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Simulated chronic NO_3^- deposition reduces soil respiration in northern hardwood forests

ANDREW J. BURTON*, KURT S. PREGITZER*, JEFFREY N. CRAWFORD*, GREGORY P. ZOGG† and DONALD R. ZAK‡

*School of Forest Resources & Environmental Science, Michigan Technological University, Houghton, MI 49931, USA, †Department of Biological Sciences, University of New England, Biddeford, ME 04005, USA, ‡School of Natural Resources & Environment, University of Michigan, Ann Arbor, MI 48109, USA

Abstract

Chronic N additions to forest ecosystems can enhance soil N availability, potentially leading to reduced C allocation to root systems. This in turn could decrease soil CO_2 efflux. We measured soil respiration during the first, fifth, sixth and eighth years of simulated atmospheric NO_3^- deposition ($3\,g\,N\,m^{-2}\,yr^{-1}$) to four sugar maple-dominated northern hardwood forests in Michigan to assess these possibilities. During the first year, soil respiration rates were slightly, but not significantly, higher in the NO_3^--amended plots. In all subsequent measurement years, soil respiration rates from NO_3^--amended soils were significantly depressed. Soil temperature and soil matric potential were measured concurrently with soil respiration and used to develop regression relationships for predicting soil respiration rates. Estimates of growing season and annual soil CO_2 efflux made using these relationships indicate that these C fluxes were depressed by 15% in the eighth year of chronic NO_3^- additions. The decrease in soil respiration was not due to reduced C allocation to roots, as root respiration rates, root biomass, and root turnover were not significantly affected by N additions. Aboveground litter also was unchanged by the 8 years of treatment. Of the remaining potential causes for the decline in soil CO_2 efflux, reduced microbial respiration appears to be the most likely possibility. Documented reductions in microbial biomass and the activities of extracellular enzymes used for litter degradation on the NO_3^--amended plots are consistent with this explanation.

Keywords: atmospheric nitrate deposition, nitrogen fertilization, root biomass, root respiration, soil CO_2 efflux, temperature and moisture effects

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Introduction

In many forests, ecosystem respiration is the main determinant of net C exchange (Valentini *et al.*, 2000). Because soil respiration is a large flux of C to the atmosphere (Houghton & Woodwell, 1989; Janssens *et al.*, 2001), any anthropogenic factor that alters soil respiration has the potential to alter the strength of an ecosystem as a net C source or sink. Experimental additions of N to mature forests typically result in decreased soil respiration (Söderström *et al.*, 1983; Haynes & Gower, 1995; Bowden *et al.*, 2000; Homann *et al.*, 2001, Butnor *et al.*, 2003), although positive effects and a lack of response also have been documented (Nohrstedt & Börjesson, 1998; Kane *et al.*, 2003). Inorganic N at high application rates ($\geq 10\,g\,N\,m^{-2}\,yr^{-1}$) clearly reduces respiration rates (Haynes & Gower, 1995; Bowden *et al.*, 2000), but it is not well understood if chronic, low-level additions of inorganic N, more representative of anthropogenic N deposition, can elicit similar responses.

Changes in soil CO_2 efflux could result from either reduced respiration of roots and their associated mycorrhizae or from lower microbial respiration. At the ecosystem level, lower root respiration would occur if either specific respiration rates or root biomass declined in response to N additions. Decreases in specific respiration rates are unlikely, since enhanced root N concentrations are commonly associated with greater specific root respiration rates (Burton *et al.*, 1996,

Correspondence: Andrew J. Burton, fax (906)487-2915, e-mail: ajburton@mtu.edu

2002; Ryan *et al*., 1996). However, reductions in root biomass have occurred in response to N fertilization of mature forests (Vogt *et al*., 1990; Haynes & Gower, 1995), as the relative amount of C allocated belowground decreased in response to improved N availability.

Microbial respiration could decrease in response to N additions if either the quantity of C inputs from litter and exudates decreased or the rates of decomposition processes were altered. Foliar mass and litter inputs are likely to increase (Ryan *et al*., 1996), not decrease, following N additions, but litter inputs from root biomass turnover can change in either direction as N availability increases (Keyes & Grier, 1981; Nadelhoffer *et al*., 1985; Hendricks *et al*., 1993; Pregitzer *et al*., 1993, 1995; Burton *et al*., 2000). Root exudation also can change as nutrient availability is altered (Dakora & Phillips, 2002). In the absence of changes in the quantity of C inputs, microbial respiration could still be reduced by N additions, due to altered decomposition of N-enriched litter and soil organic matter (Fog, 1988; Berg & Matzner, 1997; Berg, 2000; Carreiro *et al*., 2000; Thirukkumaran & Parkinson, 2000).

From 1994 to 2001, we examined soil respiration in four northern hardwood ecosystems to determine if relatively low rates of NO_3^- additions ($3.0\,g\,N\,m^{-2}\,yr^{-1}$) would alter this important C flux. This N addition rate is approximately two to three times ambient N deposition over wide areas in the industrialized north central and northeastern US (MacDonald *et al*., 1992; Harding ESE, Inc., 2002). Atmospheric N inputs in portions of Europe and at high deposition locations in the US already equal or exceed $3.0\,g\,N\,m^{-2}\,yr^{-1}$ (Bredemeier *et al*., 1998; Fenn *et al*., 1998; MacDonald *et al*., 2002). Root respiration rates, root biomass, and root longevity and turnover also were measured, to assess whether changes in the amount of C allocated to root functions were responsible for any observed differences in soil respiration following chronic N additions. The specific objectives of the research were: (1) to determine if chronic NO_3^- additions altered soil CO_2 efflux; (2) to quantify the effects of soil temperature and soil moisture on soil respiration; (3) to estimate growing season and annual soil CO_2 efflux for control and NO_3^--amended soils at the four study sites; and (4) to determine if responses of soil CO_2 efflux to simulated chronic NO_3^- deposition resulted from altered ecosystem root respiration or changes in belowground C inputs from annual root turnover.

