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Short-term soil respiration and nitrogen immobilization response to nitrogen applications in control and nitrogen-enriched temperate forests

Patricia Micks[a,*], John D. Aber[b], Richard D. Boone[c], Eric A. Davidson[d]

[a]*Marine Biological Laboratory, The Ecosystems Center, Woods Hole, MA 02543, USA*
[b]*Complex Systems Research Center, Institute for the Study of Earth, Oceans and Space, University of New Hampshire, Durham, NH 03824, USA*
[c]*Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK 99775, USA*
[d]*Woods Hole Research Center, Woods Hole, MA 02543, USA*

Abstract

Forest stands at the Harvard Forest, Petersham, MA, receiving experimentally elevated N inputs have shown greatly increased N leaching loss yet still retain over 70% of the added N in soils, presumably in organic form. Whether microbial or abiotic mechanisms are responsible for the high N retention is not well understood. We monitored soil respiration and extractable NH_4-N and NO_3-N following monthly applications of NH_4NO_3 to a hardwood forest and a pine plantation during the fifth year of chronic fertilizer applications (15 g N as NH_4NO_3 m^{-2} per year). We hypothesized that individual N applications would increase short-term soil respiration (within 1 month) in previously unamended and N-limited soil, but that little or no increase would occur following N applications to chronically N-amended soils, assumed to be carbon-limited to some degree after 5 years of N additions. Short-term soil respiration did not increase after N additions in either the chronically amended or previously untreated soils except for one instance in the latter. However, extractable N levels in both previously unamended plots returned to pre-application levels within 2 weeks of the N addition. This rapid disappearance of the applied N suggests microbial immobilization, but in all but one instance there was no accompanying CO_2 efflux increase indicating increased microbial biomass growth. A model of N immobilization through microbial biomass production, driven by the observed apparent net N immobilization, predicted soil CO_2 efflux 4–17 times greater than measured rates. Microbial biomass production does not appear to be the mechanism by which the fertilizer N immobilization occurred, according to our assumptions about microbial C:N ratios and carbon use efficiency. Hardwood stand average soil respiration rates over the study period were significantly higher in the previously unamended plot than in the control, and the control and chronically N-treated plot respiration rates were similar. Soil respiration rates for all pine stand treatments were similar. These results are insufficient to support our hypotheses concerning carbon versus nitrogen limitation in these soils. Our results, along with evidence from other studies, suggest that abiotic mechanisms play a role in the high retention of long-term N additions in these soils.

Keywords: Soil respiration; Nitrogen deposition; Forest soil; Ammonium nitrate; Nitrogen immobilization

1. Introduction

Atmospheric nitrogen deposition to temperate forests in the northern hemisphere ranges from 0.3 to

* Corresponding author. Tel.: +1-508-289-7537; fax: +1-508-457-1548.
E-mail address: pmicks@mbl.edu (P. Micks).

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7.5 g N m^{-2} per year, and these rates are predicted to increase (Dise and Wright, 1995; Galloway et al., 1994, 2003). A growing body of evidence demonstrates the harmful consequences of long-term N deposition as forests move from an initially N-limited state towards N saturation (Aber et al., 1995; Fenn et al., 1998; Gundersen et al., 1998).

The long-term effects of elevated atmospheric N inputs on soil N dynamics will be a critical factor in forest ecosystem response to N deposition. In order to predict this response, it is necessary to investigate how forest soils process and retain N deposition inputs. N addition experiments have shown that most of the atmospherically deposited N in temperate forest ecosystems is retained in soil, largely in soil organic matter (Koopmans et al., 1996; Nadelhoffer et al., 1999a,b; Tietema et al., 1998). Results from long-term experimental N additions at the Harvard Forest chronic nitrogen amendment study (Petersham, MA, USA) demonstrate that after 9 years of N additions (5 or 15 g N m^{-2} per year as NH_4NO_3), a mixed hardwood stand and a red pine plantation have retained 85–99% of the added N, with an estimated 70–84% retained in soil organic matter (Magill et al., 2000). These results are supported by a ^{15}N tracer study tracking the fate of ambient N deposition and NH_4NO_3 additions, which demonstrated that soils, primarily the O horizon, were the dominant sinks for N deposition in these forests (Nadelhoffer, 1999b; Micks et al., 2004; Nadelhoffer et al., 2004).

It was hypothesized that chronic N additions to N-limited forest soil would initially stimulate soil microbial activity, but over time would result in a carbon-limited state after microbial demand for N was satisfied (Aber et al., 1989). Results from the first 3 years of the chronic nitrogen amendment study indicated that both forests were initially N-limited, the hardwood forest to a greater degree than the pine forest (Aber et al., 1993). Pre-amendment soil microbial activity and microbial biomass N pools would be expected to decline over time as a result of chronic fertilizer applications. Yet, storage rates of N additions in soils remained high even after the onset of N saturation in both the pine and hardwood forests, which occurred after 1 and 7 years of N additions, respectively. It is not clear what mechanisms are responsible for the high retention of N additions in these soils. While microbial uptake of NH_4^+ and NO_3^- is a primary process in forest soil N dynamics (Stark and Hart, 1997; Davidson et al., 1992; Tietema, 1998), the sustained high retention rate of N additions suggests that abiotic mechanisms may also be important in N uptake and storage in these soils (Axelsson and Berg, 1988; Azhar et al., 1986; Dail et al., 2001; Johnson et al., 2000; Nommik and Vahtras, 1982; Schimel and Firestone, 1989). Aber et al. (1998) proposed that a combination of microbial (free-living or mycorrhizal) and abiotic mechanisms may be responsible for the high storage capacity for N additions by these soils.

