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Microbial biomass and nitrogen cycling responses to fertilization and litter removal in young northern hardwood forests

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Abstract. The influence of site fertility on soil microbial biomass and activity is not well understood but is likely to be complex because of interactions with plant responses to nutrient availability. We examined the effects of long-term (8 yr) fertilization and litter removal on forest floor microbial biomass and N and C transformations to test the hypothesis that higher soil resource availability stimulates microbial activity. Microbial biomass and respiration decreased by 20–30% in response to fertilization. Microbial C averaged 3.8 mg C/g soil in fertilized, 5.8 mg C/g in control, and 5.5 mg C/g in litter removal plots. Microbial respiration was 200 μg CO_2-C g^{-1} d^{-1} in fertilized plots, compared to 270 μg CO_2-C g^{-1} d^{-1} in controls. Gross N mineralization and N immobilization did not differ among treatments, despite higher litter nutrient concentrations in fertilized plots and the removal of substantial quantities of C and N in litter removal plots. Net N mineralization was significantly reduced by fertilization. Gross nitrification and NO_3^- immobilization both were increased by fertilization. Nitrate thus became a more important part of microbial N cycling in fertilized plots even though NH_4^+ availability was not stimulated by fertilization.

Soil microorganisms did not mineralize more C or N in response to fertilization and higher litter quality; instead, results suggest a difference in the physiological status of microbial biomass in fertilized plots that influenced N transformations. Respiration quotients (qCO_2, respiration per unit biomass) were higher in fertilized plots (56 μg CO_2-C mg C^{-1} d^{-1}) than control (48 μg CO_2-C mg C^{-1} d^{-1}) or litter removal (45 μg CO_2-C mg C^{-1} d^{-1}), corresponding to higher microbial growth efficiency, higher proportions of gross mineralization immobilized, and lower net N mineralization in fertilized plots. While microbial biomass is an important labile nutrient pool, patterns of microbial growth and turnover were distinct from this pool and were more important to microbial function in nitrogen cycling.

Introduction

Microbial biomass and N transformations are key components of plant-soil feedbacks that should respond to variation in nutrient availability. Microbial

biomass and the processes carried out by soil microorganisms depend upon complex interactions with plants (Singh et al. 1989; Aber et al. 1991; Bohlen et al. 2001). Carbon supply to microorganisms depends not only on primary productivity, but also on carbon allocation patterns (Aerts et al. 1992; Fisk et al. 1998), rhizosphere activity (Newman 1985; Bottner et al. 1988; Smith & Paul 1990), and litter substrate quality (Flanagan & VanCleve 1983; Clein & Schimel 1995), all of which can vary with soil fertility (Vitousek 1982; Bloom et al. 1985; Fahey et al. 1998). Microbial biomass can be a good general indicator of N cycling processes at landscape or regional scales in northern hardwood forests (Bohlen et al. 2001), where vegetation composition, soil characteristics, and microbial processes covary (Pastor et al. 1984; Reich et al. 1997). However, a better understanding of the specific influence of nutrient availability on feedbacks between plants and microorganisms requires experimental studies of microbial biomass and nutrient transformations. We measured microbial responses to long-term fertilization and litter removal across replicate northern hardwood forest stands in central New Hampshire, in order to learn more about how microbial biomass and activity regulate N cycling processes within this ecosystem.

Soil microorganisms generally are C limited (Powlson et al. 1987; Anderson & Domsch 1985; Smith & Paul 1990; Wardle 1992), and as a result microbial biomass depends upon soil organic matter (Zak et al. 1990; Wardle 1992) and net primary productivity (Zak et al. 1994; Fisk et al. 1998). Nutrient limitation of microbial biomass and activity also is possible (Scheu 1990; Wardle 1992; Gallardo & Schlesinger 1994; Fisk & Schmidt 1996; Hart & Stark 1997). Both C and nutrients should be more available to soil microorganisms in more fertile and productive sites; hence it is surprising that nitrogen fertilization often supresses microbial biomass, even in forest ecosystems where productivity is primarily N limited (Söderström et al. 1983; Prescott et al. 1992; Smolander et al. 1994; Fahey et al. 1998; Scott et al. 1998). This effect can be more pronounced in less productive forest sites (Arnebrandt et al. 1996). The implications for N turnover and availability are unclear, and we need a better understanding of the role of microbial biomass and activity in either buffering or stimulating nutrient cycling rates.