Materials and methods

Study site descriptions

The research was conducted in four second-growth northern hardwood forests located along a 3° latitudinal transect in Michigan. The forests are approximately 90 years of age, are dominated by sugar maple (*Acer saccharum* Marsh.), and have sandy, well-drained Spodosol soils (Table 1). The mean annual air temperature increases by about 3 °C from north to south (Sites A–D) along the latitudinal gradient, and differences among the sites in inherent N availability have been documented (Burton *et al*., 1996; Zogg *et al*., 1996), with higher native N availability occurring at Sites B and C (Table 1). Three control and three NO_3^--amended plots

Table 1 Selected characteristics of four northern hardwood forests in Michigan, USA

Characteristic	Site A	Site B	Site C	Site D
Latitude (N)	46°52′	45°33′	44°23′	43°40′
Longitude (W)	88°53′	84°51′	85°50′	86°09′
Mean annual precipitation* (mm)	821	828	856	793
Mean annual temperature† (°C)	4.8	6.1	6.9	7.6
Total basal area ($m^2\,ha^{-1}$)	35	33	33	36
Sugar maple basal area (%)	91	86	79	71
Overstory age	94	88	89	93
Net N mineralization‡ ($\mu g\,N\,g\,soil^{-1}$)	0.29b	0.46a	0.48a	0.32b

Overstory data are from the year 2001. Site means for N mineralization followed by a different letter are significantly different at the 0.05 level of probability.

*Mean annual precipitation, for the years 1994–2001, was recorded using weighing rain gages (Model 5-780, Belfort Instrument Co., Baltimore, MD, USA) located in open areas within 5 km of each site.

†Mean annual temperature, for the years 1994–2001, was recorded on site at 2 m using thermistors that were read every 30 min throughout the year, with averages recorded every 3 h using data loggers (EasyLogger Models 824 and 925, Data Loggers, Inc., Logan, UT, USA).

‡Net N mineralization data from Zogg *et al*. (1996) are for the top 10 cm of soil and organic matter occurring beneath the surface litter (Oi) layer, determined using the buried bag technique for the period of May through November, 1994.

(30 m × 30 m) were located at each site. Since 1994, the NO_3^--amended plots have received 3.0 g N m^{-2} yr^{-1} as $NaNO_3$, applied in six monthly 0.5 g N increments from April through September. Nitrogen was also applied to a 10 m wide buffer area surrounding each treated plot. Nitrogen additions to the study sites are cycling through the ecosystem, resulting in enhanced foliar and litter N concentrations (Zak *et al.*, 2004).

Soil temperature and matric potential were continuously monitored at the sites. In all plots, soil moisture blocks (Model 5201, Soilmoisture Equipment Corporation, Goleta, CA, USA) were placed at a depth of 15 cm, and temperature thermistors (Model ES-060-SW, Wescor Inc., Logan, UT, USA) were placed at 5 and 15 cm. Moisture blocks and thermistors were read every 30 min by data loggers (EasyLogger Models 824 and 925, Wescor Inc., Logan, UT, USA), with average values recorded every 3 h. Moisture block resistance readings (ohms) were converted to matric potential (MPa) using relationships developed for each site using intact soil cores equilibrated with soil moisture plates at potentials ranging from −0.01 to −1.5 MPa.

Soil respiration

In 1994, soil respiration was measured at all four sites at 4–5-week intervals from mid-May to early November, using a static chamber technique. In 1998, 1999, and 2001, soil respiration was measured using a dynamic chamber, infra-red gas analyzer (IRGA) system (SRC-1 chamber and EGM-2 IRGA, PP Systems, Haverhill, MA, USA). Measurements were made 22 times at Site A during 1998, at weekly intervals from late April through early November. In 1999 and 2001, soil respiration measurements were made at all four study sites to determine if treatment responses observed at Site A in 1998 were occurring consistently across sites. Seven measurement periods from late June to late September were used during 1999, and eleven measurement periods from mid-May to early November were used during 2001. During the conversion of methods from static chamber to dynamic chamber-IRGA, one set of soil respiration measurements utilizing both techniques was made at all four sites in August, 1998.

Soil respiration measurements with static chambers in 1994 utilized two to three 25 cm × 25 cm aluminum frames, located on the outer edge of each 30 m × 30 m plot. Frames were permanently established in November, 1993 by inserting them into the forest floor and surface soil to a depth of 5 cm. On each sample date, a plastic box (25 cm × 25 cm, 15 cm in height) was inserted in gutters on the top of the aluminum frames to collect gases. Water (ca. 10 mL) was placed in the gutter to ensure an air-tight seal. Using needles and syringes, four gas samples were withdrawn from each box at 15 min intervals. These were stored in 3 mL glass vials sealed with buytl rubber septa and analyzed for CO_2 within 2 weeks of collection by gas chromatography (Tracor 540, Tracor Instruments, Austin, TX, USA). Soil respiration rates for each sample location were determined by regression analyses of CO_2 concentration vs. time, with values expressed as μmol CO_2 m^{-2} s^{-1}.

Soil respiration measurement with the dynamic chamber – IRGA system utilized 10 soil respiration collars, located in the interior 20 m × 20 m portion of each plot. The respiration collars (10.2 cm diameter, schedule 40 PVC) were inserted 2.5 cm into the forest floor at least 2 weeks prior to initial measurements each year and were left in place for the duration of the growing season. A portable thermometer was used to measure soil temperature at 5 cm concurrently with soil respiration measured at each collar. Barometric pressure readings were taken during each measurement interval to correct for pressure broadening of infrared absorption bands and the effects of atmospheric pressure on gas volumetric concentrations (μmol L^{-1}).