Here we report a study in which we sought to determine whether continued N applications to the Harvard Forest chronic N plots would stimulate short-term microbial biomass growth in soils after 4 years of N additions, and if so indicate whether microbial uptake may be responsible for the high retention of added N. We hypothesized that chronic N additions would stimulate soil microbial growth early in the experiment, causing labile C stocks to decline and resulting in microbial populations becoming increasingly energy-limited over time. Assuming that microbial biomass growth is accompanied by an increase in respiration, we expected that if soil microbiota were not C-limited, microbial growth would be stimulated and a pulse of soil CO_2 efflux would occur following each regular monthly N application. If the microbes were C-limited, however, we did not expect soil respiration to respond to individual N applications.

To test our hypothesis we measured soil respiration before and after two successive monthly fertilizer applications in the summer of the fifth year of N additions. We measured soil respiration in the high-N treatment plots (15 g m^{-2} per year) of both stands and compared these results to soil respiration rates measured in control plots. We also measured respiration in previously untreated plots that were given the same N applications (plus one higher-dose application) as the chronically treated plots during the 2-month study period. We hypothesized (1) that the previously untreated, N-limited soils would respond more to the N applications (via increased short-term CO_2 efflux) than soils that had received 4 years of N additions; and (2) that the chronically treated plot in the more N-saturated red pine stand would respond less than its counterpart in the hardwood forest. We also compared the observed soil respiration rates and apparent net uptake of nitrogen in the previously

unamended plots to a simple model of microbial C and N dynamics which predicts maximum potential soil respiration rates if all the applied N is immobilized and allocated to growth of new microbial biomass. Our goal was to determine whether short-term microbial growth response to N addition is a potentially important factor in the retention of chronic N inputs in these forests.

2. Methods

2.1. Study site

The chronic N Addition experiment is a core experiment of the Harvard Forest Long-Term Ecological Research (LTER) program, located at the Harvard Forest in Petersham, Massachusetts, USA (42°32′N, 72°10′W). Vegetation is typical of the hardwood–white pine–hemlock zone (Westveld, 1956). The climate is cool and humid with mean monthly temperatures ranging from −7 °C (January) to 20 °C (July). Annual precipitation averages 110 cm and is distributed evenly throughout the year (Van Cleve and Martin, 1991). Atmospheric wet + dry N deposition is estimated to be 0.8 g m^{-2} per year (Ollinger et al., 1993).

The experimental plots are located in two adjacent forest stands: an even-aged red pine (*Pinus resinosa* Ait) plantation and a mixed hardwood stand ca. 65 and 50 years old, respectively, and were in the fifth year of N additions at the time of this experiment (1992). Pretreatment soil characteristics are given in Table 1. Black and red oak (*Quercus velutina* Lam. and *Q. borealis* Michx. f.) dominate the hardwood stand which also contains black birch (*Betula lenta* L.), red maple (*Acer rubrum* L.), and American beech (*Fagus grandifolia* Ehrh.). The presence of an Ap horizon in the red pine plantation soil profile indicates this site was previously cultivated, while the hardwood stand was formerly cleared and pastured. Soils are stony to sandy Dystrochrepts. Pine stand soil is a Montauk variant and the soil in the hardwood stand is a Canton variant.

Chronic N treatments, begun in 1988, include low-N (5 g N m^{-2} per year), high-N (15 g N m^{-1} per year), N + S (5 g N m^{-2} per year and 7.4 g S m^{-2} per year) and an untreated control. Plots are 0.09 ha (30 m × 30 m). N is added as NH_4NO_3, and S as Na_2SO_4, in six equal monthly applications during the growing season (Magill et al., 2000). Each application is dissolved in 20 l of water and delivered via a backpack sprayer. During this study, on dates when fertilizer was applied to high N plots, control plots received an equivalent application of water only. Only the control and high-N treatments were used in the present study.

Table 1
Initial soil characteristics of the chronic nitrogen experiment site

	Pine stand	Hardwood stand
Forest floor		
Mass (g m^{-2})	8428	9471
Carbon (%)	26.8	19.4
Nitrogen (%)	1.1	0.8
C:N	24.1	24.2
Mineral soil (0–10 cm depth)		
Carbon (%)	5.8	7.6
Nitrogen (%)	0.3	0.4
C:N	22.2	21.1
Fine root biomass (g m^{-2})	231	276

From Aber et al. (1993).

In June 1992 a 25 m^2 plot (“new plot” treatment) was established in a previously unamended area in each stand for the purpose of measuring soil respiration and extractable NH_4^+ and NO_3^- levels following N applications to previously untreated soil.

2.2. NH_4NO_3 application

During our study, high-N plots in both stands received regular monthly applications of NH_4NO_3, at the rate of 2.5 g N m^{-2}, on 30 June, 29 July, and 24 August. The new plots received identical applications via hand-held sprayer on 30 June and 29 July. Because new plot soil respiration did not respond to the June and July applications, a third application at double the regular rate (2×) was made to the new plots on 17 August. The high-N plots did not receive a fertilizer application on that date.

2.3. Soil respiration measurement

Soil respiration was measured in the control, high-N, and new plots in both stands on 22 days between 30 June and 24 August 1992. We used the soda lime technique (Cropper et al., 1985; Edwards, 1982; Raich

et al., 1990) which allowed a large number of sites to be measured simultaneously. Fifteen white plastic chambers (20 cm tall × 28 cm diameter) were placed in each plot, the locations remaining fixed for the duration of the experiment. This number was based on a study of spatial variability of soil CO_2 efflux in the red pine stand by Raich et al. (1990), who determined that at least 13 chambers were required to estimate mean CO_2 flux with no more than 10% error 90% of the time.