Feedbacks between plant litter substrate quality and soil nutrient availability may result from the dependence of soil microorganisms on carbon supply from plants, and the coincident limitation of plant growth by nutrients supplied by microbial mineralization of plant detritus. For example, higher N returns in litterfall and higher quality litter substrates are thought to promote more rapid N turnover in more fertile forest sites, thereby perpetuating higher net N mineralization and availability for plant uptake (Vitousek 1982; Flanagan & Van Cleve 1983; Nadelhoffer et al. 1983; Chapin 1991; Clein

& Schimel 1995; Hart et al. 1997). These interactions are constrained in part by life history characteristics that control plant nutrient use and growth response to nutrient availability (Chapin 1980; Aber et al. 1991). They might likewise depend upon microbial biomass or activity; however, those possible constraints have not been characterized adequately. For instance, several fertilization studies have reported no change or decreased net N mineralization despite higher plant litter N returns to soil (Magill et al. 1996; McNulty et al. 1996; Magill et al. 1997; Aber et al. 1998), but the causes of this unexpected response remain unclear.

Only by characterizing in more detail the microbial N cycling responses will the feedbacks between soil nutrient availability and plant litter substrate quality be clarified. Net N mineralization is the balance of the counteracting process of gross N mineralization and N immobilization, and the net N mineralization response to fertilization could be more effectively interpreted if we knew the response of these underlying processes. In some ecosystems, substrate controls of gross N mineralization can have a dominant effect on net N mineralization and N cycling (Burke et al. 1989; Fisk et al. 1998; Hart et al. 1997; Scott et al. 1998). However, differences in microbial immobilization control N availability and cycling in other ecosystems (Vitousek & Matson 1985; Schimel 1986; Davidson et al. 1992; Hart et al. 1994). Gross N transformations have seldom been investigated in studies of fertility.

In this study we tested the effects of long-term fertilization and litter removal on microbial biomass and the microbial N transformations that control N availability. We hypothesized that microbial activity and N cycling rates would be higher in fertilized plots, and lower where aboveground litter inputs are removed. We expected that fertilization, by increasing nutrients and also forest productivity, would stimulate microbial N demand. However, we also expected that substrate N supply (via gross N mineralization) would be relatively more important than microbial N demand (N immobilization) in controlling plant-available N. We carried out our study in six young northern hardwood forest sites in the White Mountain National Forest, NH, in which fertilization and litter removal had been conducted for eight years (Fahey et al. 1998). This provided a replicated experimental assessment of microbial responses to fertility. The ecosystem replication of our fertility treatments also allowed us to explore the effects of inherent variation in site productivity on N cycling responses.

Methods

Study sites and treatments

The forest stands and treatment plots, described in detail by Fahey et al. (1998), are located within the White Mountain National Forest, NH. All of the stands had been clearcut 14 to 27 years prior to our study (Table 1). Pre-cut forests were in the maple-beech-birch cover type (Eyre 1980) and were dominated by a combination of American beech (*Fagus grandifolia* Ehrh.), yellow birch (*Betula alleghaniensis* Michx.), and sugar maple (*Acer saccharum* Marsh.). The naturally regenerating forests also were dominated by northern hardwoods, but with high abundance also of the pioneer tree species pin cherry (*Prunus pensylvanica* L.) and paper birch (*Betula papyrifera* Marsh). All sites were located between 300–400 m elevation on well-drained soils (Typic Haplorthods) of gentle to moderate slopes.

Three 20 $\times$ 20 m plots were established within each site. Plots were chosen to be as uniform as possible in terms of vegetation and were separated from each other by at least 30 m. Each plot was assigned to one of three treatments: control, fertilization, or nutrient depletion (litter removal). Fertilizer and nutrient depletion treatments were carried out from 1989–1998 and are described in detail in Fahey et al. (1998). Fertilization consisted of a balanced fertilizer including micronutrients, applied to the soil surface six times at 4-wk intervals throughout the growing season (May–October). The annual macronutrient additions were (in g/m^2): N = 16.7, P = 5.8, K = 25.4, Ca = 31.0, and Mg = 3.7. Nitrogen was added as NH_4NO_3 and this quantity roughly tripled N availability in forest sites of this type (Mou et al. 1993). The nutrient depletion treatment consisted of two separate approaches: sawdust addition to immobilize nutrients, and removal of the Oi litter layer to reduce nutrient recycling from aboveground litterfall. Sawdust was added in 1989, 1990, and 1992. Litter removal was carried out immediately after leaf-fall in 1989, 1991, 1993, 1995, 1996, and 1997. Litter removal also removed most added sawdust.