Root respiration

Fine root respiration rates were measured at all four sites ten times between May and November, 2001. Samples consisted of roots $\leq$ 1 mm diameter collected from the top 5 cm of organic matter and mineral soil at three to four locations within each plot and composited. The roots were brushed free of loose soil and organic matter, but were not washed or rinsed. Root samples typically consisted of five to seven excised root mats, each of which comprised an intact network of root segments containing primarily first-, second-, and third-order roots (Pregitzer *et al.*, 1998, 2002). One composite sample was analyzed from each plot at a site on each measurement date. Roots were collected over a 15 min period, after which the sample (ca. 2–4 g fresh weight) was immediately placed in a respiration cuvette (Burton *et al.*, 2002, Burton & Pregitzer, 2003) attached to an IRGA (CIRAS-1 portable gas analyzer, PP Systems, Haverhill, MA, USA) in an open system. Steady respiration rates were achieved within 15–20 min after placing a sample in the cuvette. At each site, a 17 cm long aluminum cuvette base was installed in the soil, with only the upper 1 cm of the base above the soil surface. These were left in place for the duration of the experiment and allowed roots inside the cuvette to be maintained at ambient soil temperature during respiration measurements (Burton & Pregitzer, 2003). The input [CO_2] for the cuvette was maintained at

$1000\,\mu L\,L^{-1}$ in order to approximate surface soil $[CO_2]$. We tested a subset of our field root respiration samples for a possible $[CO_2]$ effect (Qi *et al*., 1994; Burton *et al*., 1997) by determining respiration rates at both 350 and $1000\,\mu L\,L^{-1}$, and found no effect of $[CO_2]$ (Burton & Pregitzer, 2002).

The interruption of C flow to roots following excision could lead to decreased root respiration over time (Bloom & Caldwell, 1988; McDonnell & Farrar, 1992). To minimize this potential effect, we limited the damage to the sample by using the five to seven intact root mats described above and completing respiration measurements within 35 min of excision. In previous work with sugar maple roots, we have found no immediate effect of excision on respiration rates (Burton *et al*., 1998), and only small declines over longer time periods of up to four hours.

Following respiration measurements, root samples were placed on ice until they could be returned to the laboratory (<3 h). In the lab, root samples were frozen until a later date when they were cleaned of any adhering soil and organic debris ($\leq 5\%$ of sample mass), dried, and analyzed for N using an elemental analyzer (Carlo Erba NA 1500 NC, CE Elantech, Lakewood, NJ, USA).

Respiration rates of larger diameter roots (1–2, 2–5, and 5–10 mm) were measured during two sampling periods in the summer of 2002, using the same analytical procedures as described above for roots ≤ 1.0 mm in diameter.

Root biomass

Root biomass was determined for all four sites during May 1994, June 1996, September 2000, and August 2001. During each sampling, eight to ten soil cores (5 cm diameter $\times$ 25 cm deep) were collected from each plot at each site. Roots were sorted from the cores by hand, with live roots placed into one of five diameter classes: <0.5, 0.5–1,1–2, 2–5, and 5–10 mm. Root mass was determined after oven drying for 48 h at 70 °C. The residual soil from one third of the cores was elutriated (Hydropneumatic Root Washer, Gillison's Variety Fabrication, Benzonia, MI, USA) to capture very fine roots that were missed by hand sorting (Smucker *et al*., 1982; Hendrick & Pregitzer, 1993). The elutriated root slurry was placed in a clear plastic tray with a grid pattern on the bottom. This was then placed on a light table, and the total number of line intercepts was recorded. For a subset of these samples, all elutriated very fine roots (<0.5 mm) were retrieved by hand from the line-intercept tray, dried, and weighed. This allowed us to develop a relationship between line-intercept count and very fine root mass, which was used to convert all line-intercept counts to masses.

Root longevity and turnover

Fine root production and mortality data were collected from Site A over a 3-year period, using five clear polybutyrate minirhizotron tubes (2 m long $\times$ 5.08 cm inside diameter) per plot. Sampling for root longevity and turnover for the three-year period was limited to Site A due to the time-consuming nature of image processing and analysis. Minirhizotron tubes utilized in this study were installed in October 1993 at a 45° angle to the soil. Rectangular, numbered image frames (0.9 cm $\times$ 1.3 cm) were scribed every 0.9 cm along a transect on the exterior surface of each minirhizotron tube prior to installation to enable videotaping of the same locations within the soil on all sampling dates (Hendrick & Pregitzer, 1992; Burton *et al*., 2000).

Root video images were collected at approximately 5-week intervals from mid-May through early November during 1999, 2000, and 2001 using a model BTC 1.125 Minirhizotron Research Color Camera (Bartz Technology Co., Santa Barbara, CA, USA). During quantification of root demography, all images of a given frame were displayed together to allow individual roots to be followed throughout their lifespan. This ensured that dead or missing roots did not reappear at a later date with better image quality. Each root was given a unique identification number on the date it first appeared; on all subsequent image dates, the root was reclassified as living or dead (based upon color and consistency in the image), and root length was recorded.