CO_2 efflux was measured simultaneously in all 90 sampling locations on each sampling date. An open metal tin containing 60 g of oven-dried 6–12 mesh soda lime was placed on the surface of the forest floor and covered with the white plastic chamber. Chambers were seated no more than 1 cm deep in the forest floor to avoid severing live roots. Aluminum foil covered the chambers' upper surface to minimize solar heating. After 24 h, the soda lime tins were removed, oven-dried (105 °C) 24 h, and weighed. Average 24 h CO_2 efflux was calculated for each measurement date and chamber, based on the weight of CO_2 absorbed by the soda lime during the 24 h period and corrected for background CO_2 absorption by 12 soda lime blanks.

One-way ANOVA was performed separately for each stand and measurement date to determine whether efflux differed significantly among the new plot, high N, and control plots. The non-parametric Kruskal–Wallis test was used for one case in which the equal variance assumption was violated. For dates when a significant difference in soil respiration rate was indicated among treatments within a stand ($P < 0.05$), Fisher's least significant difference (LSD) was used to identify which treatment means differed significantly. Repeated measures ANOVA was used to test whether treatments within each stand differed across the study period. Where a treatment differed significantly from the other two, soil respiration rates were averaged over the 15 chambers and 22 dates, and a treatment mean respiration rate was calculated. Significant differences among treatment means within stands were then tested with Fisher's LSD post-hoc comparison.

2.4. Soil extractable NH_4^+ and NO_3^-

Extractable NH_4^+ and NO_3^- were measured in the new plot soils following the second regular (29 July) and the 2× fertilizer applications (17 August). Five samples of the organic horizon and mineral soil 0–10 cm depth were collected with a metal corer (6 cm diameter) within 24 h before each application, 2 days after, then at 5–6-day intervals for a total of seven samplings over 28 days. Control and high N plot soils were not collected for extraction due to sampling restrictions on those plots. Organic horizon and mineral soil were passed separately through a 5.6 mm sieve, resulting in removal of some Oi material. Ten grams subsamples (<5/6 mm fraction) were extracted with 100 ml 2 M KCl for 1 h on a rotary shaker. Extracts were filtered through glass fiber filters (Gelman GFA) and immediately frozen for later analysis on a Braun and Lubbe (Technicon) Traacs 800 Autoanalyzer. NH_4-N was determined by the Berthelot reaction (Technicon Method 780-86T) and NO_3-N by hydrazine sulfate reduction (Technicon Method 782-86T). Detection limit for both NH_4-N and NO_3-N was 0.01 mg l^{-1}. Concentrations less than 0.02 mg l^{-1} were considered zero.

2.5. Apparent net immobilization of applied N

We used the extractable N data to calculate the apparent net immobilization rate (ANIR) of the N applied to the new plots on 29 July and 17 August. Applications were 2.5 g N m^{-2} on 29 July and 5.0 g N m^{-2} on 17 August. Background NH_4^+-N and NO_3^--N (levels present on the day before each fertilization event) were subtracted from subsequent data. Above-background extractable NH_4^+-N and NO_3^--N were assumed to represent fertilizer N remaining in the extractable soil pool. The amount of fertilizer N immobilized was calculated as the difference in the background-corrected NH_4-N + NO_3-N concentrations between successive sampling dates. ANIR per day was obtained by dividing the mass of applied N immobilized between consecutive soil sampling dates by the number of days in the interval. Percent of fertilizer N remaining at the time of soil sampling was then calculated for all sampling dates after the most recent fertilizer application, assuming that 100% of the fertilizer N was present in the soil on the date of application.

We assumed that any short-term disappearance of the applied N from extractable soil pools was a result of microbial uptake only. Uptake by roots cannot be

discounted, but we assumed that microbes were stronger competitors for inorganic N than plants in the two forests based on the evidence that soils retain a much higher proportion of N additions than does plant biomass (Magill et al., 2000; Nadelhoffer et al., 1999b). We also assumed that leaching loss of NO_3^- and NH_4^+ was negligible, as no loss was detected below 60 cm depth during the first year of the chronic N experiment (Magill et al., 2000). ANIR is assumed to represent a net increase in immobilization beyond the gross background fluxes of mineralization and immobilization.

2.6. Model to predict respiration from apparent nitrogen immobilization

To test whether the ANIR and CO_2 effluxes we measured in the new plots could be the result of new microbial biomass growth, CO_2 efflux was predicted from ANIR data using a model similar to that of (Schimel, 1988). The model assumes that microbes are stronger competitors for inorganic N than plant roots, and that N immobilization results in microbial biomass growth and that a net increase in respiration, proportional to that growth, will occur. The model is

$$C_R = C_B(Y_C^{-1} - 1)$$

where Y_C is the microbial carbon use efficiency (fraction of C assimilated per unit C respired); C_B is the growth accumulation of microbial biomass carbon (ANIR (g N per day) × microbial C/N ratio); C_R is the mass of carbon respired (g CO_2-C m^{-2} per day).

CO_2 efflux was predicted using three sets of microbial parameters. Scenario 1 ($Y_C = 0.50$ and C:N = 8) describes average conditions for a fungal-dominated soil microflora typical of acidic forest soils (Alexander, 1977; Tietema and Wessel, 1992). Values for Scenario 2 ($Y_C = 0.35$ and C:N = 12) were chosen to estimate an upper limit for CO_2 production (Alexander, 1977; Babel and Müller, 1985; Hadas et al., 1992; Schimel, 1988; Tietema and Wessel, 1992). We assumed that NO_3^- assimilation carries a higher energy cost to microorganisms than assimilation of NH_4^+, resulting in higher CO_2 output (Penning de Vries et al., 1974). Therefore in Scenarios 1 and 2 we multiplied the mass of CO_2 respired due to NO_3^- assimilation by a factor of 2.3 (as calculated by Penning de Vries et al. (1974)), before adding it to the CO_2 produced by NH_4^+ uptake, to obtain total estimated CO_2 efflux. Scenario 3 provided a lower limit estimate, using the conservative Y_C and C:N values of Scenario 1 without the added NO_3^- assimilation cost.

