) also detected a significant reduction in the activity of the enzyme phenol oxidase, a lignin-degrading enzyme produced by white-rot fungi. Along a N deposition gradient in the California coastal scrubland, N fertilization reduced mycorrhizal infection rates and survival (Egerton-Warburton and Allen, 2000), and Ruhling and Tyler (1991) observed that following N additions, mycorrhizal species nearly ceased producing fruiting bodies. Alterations in the microbial community may also change enzyme production and decomposer efficiency, reducing rates of respiration. It is also possible that decreases in soil pH have reduced microbial activity. In both stands, soil pH measured in the O-horizons in 2002 was lower in the fertilized plots than in control plots. In the hardwood stand control pH was 3.35, and the high N plot was 3.15. In the pine stand, control pH was 3.54, and the high N was 3.15 (A. Magill, University of New Hampshire, personal communication). Additions of proton-producing NH_4, along with the acidity generated by accelerated rates of nitrification in the fertilized plots, may well have reduced soil microbial activity as others have suggested (Aerts and de Caluwe, 1999).

After 13 years, root activity may also be reduced. Root biomass (A. Magill, personal communication) in organic horizons and mineral horizon to 20 cm in 1999 was highest in the control plot and lowest in the high N plot; biomass in the pine stand, however, did not show a discernable pattern. In year 13, tree mortality was noticeable in the pine stand, and substantial crown dieback was observed in the hardwood stand. Insofar as microbes preferentially use the short-lived fractions of soil organic matter (Parton et al., 1987; Schimel

et al., 1994), and that the average age of C contributing to soil respiration at the Harvard Forest is 8 years (Trumbore, 2000), loss of both aboveground and belowground litter would reduce the microbial contribution to soil respiration. Furthermore, as shown by Uselman et al. (2000), rhizodeposition is rapidly used by soil microbes, and loss of this organic matter input due to declines in root activity would also depress soil respiration.

This study indicates that the microbial community has undergone long-term change in response to chronic N additions, strongly affecting an important carbon flux. Our work suggests that different processes within forest soils may be altered over the course of N deposition, and that initial, short-term measures of soil respiration in response to N fertilization will inadequately indicate long-term soil responses to altered resource availability. Given the importance of soil respiration in net ecosystem C balance, it is critical to understand and to be able to predict long-term responses of soil processes to N deposition.

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