The effect of NO_3^--additions on fine root lifespan was assessed by studying the survival of fine roots (≤ 1.0 mm) from the 0–10 cm depth. Sixty randomly selected image frames from this depth range were analyzed for both the control and NO_3^--amended plots, producing life-history data for over 250 individual roots per treatment. Fine root production for each sampling interval was determined by summing the length of all new roots and adding the extension growth of all previously existing roots. Fine root mortality for each sampling interval was determined by summing the lengths of all roots that had died during that interval and adding root length lost by existing roots due to herbivory or dieback. Production and mortality were both expressed as root length per minirhizotron tube area observed ($mm\,cm^{-2}$). The annual fine root turnover (proportion of standing root biomass replaced annually) was estimated by dividing the average of root length production and root length mortality by the mean live root length observed, as described in Burton *et al*. (2000).

Statistical analyses

The effects of N additions on soil and root respiration rates were tested using two-factor (study site and N addition) repeated measures analysis of variance (ANOVA). Respiration rates recorded on different dates were used as the repeated measure. Soil respiration data from 1994 were analyzed separately due to the different methodology used that year. Regression analysis was used to model the effects of temperature and moisture on soil respiration rates during 1999 and 2001. Plot-level means from each sample date were used in these analyses, with the natural log of respiration serving as the dependent variable. The effect of N additions on root biomass was examined by repeated measures ANOVA, with values from the four different years of record used as the repeated measure. This analysis was conducted for each individual root size class and for composite classes of all roots ≤ 1, ≤ 2, and ≤ 10 mm.

Estimates of growing season and annual soil CO_2 efflux for 2001 were made for each plot at each site using measured values of daily mean soil temperature and moisture in conjunction with regression relationships for each individual plot at each site. Daily estimates of soil CO_2 efflux for a plot were then summed to determine growing season (May–October) and annual soil CO_2 efflux. Two-factor ANOVA (study site and N addition) was used to study the effects of N additions on these cumulative CO_2 effluxes.

Fine root survival functions were determined using the life table method of failure-time analysis (Fox, 1993), which is appropriate for right censored data (Lee, 1992). The SAS lifetest procedure (SAS Institute, Inc., 1989) was used to perform these analyses and to determine product-limit survival estimates of mean and median root lifespans. Differences among treatments in annual root turnover were determined using ANOVA.

Results

Soil respiration was not affected by NO_3^- additions during 1994, the first year of our experiment, but during 1998, 1999, and 2001, a reduction in soil respiration rates on N-amended plots was clearly apparent (Figs 1 and 2, Table 2). The decline in soil respiration was similar for both 1999 and 2001 (circa 15%). The lack of response in 1994 was not an artifact of the different methodology used that year, as a one-time sampling at all sites during August 1998 detected reduced soil respiration rates for NO_3^--amended plots using either method. For static chambers, the mean reduction across sites was 4.3% (not statistically significant), and for the dynamic chamber – IRGA method a significant reduction of 9.5% occurred. There were differences between the methods in the magnitude of observed respiration rates, with the static chamber method producing rates in 1994 that were consistently around 45% of those measured by the dynamic chamber – IRGA system under the same soil temperature and moisture conditions.

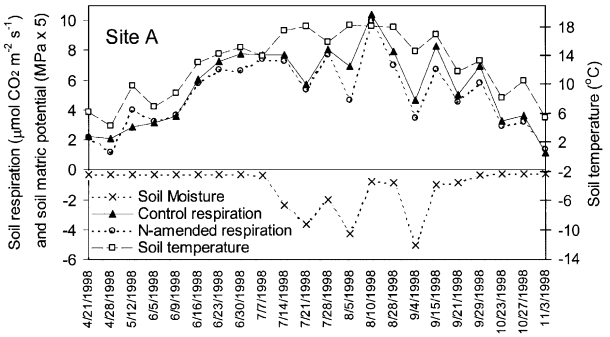

Fig. 1 Temporal patterns of soil respiration, soil temperature, and soil moisture during 1998 at Site A. Average soil respiration on NO_3^--amended plots was 13% less than on control plots.

Differences in soil respiration rates also occurred among sites (Table 2), with Sites A and D tending to have greater soil respiration rates for a given set of temperature and moisture conditions. Within sites, differences among measurement dates in soil respiration rates were strongly related to soil temperature and soil moisture (Fig. 1). For 1999 and 2001, soil respiration rates for each site and treatment could be predicted by the combination of soil temperature and soil matric potential (Table 3). Using daily soil temperature and matric potential data from the sites, we estimated daily soil CO_2 efflux for all control and NO_3^--amended plots for 2001. Relative to the control, cumulative soil CO_2 efflux during the growing season (May to October) was reduced by an average of 136 g C m^{-2} in NO_3^--amended plots (Fig. 3). When similar estimates are made for the entire year, assuming the growing season regression relationships continue to hold over winter, the average reduction in soil CO_2 efflux is estimated to be 177 g C m^{-2} (1071 vs. 894 g C m^{-2}).

Unlike soil respiration rates, fine root respiration rates were not affected by NO_3^- additions, although differences among sites and measurement dates did exist (Table 4, Fig. 4). These were related to inherent differences among sites in fine root N concentration and variation among sites and sample dates in soil temperature and soil moisture, as has been documented previously for these sites (see Burton *et al*., 1996, 1998; Zogg *et al*., 1996; Burton & Pregitzer, 2003). Coarse-root respiration also was unaffected by N amendments (*P* for treatment effect in ANOVA varied from 0.31 to 0.85, depending on coarse-root size class).