The mass of carbon respired (C_R) was determined according to the three scenarios for the 28-day period over which soil extractable N was measured, using the daily ANIR values determined for NO_3-N and NH_4-N. Control soil CO_2-C efflux was added to the predictions to provide a basal level from which fertilizer-induced changes would arise. The results were then compared to field-measured new plot soil respiration.

For the interpretation of both soil respiration data and model results, we assumed that the relative contributions of roots and soil microbes to total soil respiration in the high N plots were not affected by chronic N additions, such that the proportions were constant across treatments within each stand. The effects of chronic N additions on microbial and root respiration in these soils are further addressed in the discussion.

3. Results

3.1. Soil respiration

Plot mean soil CO_2 efflux ranged from 42 to 205 mg CO_2-C m^{-2} h^{-1} over the 22 measurement dates (Fig. 1). Rates are consistent with those measured during the first 2 years of the chronic N experiment (Bowden et al., 2004), and are consistent with rates determined for temperate deciduous forests around the world at similar soil temperatures (25–225 mg CO_2-C m^{-2} h^{-1}) (Kicklighter et al., 1994). Minimum detectable efflux varied from 14 to 39 mg CO_2-C m^{-2} h^{-1}, calculated as one standard deviation from the lowest and highest daily mean weight gain of the soda lime blanks. The smallest detectable difference among pine and hardwood stand treatments was 19 and 21 mg CO_2-C m^{-2} h^{-1}, respectively. Rates were lowest during the first few dates in July, possibly due to incomplete drying of the soda lime. Mean efflux over the study period was 42% higher in the hardwood stand than in the pine stand (117 mg CO_2-C m^{-2} h^{-1} versus 82 mg CO_2-C m^{-2} h^{-1}, Table 2) (one-way ANOVA and Fisher's LSD, $P < 0.001$).

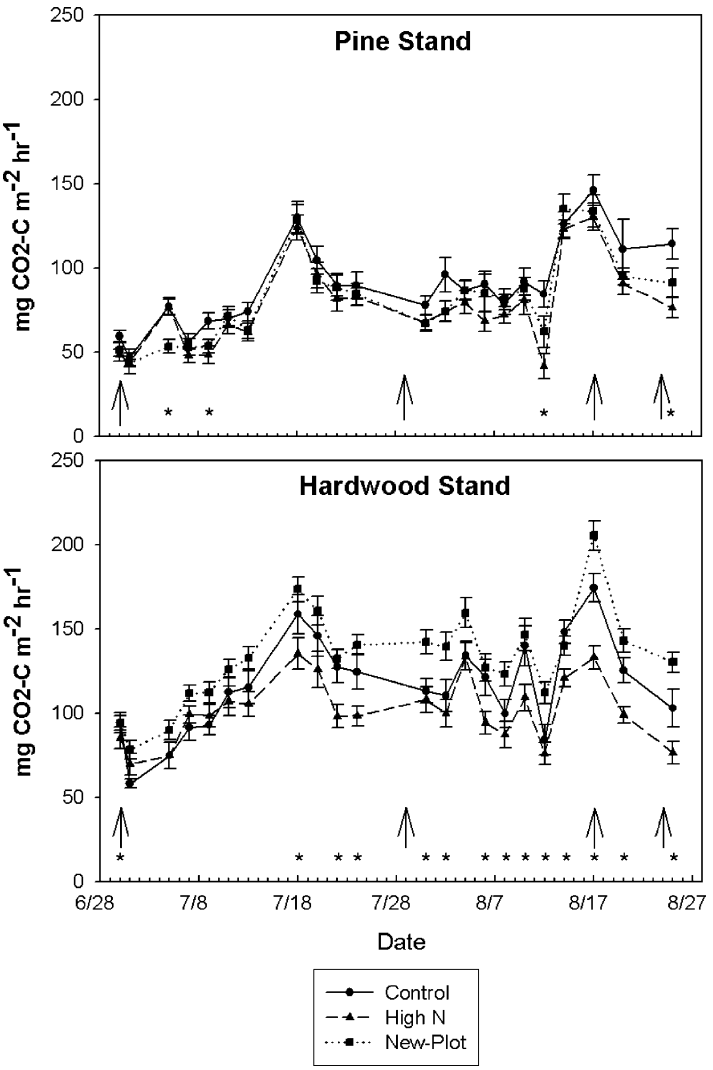

Fig. 1. Soil respiration measured in the red pine and hardwood stands in July and August 1992. Points represent plot mean hourly CO_2 efflux; $n = 15$. Arrows indicate dates of fertilizer applications: high-N plots received a regular N application on 6–30, 7–29, and 8–24; new plots received a regular N application on 6–30 and 7–29 and an application at double the regular rate on 8–17. Asterisks denote dates on which at least two plot means were significantly different (one-way ANOVA, $P > 0.05$).

Respiration from new plot and high-N plot soils did not increase significantly over control levels following fertilizer applications in either stand except in the hardwood new plot following the 2× fertilizer application on 17 August (Fig. 1). On that date the new plot soil respiration rate was significantly greater than the control.