Fahey et al. (1998) studied 9 stands. Three of those stands were disturbed by an ice storm subsequent to their work, and we used the remaining 6 in the present study. We refer to these stands as sites 1–6 (Table 1). These numbers correspond to the sites of Fahey et al. (1998) as follows: 1 = Y2, 2 = M1, 3 = M2, 4 = O1, 5 = O2, 6 = O3.

Litterfall

We quantified nutrients in litterfall as an indicator of treatment effects on litter quality. Litterfall mass was measured in ten 0.18 m^2 laundry baskets in each

Table 1. Aboveground NPP, litterfall, and forest floor characteristics of fertilized, control, and litter-depleted stands in six sites in the White Mountain National Forest, NH.

Site	Age (yr)	Treatment	ANPP* ($g\ m^{-2} yr^{-1}$)	leaf litterfall ($g\ m^{-2}\ yr^{-1}$)			litter	forest floor			
				mass*	N	P	lignin:N*	depth (cm)	carbon (g/m2)	C:N	pH
1	14	fertilized	1902	325	6.9	0.56	24	6.2	2640	17.0	4.3
		control	1478	323	4.4	0.34	40	4.8	2250	16.5	4.0
		depleted	1164	277	3.0	0.28	50	5.3	3520	18.1	4.1
2	20	fertilized	1389	309	5.2	0.52	24	4.6	2750	16.0	3.8
		control	1122	284	3.0	0.25	45	5.3	3380	14.9	4.0
		depleted	1087	285	3.2	0.38	42	3.8	2580	15.5	3.7
3	20	fertilized	1351	302	4.9	0.32	29	5.4	3220	15.3	4.1
		control	690	242	2.4	0.29	43	6.9	3720	16.4	4.2
		depleted	916	297	3.1	0.33	43	3.8	2360	15.0	3.9
4	27	fertilized	1778	371	6.4	0.49	28	4.9	2480	16.2	4.0
		control	974	463	4.5	0.29	50	4.5	2800	18.8	4.0
		depleted	962	326	3.5	0.25	50	3.8	3040	16.1	4.3
5	27	fertilized	1270	334	5.9	0.76	24	5.3	1970	17.9	4.1
		control	1077	317	3.8	0.62	45	4.4	2420	13.9	4.2
		depleted	1164	342	3.1	0.61	47	3.5	2390	14.6	4.3
6	27	fertilized	1990	303	5.3	0.82	26	4.9	4950	16.8	3.8
		control	1938	408	3.4	0.22	51	4.5	4580	17.2	3.6
		depleted	1385	330	3.3	0.23	52	3.8	4160	16.2	3.8

*data from Fahey et al. 1998.

treatment plot. Litterfall was collected 4 times a year in 1995, 1996, and 1997, sorted to species, dried at 55 °C, and weighed. Fresh leaf-litter samples were collected for chemistry analyses from each plot during the middle of peak litterfall in October 1998. Leaves were collected on plastic sheeting spread in each plot over 3–4 rain-free days. Samples were sorted by species, dried at 55 °C, and ground. N was analyzed on a Carlo Erba CHN analyzer. P, Ca, Mg, and K were measured by argon plasma spectroscopy following dry ashing and digestion in concentrated HNO_3 plus 30% H_2O_2 (Mou et al. 1993). We estimated total nutrients in litterfall with nutrient concentrations from 1998 and average litterfall mass from 1995, 1996, and 1997.

Sample collection

Northern hardwood forests in the northeastern United States typically have a thick surface organic horizon (Federer 1984) in which root biomass is concentrated and most nutrient uptake by roots occurs (Yanai 1992; Fahey & Hughes 1994). We chose to study microbial responses to fertility in the forest floor horizon because of its importance in N cycling (Federer 1983; Bohlen et al. 2001), and because the forest floor should be influenced to a greater extent than the underlying mineral soil by any changes in plant litter quality resulting from fertilization.

Forest floor samples were collected four times: July 1997, October 1997, May 1998, and August 1998. Multiple 2-cm diameter cores were taken along six transects randomly located across the inner 15 × 15 m of each plot. The forest floor horizon was separated from mineral soil by standard visual criteria, and Oe and Oa horizons of the forest floor were pooled into one composite sample within each plot. Samples were refrigerated at 2–4 °C for approximately 24 hr before processing and incubations. Samples were homogenized by hand and fine roots and coarse fragments (> 2mm) were removed. All of the following measurements were made on subsamples of these forest floor samples.