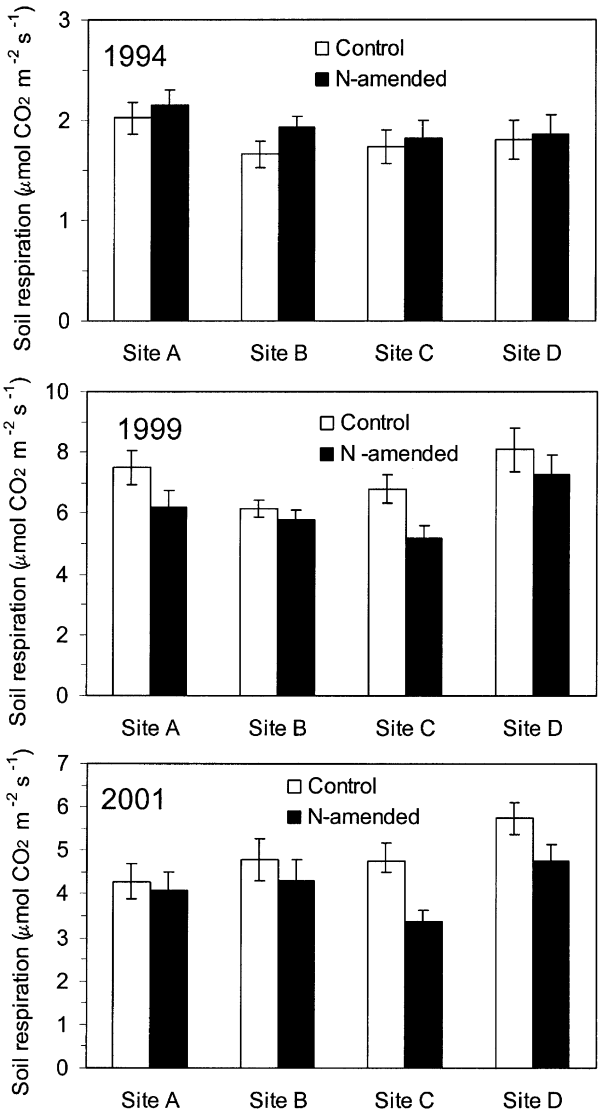

Fig. 2 Mean soil respiration rates by site for control and NO_3^--amended treatments for six dates during 1994, seven dates during 1999, and seven dates during 2001. Error bars are one standard error of the mean for all plots and dates within a treatment and site. In 1999 and 2001, soil respiration rates for NO_3^--amended plots were significantly lower than for control plots ($P<0.001$). Differences among years in average soil respiration are largely a consequence of differences in average soil temperature on the dates sampled (11.4 °C in 1994, 16.3 °C in 1999 and 15.1 °C in 2001). Rates for 1994 are also lower due to underestimation of actual rates by the static chamber method employed during that year.

Root biomass was unaffected by chronic NO_3^- additions (Table 5), with the average root biomass for control and N-amended treatments being nearly identical from 1994 to 2001 (Fig. 5). Similar results existed for all size classes examined. A majority of the ≤ 10 mm root biomass at these sites was comprised of fine roots ≤ 1 mm in diameter (52%), with ≤ 0.5 mm roots alone contributing 42% of the total biomass (Fig. 6). Root biomass did vary by site, with Sites A and D having greater root biomass than Sites B and C (Fig. 6).

Fine root (≤ 1 mm) lifespans in N-amended plots at Site A also were not significantly affected by N additions (Fig. 7). The average rates of fine root turnover determined from over 800 days of root observations were $0.57\,yr^{-1}$ for the NO_3^--amended treatment and $0.62\,yr^{-1}$ for the control treatment (difference not statistically significant).

Discussion

Our static-chamber technique underestimated soil respiration rates relative to the dynamic chamber method, in agreement with the static vs. dynamic chamber comparisons of Nay *et al.* (1994) and with the numerical simulations performed by Healy *et al.* (1996). In comparison with dynamic chambers, static methods have been shown to be most biased when soil CO_2 efflux rates are high (Jensen *et al.*, 1996), which often was the case during the 1994 measurements. Our assumption of linear accumulation of CO_2 for the 45 min period during which headspace CO_2 was accumulating may have added to the static chambers' degree of underestimation relative to the dynamic chamber system. Still we feel that the comparison among treatments in 1994 is valid, and it is clear that soil respiration was not reduced during the first year of NO_3^- additions, unlike the later years of the study.

Over time, soil respiration was significantly depressed by the chronic NO_3^- additions at all four study sites. The decline in soil respiration in 1999 and 2001 represented a significant portion (ca. 15%) of annual soil CO_2 efflux and agrees with the findings of others who have amended forests with higher levels ($\geq 10\,g\,N\,m^{-2}\,yr^{-1}$) of inorganic N (Haynes & Gower, 1995; Bowden *et al.*, 2000) and urea (Söderström *et al.*, 1983). The several-year delay in treatment response at our sites illustrates the importance of longer-term field studies for assessing the impact of chronic, low levels of N addition, such as those resulting from high rates of atmospheric deposition. A similar temporal response to long-term N additions has been observed for oak-dominated hardwoods at Harvard Forest, with increased soil respiration occurring in the first year of N additions (5 and $15\,g\,N\,m^{-2}\,yr^{-1}$), followed ultimately by reductions in soil CO_2 efflux after many years of treatment (Bowden *et al.*, 2004). In a pine stand at Harvard Forest, decreases in soil respiration were apparent in the first year of treatment, and became

Table 2 Repeated measures analysis of variance for the effects of NO_3^- additions on soil respiration rates in four Michigan sugar maple forests during the 1999 and 2001 growing seasons

Source	SS	df	MS	*F*-ratio	$P>F$
Between subjects					
Study site	102.6	3	34.21	10.02	0.001
N addition ($30\,kg\,N\,ha^{-1}\,yr^{-1}$ as $NaNO_3$)	69.8	1	69.79	20.45	<0.001
Study site × N addition	14.3	3	4.77	1.40	0.280
Error	54.6	16	3.41		
Within subjects					
Measurement date	1435.5	14	102.53	154.19	<0.001
Measurement date × study site	359.3	42	8.55	12.86	<0.001
Measurement date × N addition	12.9	14	0.92	1.38	0.162
Measurement date × N addition × study site	33.5	42	0.80	1.20	0.202
Error	149.0	224	0.66		