Throughout the study period, daily differences among treatments were generally greater in the hardwood stand than in the pine stand. At least two of the three hardwood treatments were significantly different from each other ($P < 0.05$) on 13 of the 22 dates, with new plot flux significantly highest on 7 dates and the high-N plot significantly lowest on 7 dates. Significant differences among treatments occurred on only three dates in the pine stand, and in these cases the new plot soil CO_2 efflux was significantly lower than the control. When averaged over the entire study period, the

Table 2
Mean soil CO_2 efflux (g CO_2-C m^{-2} h^{-1}) for the 2-month study period

	Mean	S.E.
Pine stand		
Control plot	89.4 a	5.8
High N plot	76.5 a	5.1
New plot	81.0 a	5.3
Stand mean	82.3	3.2
Hardwood stand		
Control plot	116.3 a	7.8
High N plot	101.5 a	6.2
New plot	132.2 b	5.6
Stand mean	116.7	4.2

Notes: Plot means were calculated as the mean of 15 buckets, each bucket averaged across all measurement dates. S.E. = 1 standard error of the mean. Significant differences within each stand are indicated by different letters (one-way ANOVA, Fisher's LSD post-hoc test, $n = 15$ per plot). The only significant difference among treatments was that the hardwood stand new plot was significantly different than the control and high N treatments ($P = 0.002$). The two stand means are significantly different (t-test, $P < 0.001$).

hardwood new plot mean efflux was significantly higher than the control and high-N means (ANOVA and Fisher's LSD, $P = 0.002$), the latter two not significantly different from each other (Table 2). The 2-month pine stand treatment means were not significantly different from each other.

3.2. Extractable N in previously unamended soils

Extractable N declined rapidly in the new plots following N applications in both stands. Levels declined faster in the hardwood soil and were consistently lower in that stand (Table 3, Fig. 2). Two days after the regular 29 July fertilization, 50% of the N applied to the hardwood new plot was recovered, but almost none thereafter. Seventy-four % of the N applied to the pine soil was extracted after 2 days, dropping to 20% after 13 days (Table 3). Similar amounts of the 17 August 2× application were recovered 2 days later (38 and 36%, pine and hardwood stands, respectively), but after 7 days only 4% of the applied N remained in the hardwood soil, while pine levels were unchanged. In general, slightly over half of the extractable ammonium came from the organic layer while most of the nitrate was in the mineral soil.

3.3. Model predictions of soil respiration

All three model scenarios greatly over-predicted soil respiration rates following fertilizer applications (Fig. 3). Predicted rates were generally higher for the hardwood new plot, which showed stronger ANIR than the pine new plot. Scenario 1 doubled the observed rates in both stands. The lower carbon use efficiency and larger C:N ratio of Scenario 2 predicted rates roughly twice those of Scenario 1. Scenario 3, without the energy cost of nitrate reduction, gave the lowest prediction but was still nearly twice the observed rates. The greatest difference between model results and observations occurred on 17 August when ANIR was greatest: predicted rates ranged from 4 (hardwood, Scenario 3) to 17 (pine, Scenario 2) times greater than measured respiration.

4. Discussion

4.1. Short-term respiration response to N applications

We hypothesized that individual applications of NH_4NO_3 would lead to short-term increases in soil CO_2 efflux, depending upon the degree of amendment-induced C limitation. We hypothesized that microbial activity in unamended soils was N- rather than C-limited, and therefore expected that short-term soil respiration would increase in response to fertilizer application in the new plots more strongly than in chronically N-treated plots. Overall, our results do not fully support these hypotheses. Aside from the short-term soil respiration increase observed in the hardwood new plot after the 2× N application, there was no other evidence of short-term increases in soil respiration resulting from N applications to chronically or previously unamended soils. Rapid disappearance of the applied nitrogen from previously unamended soils suggests that immobilization of applied ammonium and nitrate did occur, but only in the one instance (hardwood new plot after the 2× N application on 17 August) did we detect an accompanying CO_2 efflux increase that could indicate microbial biomass growth. Results from this one case support evidence of stronger N limitation in the previously unamended hardwood versus pine stand soils,

Table 3
Total extractable DIN (NH_4^+-N + NO_3^--N) in new plot soils

Date	Measured extractable DIN (g NH_4^+-N + NO_3^--N m^{-2})	Applied N remaining (g NH_4^+-N + NO_3^--N m^{-2})	ANIR[a] (g N m^{-2} per day)	Applied N remaining (%)
Pine stand				
7–28	0.838	0.000	–	–
7–29	2.5 g NH_4NO_3 m^{-2} applied		–	100
7–31	2.698	1.860	0.320	74
8–05	2.224	1.386	0.095	55
8–11	1.342	0.504	0.147	20
8–17[b]	0.654	0.000	0.084	0
8–17	5.0 g NH_4NO_3 m^{-2} applied		–	100
8–19	2.723	2.182	1.409	44
8–24	2.866	2.212	0.017	44
Hardwood stand				
7–28	0.000	0.000	–	–
7–29	2.5 g NH_4NO_3 m^{-2} applied		–	100
7–31	1.244	1.244	0.628	50
8–05	0.025	0.025	0.244	1
8–11	0.045	0.045	0	2
8–17[b]	0.064	0.064	0	3
8–17	5.0 g NH_4NO_3 m^{-2} applied		–	100
8–19	1.808	1.744	1.628	35
8–24	0.217	0.153	0.318	3

Notes: Results are for organic and mineral horizons combined. Measured extractable DIN values are the sum of the average NH_4-N and average NO_3-N extracted from five cores. Background DIN found on 28 July was subtracted from DIN data through 17 August, and background DIN found on 17 August (prior to fertilizer application later that day) was subtracted from DIN data through 24 August. The resulting values ('applied N remaining') are assumed to represent the amount of extracted N that derived from the fertilizer. ANIR on 7–31 and 8–17 is the difference between the amount of fertilizer N applied and the amount remaining on those dates, divided by the number of days in the interval. ANIR for other dates is the difference between the amount of applied N remaining on two successive dates, divided by the number of days in the interval. ANIR is assumed to be linear between consecutive sampling dates.