Table 3 Regression coefficients for predicting the natural log of soil respiration ($\mu mol\,CO_2\,m^{-2}\,s^{-1}$) from soil temperature (°C) and soil moisture potential (MPa) during the 1999 and 2001 growing seasons

Study site	Treatment	Constant	Temperature	Moisture	R^2	*P*
Site A	Control	−0.240	0.134	0.366	0.787	<0.001
Site A	NO_3^--amended	−0.278	0.124	0.419	0.708	<0.001
Site B	Control	0.041	0.113	1.710	0.794	<0.001
Site B	NO_3^--amended	−0.113	0.114	1.099	0.768	<0.001
Site C	Control	0.032	0.113	0.657	0.718	<0.001
Site C	NO_3^--amended	−0.194	0.104	0.506	0.717	<0.001
Site D	Control	0.437	0.098	0.783	0.842	<0.001
Site D	NO_3^--amended	0.189	0.104	0.639	0.639	<0.001

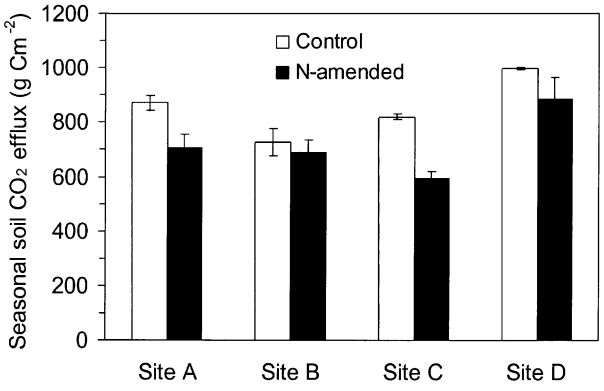

Fig. 3 Estimated growing season (May–October) soil respiration for 2001 for the control and NO_3^--amended treatments. Soil respiration is significantly lower ($P<0.001$) for the NO_3^--amended plots. Error bars indicate one standard error of the mean for three plots per treatment at each site.

much more pronounced after additional years of treatment (Bowden *et al.*, 2004).

The reduction in soil respiration does not appear to result from a change in ecosystem-level root respiration, because neither specific root respiration rates nor root biomass were altered by the NO_3^- additions. The lack of a change in root respiration rates in 2001 agrees with our findings for these sites since 1994 (Burton *et al.*, 1996, 1998; Zogg *et al.*, 1996; Burton & Pregitzer, 2003). In mature forests, others have found reductions in root biomass associated with N additions (Vogt *et al.*, 1990; Haynes & Gower, 1995), in contrast to the results of our study.

Differences among our sites in root biomass (Fig. 6), specific root respiration rate (Fig. 4, Burton *et al.*, 1996), and fine root N (Burton *et al.*, 1996) correspond to differences among sites in inherent N availability, and thus we were somewhat surprised that 8 years of N additions did not elicit responses in these variables. It is possible that our sites were not strongly N limited, and the observed patterns among sites in root biomass, specific root respiration rate, and fine root N concentration resulted from a complex response to multiple factors, not just N availability. If other elements, such as P, are now most limiting on the NO_3^--amended plots, we would not necessarily expect root biomass, N

Table 4 Repeated measures analysis of variance for the effects of NO_3^- additions on fine root (≤1.0 mm) respiration rates in four Michigan sugar maple forests during the 2001 growing season

Source	SS	df	MS	*F*-ratio	*P* > *F*
Between subjects					
Study site	257.8	3	85.95	62.34	<0.001
N addition (30 kg N ha^{-1} yr^{-1} as $NaNO_3$)	2.4	1	2.36	1.72	0.209
Study site × N addition	3.7	3	1.24	0.90	0.464
Error	22.1	16	1.38		
Within subjects					
Measurement date	851.4	9	94.60	38.27	<0.001
Measurement date × study site	248.1	27	9.19	3.72	<0.001
Measurement date × N addition	14.7	9	1.63	0.66	0.744
Measurement date × N addition × study site	79.4	27	2.94	1.19	0.254
Error	356.0	144	2.47		

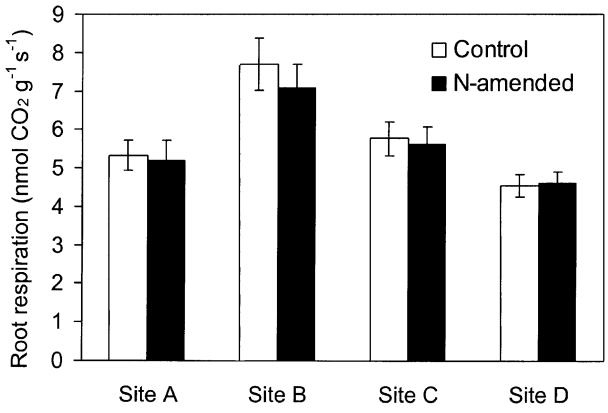

Fig. 4 Average fine root (≤1 mm) respiration rates by treatment and site for ten dates during 2001. Error bars are the standard error of the mean. Root respiration rates were not significantly different between control and NO_3^--amended treatments.

concentration, or specific respiration rate to change. Previous work with the ecosystem type existing at our sites suggests that it is not strongly N limited (Zak *et al.*, 1989, sugar maple-red oak/*Maianthemum* ecosystem type). Additionally, the NO_3^--amended plots at all four sites quickly became N saturated as the N additions proceeded, resulting in the rapid onset of high levels of NO_3^- and dissolved organic N leaching (Pregitzer *et al.*, 2004).

An increase in root lifespan in response to N additions could lead to a lower annual root turnover (i.e. a smaller proportion of roots dying and being replaced each year). Such a reduction in root litter C inputs would decrease the supply of substrate for microbial activity, lowering total soil respiration. We have previously documented slightly lower rates of root turnover for the control plots at the two sites that have higher natural levels of N availability (Sites B and

Table 5 Repeated measures analysis of variance for the effects of NO_3^- additions on fine root (≤1.0 mm) biomass in four Michigan sugar maple forests

Source	SS	df	MS	*F*-ratio	*P* > *F*
Between subjects					
Study site	417 683	3	139 228	6.053	0.006
N addition (30 kg N ha^{-1} yr^{-1} as $NaNO_3$)	51	1	51	0.002	0.963
Study site × N addition	40 460	3	13 487	0.586	0.633
Error	368 014	16	23 001		
Within subjects					
Year	41 702	3	13 901	3.396	0.025
Year × study site	79 111	9	8790	2.148	0.060
Year × N addition	3810	3	1270	0.310	0.818
Year × N addition × study site	59 850	9	6650	1.625	0.135
Error	196 469	48	4093		

Years of record include 1994, 1996, 2000, and 2001. Nitrogen additions were initiated in 1994. Similar results existed for all individual and cumulative root size classes (<0.5 0.5–1.0, 1.0–2.0, 2.0–5.0, 5.0–10.0, ≤2.0, ≤10.0 mm).

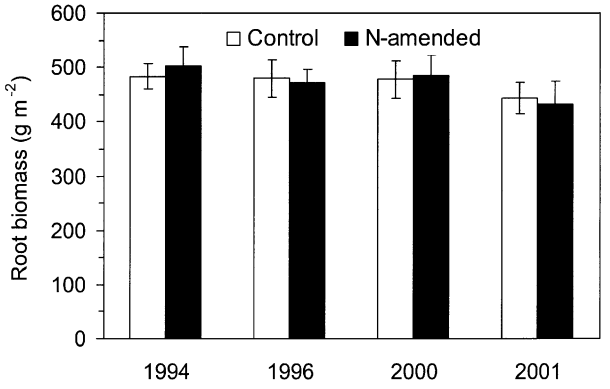

Fig. 5 Average fine root (≤1 mm) biomass across sites, by year, for the control and NO_3^--amended treatments. Values for each year are means and standard errors for all plots of a treatment across all four sites ($n = 12$). Fine root biomass was not significantly different between control and NO_3^--amended treatments for any year.

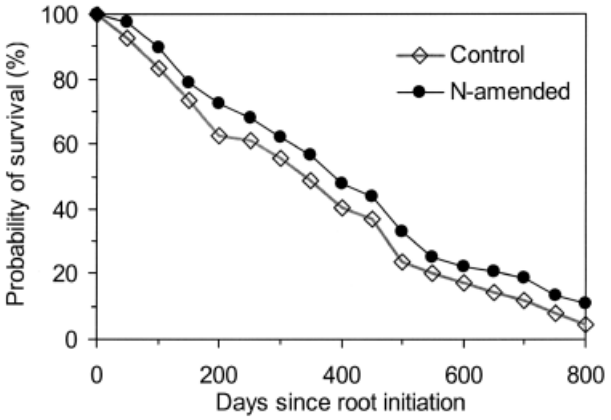

Fig. 7 Survival of fine (≤1.0 mm) roots for control and NO_3^--amended treatments at Site A during the period 1999–2001. No significant treatment effect existed. Root turnover rates calculated as described in Burton *et al.* (2000) were 0.57 yr^{-1} for the NO_3^--amended treatment and 0.62 yr^{-1} for the control.

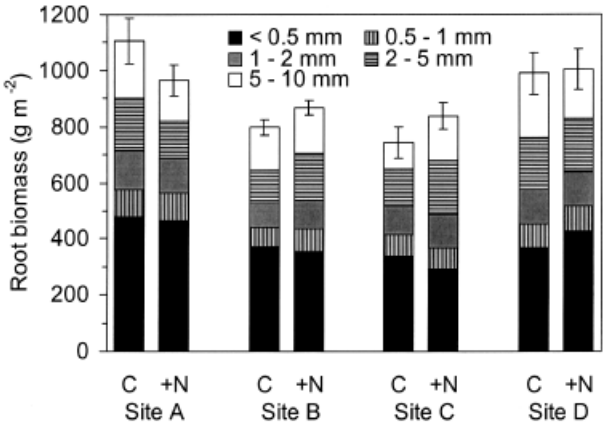

Fig. 6 Root biomass by root size class, treatment, and site for 2001. Error bars indicate the standard error for three plots per treatment for total root biomass. Root biomass for Sites B and C is significantly lower than for Sites A and D. NO_3^- additions did not affect root biomass.

C, Burton *et al.*, 2000), suggesting the possibility that root life spans might increase on N-amended plots. Minirhizotron images collected over 800 days to assess fine root dynamics at Site A show a slight, but non-significant, decrease in fine root turnover for the NO_3^--amended plots (Fig. 7). Given a fine root (≤1 mm) biomass of 570 g C m^{-2} for Site A (Fig. 6), this difference in root biomass turnover, if real, would only reduce soil C inputs by 28 g C m^{-2} yr^{-1}, accounting for no more than 15% of the observed reduction in soil respiration on the NO_3^--amended plots. It appears that changes in root litter inputs resulting from altered root lifespan have not made a major contribution to the observed decline in annual soil respiration for the NO_3^--amended treatment. Reductions in the quantity of aboveground litter inputs also could decrease soil CO_2 efflux by lowering substrate availability for microbes, but annual leaf litter mass has not changed in response to NO_3^- additions (K.S. Pregitzer, unpublished results).