[a] Apparent nitrogen immobilization rate.

[b] Measurement made prior to fertilizer addition on same date.

and are consistent with our hypotheses. However, because the high-N plots did not receive fertilizer on the date the new plots received the 2× application, we cannot draw any conclusions regarding changes in C limitation as a consequence of long-term N additions. Also, the observed soil respiration increase would account for only a small fraction of the apparent N immobilization rate, according to our model assumptions.

Additions of NH_4^+ and NO_3^- have been found to either decrease or cause no change in soil respiration and microbial activity in forest soils. Our results are consistent with the short-term studies of Fessenden et al. (1971), Salonius (1972), and Flanagan and Van Cleve (1983), in which no change in respiration was observed in laboratory-incubated forest soils treated with NH_4NO_3. Söderström et al. (1983) observed a short-term respiration increase (1–4 days) after NH_4NO_3 addition to laboratory-incubated humus, but rates dropped below controls afterward. Depressed soil respiration following $(NH_4)_2SO_4$ or NH_4NO_3 treatment in laboratory incubated soils was seen by Roberge (1976) and Foster et al. (1980). Thirukkumaran and Parkinson (2000) also observed reduced microbial respiration and biomass. Studies of longer-term effects of NH_4NO_3 treatment have also shown decreased (Bååth et al., 1981; Bowden et al., 2001; Nohrstedt et al., 1989; Söderström et al., 1983) or unchanged (Flanagan and Van Cleve, 1983) soil respiration and microbial biomass.

The supply of available soil C has long been considered a limiting factor in the ability of soil microbes to immobilize N additions (Flanagan and Van Cleve, 1983; Söderström et al., 1983). The contradictory

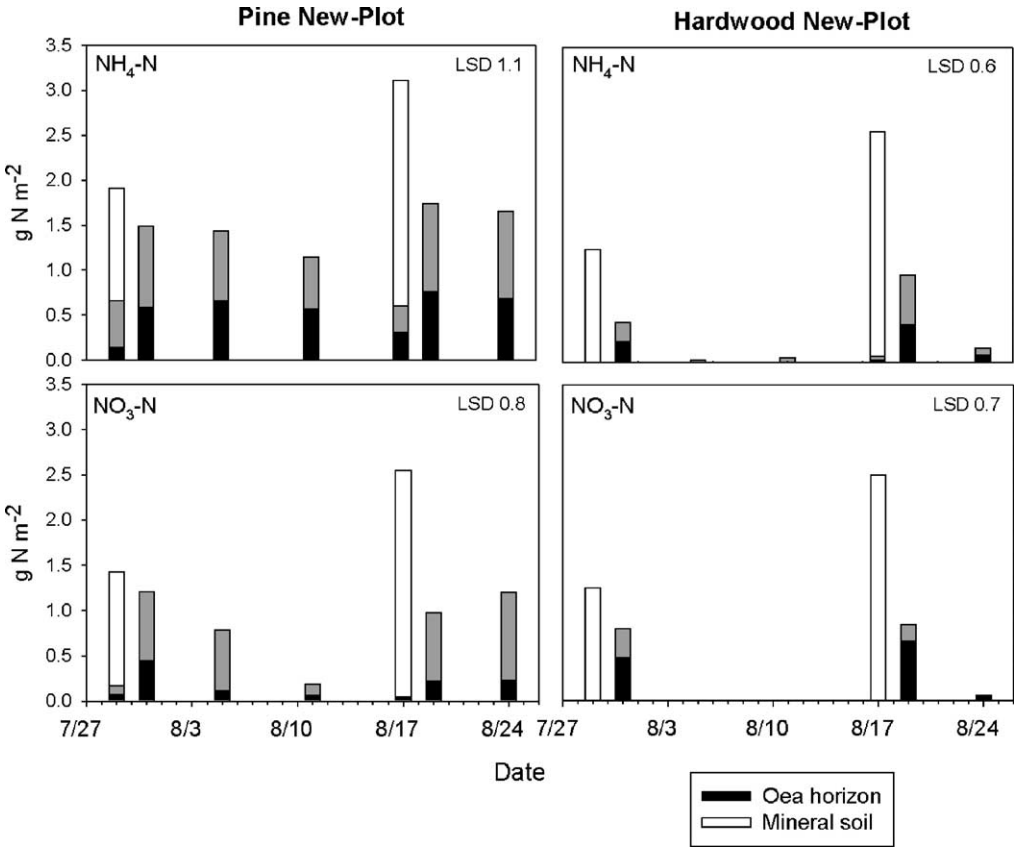

Fig. 2. Extractable NH_4-N and NO_3-N in new plot soils. Fertilizer applications (white bars) added 1.25 g m^{-2} each of NH_4-N and NO_3-N on 29 July and 2.5 g m^{-2} each on 17 August. Means for organic (Oe + Oa) and mineral (0–10 cm) soils are shown; $n = 5$. LSD (Fisher's least significant difference) is the value by which any two bars must differ in order to be significantly different ($P < 0.05$).

results among previous studies may have been influenced by the degree of initial soil N or C limitation. Carbon limitation, either pre-existing or induced as a consequence of N additions, was suggested by Söderström et al. (1983) and Flanagan and Van Cleve (1983) to explain long-term depression of soil respiration and microbial biomass in N-fertilized forest soils. Labile C and N availability may be further reduced by abiotic condensation of fertilizer N with soil C, as proposed by Söderström et al. (1983), and by lignolytic enzyme suppression due to high NH_4^+ availability (Keyser et al., 1978) which could decrease availability of easily degradable C compounds embedded within the lignin matrix (Fog, 1988).