Direct measurements at the study sites have eliminated changes in specific root respiration rate, root biomass, root biomass turnover, and the quantity of aboveground litterfall as primary mechanisms behind the observed decline in soil respiration following chronic NO_3^- additions. Remaining possible sources for the decline in soil CO_2 efflux include: (1) reductions in mycorrhizal respiration or biomass turnover; (2) a decrease in soil C inputs from root exudation; and/or (3) lower microbial respiration associated with reduced decomposition of annual soil C inputs and existing organic matter.

The proportion of total belowground C allocation (TBCA) utilized by mycorrhzae and exudation is generally assumed to be around 20% (Vogt *et al.*, 1982; Lambers, 1987; Paul & Clark, 1989; Eissenstat & Yanai, 1997; McDowell *et al.*, 2001). The contribution of exudates and mycorrhizae to total soil respiration then would be somewhat less than 20%, since a portion of soil respiration is derived from aboveground litter inputs rather than TBCA. If C allocation to mycorrhizae and exudation in our stands were of this relative magnitude, a very large reduction would be needed to explain the observed 15% decrease in soil respiration on the NO_3^--amended plots. Therefore, it seems unlikely that changes in exudation and mycorrhizal biomass alone can account for the decrease in soil respiration. However, experimental N additions have been shown to both increase and decrease the infection rate and

biomass of arbuscular mycorrhizal fungi (Hayman, 1982; Heijne *et al*., 1992, 1994; Egerton-Warburton & Allen, 2000; Treseder & Allen, 2000, 2002), and altered nutrient availability can affect root exudation in a variety of ways (Dakora & Phillips, 2002). Given the wide variation in their potential responses, declines in mycorrhizal infection and root exudation both remain as possible contributors of some portion of the reduction in soil CO_2 efflux.

For additions of inorganic N forms, decreases in microbial biomass and respiration have commonly been documented (Insam & Palojärvi, 1995; Ettema *et al*., 1999; Thirukkumaran & Parkinson, 2000). These findings have generally been for rates of N addition ($7.5–30\,g\,N\,m^{-2}$) much higher than those in our study. After 8 years of chronic NO_3^- additions at $3\,g\,N\,m^{-2}\,yr^{-1}$, we also have documented decreases in microbial biomass (ca. 20%; DeForest *et al*., 2004). If the reduction in microbial biomass creates a proportional decline in microbial respiration, then much of the observed 15% decline in soil CO_2 efflux could be explained. A 20% decrease in microbial respiration would cause an 8–14% decrease in soil respiration, if root and mycorrhizal respiration account for 30–60% of soil respiration as is typically reported (Edwards & Harris, 1977; Nakane *et al*., 1983; Behera *et al*., 1990; Bowden *et al*., 1993). Decreases in soil respiration following 13 years of N additions to hardwood and pine stands at Harvard Forest also have been associated with decreases in microbial respiration (Bowden *et al*., 2004).

The exact cause for the decline in microbial biomass at our sites has not yet been identified, but potentially could be explained by several possible mechanisms through which excess N can limit substrate availability. Others have observed less complete decomposition of litter that is high in N, as excess N, primarily in amino forms, reacts with degrading lignin creating more recalcitrant aromatic compounds (Fog, 1988; Berg & Matzner, 1997; Berg, 2000) or directly represses the synthesis of lignin-degrading enzymes (Berg, 2000; Carreiro *et al*., 2000; Saiya-Cork *et al*., 2002). The first mechanism is unlikely to occur under the acidic soil conditions existing at our sites. However, litter N concentrations at our sites have increased in response to N additions (Zak *et al*., 2004), and the activity of enzymes responsible for the degradation of plant litter have been suppressed (DeForest *et al*., 2004). Both these findings are consistent with the second possible mechanism. Decreased growth rate of decomposers and changes in decomposer efficiency, possibly associated with altered microbial community composition, have also been suggested as causes for reduced soil respiration following chronic N additions (Ågren *et al*., 2001). At our sites, no evidence for a change in microbial community composition has been found (DeForest *et al*., 2004). Instead, biomass of all microbial components decreased (DeForest *et al*., 2004), which may reflect reduced decomposer growth rate.

Conclusions

Soil respiration rates in northern hardwood forests were unchanged during the first year of simulated NO_3^- deposition, but were significantly reduced after five to eight years of chronic N additions. On an annual basis, soil CO_2 efflux on NO_3^--amended plots averaged 15% less than that for control plots, an amount equivalent to $177\,g\,C\,m^{-2}\,yr^{-1}$, during the eighth year of NO_3^- additions. This decline in soil CO_2 efflux was not the result of reduced allocation of C to root respiration, as specific root respiration rates, and root biomass remained unchanged. Reductions in C allocated to annual root biomass turnover also could not account for any significant portion of the decline in soil respiration following chronic NO_3^- additions. Decreases in mycorrhizal infection and root exudation remain potential explanations for at least a portion of the decrease in soil CO_2 efflux, but it appears that declines in microbial biomass and respiration are responsible for much of the observed decline. Measured decreases in microbial biomass and extracellular soil enzyme activities at the sites, in response to chronic NO_3^- additions (DeForest *et al*., 2004), are consistent with reduced decomposition activity of the microbial community being responsible for the observed decline in soil respiration. This has potential implications for ecosystem C storage, since a decline in microbial respiration, in the absence of reductions in litter C inputs, would result in increased rates of soil C accrual on the NO_3^--amended plots.

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