Interpreted according to traditional thought concerning soil C and N limitation, our results indicate a pre-existing C limitation in the two forest stands. Long-term depression of soil respiration (Bowden et al., 2004) and litter decomposition rates (Magill and Aber, 1998) in the fertilized plots lend support to this idea, as do the results of Magill and Aber (2000) who observed net N immobilization when labile C was added to unamended soil from the hardwood stand. Schimel and Weintraub (2003) point out that the literature on C and N limitation in soils contains contradictory results because data based on C addition studies suggest C limitation while data based on N addition studies suggest N limitation. They propose a model based on microbial exoenzyme kinetics in which adding N to an N-limited system decreases soil respiration, and adding C to a C-limited soil increases respiration, contrary to traditional logic. Clearly, the causes and effects of C versus N limitation in soil require further study.

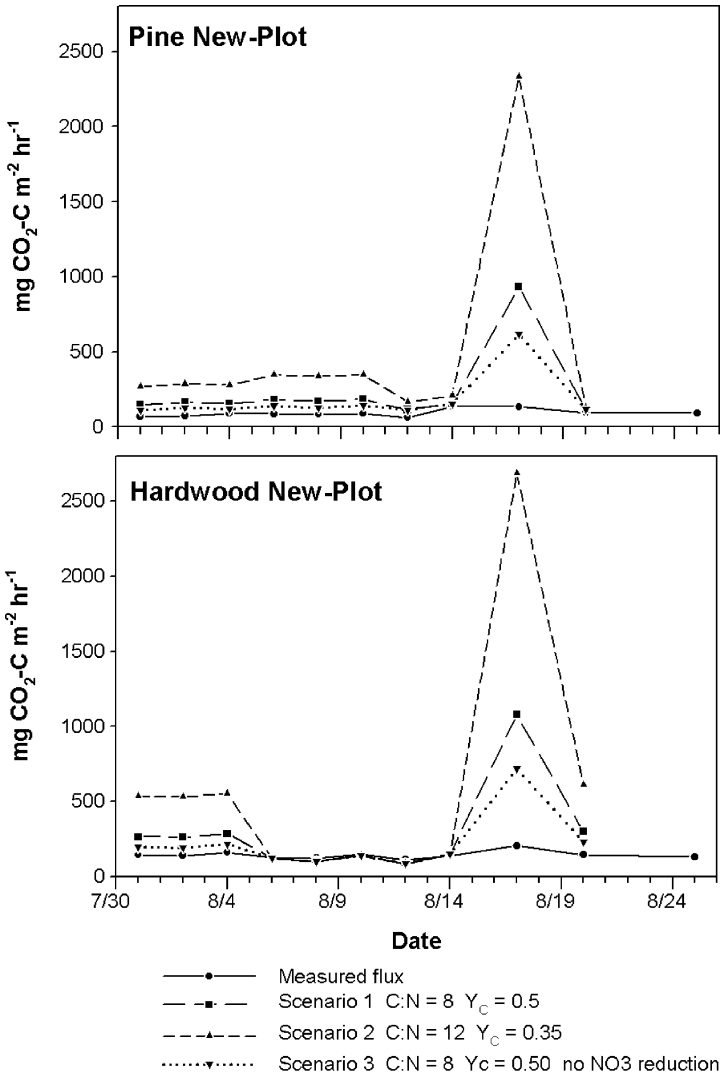

Fig. 3. Measured and predicted new plot CO_2 efflux. Predicted = control plot soil CO_2 efflux plus efflux predicted under three sets of conditions: Scenario 1 (C:N = 8, $Y_C = 0.5$), Scenario 2 (C:N = 12, $Y_C = 0.35$), and Scenario 3 (Scenario 1 without NO_3^- reduction factor).

4.2. Soil respiration after 5 years of N additions

The patterns of soil respiration among treatments and stands that we observed in year 5 of chronic N amendments are consistent with results from the first 2 years and with hardwood stand results from year 13 (Bowden et al., 2004). Soil respiration was not measured in other years. In the hardwood stand, N treatment elevated respiration rates in the first year, but high-N and control plot rates were not different in year 2 and remained so, as we found in year 5, and again in year 13. High-N treatment depressed respiration in the pine stand in years 1 and 2, and also in year 13; however, we detected no difference between these plots in year 5. Although the lack of pre-treatment data for the new plots prevents attributing results to N addition alone, the hardwood new plot flux data appear to be consistent with first-year results in which high-N plot respiration was elevated above the control.

The relative contributions of live roots and microbes to total soil respiration, with regard to the potential effects of N additions, must be considered in the interpretation of soil respiration data and changes in soil extractable inorganic N. In the first 3 years of the chronic N experiment there was no evidence to suggest that fine root biomass was affected by N additions, but in year 6, higher fine root masses in the high N plots (∼45% greater than controls) along with increased fine root turnover rates (Aber et al., 1993; Magill et al., 1997) suggest that during our study in year 5, microbial respiration in these plots could have been a smaller fraction of total soil respiration than in the controls and new plots. This could have potentially reduced our ability to detect a microbial signal in the high N plots following the fertilization events. However, respiration rates were not significantly different between high N and control soils on most dates in either stand, which does not support the idea of increased respiration (from live roots or root decomposition) concomitant with the observed increased root biomass and fine root turnover.

Bowden et al. (2004) found that after 13 years of N additions, soil respiration decreased by 41% in both stands. In laboratory-incubated soils collected in year 13, microbial respiration was 43% (hardwood high N) to 64% (pine high N) lower than controls. These results indicate that 13 years of N additions have reduced microbial respiration in these soils. However, root respiration may have also declined, as a consequence of measured decreases in forest productivity in both stands.

4.3. Apparent immobilization of fertilizer nitrogen

We interpreted the rapid disappearance of applied fertilizer N from the new plot soils as microbial immobilization. Gross immobilization rates of NO_3^- and NH_4^+ in forest soils can be high in contrast to low net fluxes (Davidson et al., 1992; Hart et al., 1994). Microbes were assumed to be stronger competitors for inorganic N than plant roots, as plant retention accounted for only 15–20% of inputs to the pine and hardwood high N plots through year 6 of the chronic nitrogen amendment study while retention in soils (Oe + Oa and mineral 0–20 cm) accounted for 83–78% of N inputs (Magill et al., 1997). Rapid disappearance of nearly all added nitrate in the hardwood stand soil was also observed in the first year of N additions (Aber et al., 1993). Because soil pH is low, we assumed that denitrification and NH_4^+ volatilization are negligible, and low 2:1 clay content suggests that NH_4^+ fixation to clay minerals is not important in these soils. We also assumed leaching loss to be negligible because NO_3^- was not detectable in soil solution at 60 cm depth in the pine stand until the third year of N additions, or in the hardwood stand until the fifth year. While the possibility of leaching below 10 cm cannot be discounted, previous results suggest that significant leaching below 60 cm did not occur in new plot soils during our 2-month study.

The rapid disappearance of the added N and lack of soil respiration response suggest that abiotic immobilization may have been important in the decrease of new plot extractable N pools following fertilizer applications. Abiotic fixation of NH_4^+ in soils has been well documented (Nommik and Vahtras, 1982; Axelsson and Berg, 1988; Schimel and Firestone, 1989; Strickland et al., 1992; Trehan, 1996). A study by Davidson et al. (1991) indicated abiotic immobilization of both NH_4^+ and NO_3^- in grassland and forest soils. Davidson et al. (2003) hypothesized a mechanism whereby NO_3^- is abiotically reduced to NO_2^- by Fe and subsequently fixed to soluble organic matter. Recent studies at the chronic nitrogen amendment plots support the occurrence of abiotic N immobilization. Using ^{15}N pool dilution, Berntson and Aber (2000) discovered that most NO_3^- immobilization occurred in a rapid phase (within 15–45 min) which was kinetically different than the slower phase typically associated with microbial immobilization. They demonstrated that 9 years of high-N additions reduced soil capacity to immobilize NO_3^- by 50% in the pine stand and by 42% in the hardwood stand. Dail et al. (2001) found that $^{15}NO_3^-$ added to sterilized unamended soil from a hardwood stand at the Harvard Forest was abiotically converted within minutes into dissolved organic forms. Aber et al. (1998) concluded, however, that estimated rates of abiotic N incorporation in these soils are too small to account for the high N retention observed at the chronic nitrogen amendment plots and at other N addition study sites. They propose that mycorrhizal uptake and exudation of N, which involves low C requirement and low respiration rates, may also function to sequester N in the amended soils.

4.4. Model predictions

Soil respiration and microbial N dynamics have been positively linked in several studies. Soil respiration rates were strongly correlated with gross N immobilization rates in a laboratory incubation study of old-growth forest soils by Hart et al. (1994), and Schimel (1986) observed a positive correlation between CO_2 evolution and net N immobilization rates in laboratory incubations of grassland and cropland soils. To Hart et al. (1994), these strong relationships suggested that C availability is an important control on N cycling rates. Tietema (1998) found both gross and net N immobilization rates in coniferous forest soils to be positively correlated with CO_2 efflux, indicating a close link between C and N cycling. All three processes were highest in soils from N saturated forests.

We hypothesized that the ratio of the apparent rate of N immobilization to increased CO_2 efflux could be predicted from N immobilization driven by microbial biomass production, given reasonable assumptions about microbial C:N ratios and carbon use efficiency. However, the model greatly over-predicted soil respiration rates, indicating underlying flaws in the assumptions and parameters on which it was based.

The use of a constant Y_C and C:N ratio in the model does not reflect the dynamic nature of these properties in natural systems (Paul and Clark, 1989). Microbial Y_C adapts to substrate quality, and may have responded to long-term changes in nutrient and energy availability in a way that allowed uptake of the applied N without producing a measurable pulse of CO_2. Y_C may have also undergone short-term change as N availability increased and subsequently decreased following individual N additions. Thirukkumaran and Parkinson (2000), for example, found that Y_C initially decreased, then later increased, during a 120-day incubation of NH_4NO_3-treated forest floor material, although respiration remained depressed below controls throughout the experiment. C:N ratios are also not necessarily constant; for example, fungi are known to translocate N from older to younger growing tissue where the substrate is N deficient (Dowding, 1976; Levi and Cowling, 1969). Further, allocation of microbially immobilized N to extracellular enzyme production was not included in our model, and this undoubtedly exaggerated CO_2 efflux predictions. Clearly, many factors complicate the use of microbial C:N ratios and Y_C to predict N uptake and respiration. The concept of single element limitation may be inappropriate to describe dynamic microbial processes in heterogeneous forest soils. Still, the magnitude of the differences between predicted and observed soil CO_2 efflux strongly suggests that traditional immobilization through microbial biomass production is not the primary mechanism.

5. Conclusion

Rapid microbial biomass growth is not indicated as a mechanism for immobilization of added fertilizer N in both previously untreated and chronically N-treated soils in the two forest stands studied. Our results suggest that an abiotic mechanism may be responsible. If microbial uptake occurred, it did so in a way that contradicted our assumptions about microbial carbon use efficiency, C:N ratio, biomass growth, and carbon and nitrogen limitations in these soils. As Schimel (1988) emphasized, assumptions regarding microbial C and N dynamics are difficult to test and conclusions based on them should be made with caution. Improvement in our knowledge regarding the high capacity for these forest soils to retain elevated N inputs awaits further research into both abiotic and microbial processes in natural systems.

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