Soil pH was measured on a 1:1 (soil:water) paste. Organic matter content was measured as loss on ignition by dry ashing forest floor subsamples at 550 °C for 4 hours.

Microbial biomass and respiration

Microbial C and N were estimated for forest floor subsamples using a chloroform fumigation – direct extraction procedure (Brookes et al. 1985; Vance et al. 1987). For each sample, one subsample was extracted in 0.5 M K_2SO_4. A second subsample was fumigated with chloroform for 5 d in a vacuum dessicator, followed by extraction in 0.5 M K_2SO_4. Subsamples of extracts

were sealed in glass ampoules for oxidation of DOC (Menzel & Vaccaro 1964) and CO_2 in the ampoule headspace was analyzed on a CO_2 coulometer (Huffman 1977). Microbial C was estimated as the difference in extractable DOC between fumigated and unfumigated soils using a correction factor (Kc) of 0.45 (Vance et al. 1987). Extract subsamples also were digested by persulfate oxidation (D'Elia et al. 1977) and analyzed for NO_3^- with an Orion continuous flow analyzer. Microbial N was estimated as the difference in extractable N content of the fumigated and unfumigated soils using a correction factor (K_n) of 0.54 (Brooks et al. 1985).

Microbial respiration was estimated with a base trap procedure to quantify CO_2 evolution from forest floor samples. Subsamples (20–25 g fresh wt) were incubated in Mason jars in the laboratory at 22 °C. After a 24 hour pre-incubation, vials containing 10 ml 0.1 M NaOH were sealed inside of mason jars. NaOH was replaced at 24 and 48 hr and titrated with 0.1 M HCl in the presence of 2 M $BaCl_2$ to determine how much NaOH had reacted with CO_2.

CO_2 evolution was quantified from a separate set of May 1998 subsamples, incubated over a longer time period for comparison with 2 d rates. The above procedure was followed and base traps were replaced at approximately 3 d intervals for 21 d.

Gross nitrogen transformations

Gross N mineralization, gross nitrification, and N immobilization were measured using a ^{15}N pool dilution technique in short-term laboratory incubations. Four replicate subsamples (20–25 g fresh wt) of each forest floor sample were pre-incubated in Mason jars for 24 h. Following pre-incubation, ^{15}N was added to two replicates as $K^{15}NO_3$ and to two replicates as $^{15}NH_4Cl$. Label was added to forest floor samples dropwise and was carefully mixed in for even distribution, without disrupting soil structure. One replicate that received $^{15}NO_3$ and one replicate that received $^{15}NH_4$ were extracted in 2 M KCl one hr after label addition. The second replicate subsamples were extracted in 2M KCl after 48 hr incubation at 22 °C. Independent short-term incubations (0, 10, 20, and 40 minutes) of forest floor material from a nearby northern hardwood forest site revealed no initial rapid N immobilization (M.C. Fisk unpublished data) that might indicate abiotic fixation (Davidson et al. 1991).

Net N mineralization was calculated as the difference in NH_4^+ and NO_3^- concentrations between final and initial extracts. A diffusion procedure similar to that of Brooks et al. (1989) was used to collect $^{15}NH_4^+$ and $^{15}NO_3^-$, and ^{15}N enrichment was determined at the Cornell Laboratory for Stable Isotope Analyses. Gross N mineralization, gross nitrification, and NH_4^+ and

NO_3^- immobilization were calculated using the equations of Kirkham and Bartholomew (1954).

N immobilization can be used as an index of microbial growth, allowing the calculation of microbial growth efficiency according to the method of Schimel (1988). Average microbial C:N from Table 4 (p. 211), equal to 9 in depleted and 10 in control and fertilized plots, was used in the following equation to estimate microbial growth efficiency:

> growth efficiency = (N immobilization * microbial C:N)/((N immobilization * microbial C:N) + CO_2-C respired).

Net N transformations

We quantified net N transformations in a 21 d incubation of May 1998 samples. For each sample, 4 replicate subsamples were placed in Mason jars and pre-incubated for 24 hours. Following pre-incubation, one replicate was extracted in 2 M KCl and analyzed for NH_4^+ and NO_3^- concentrations as described above. A second replicate was extracted after 2 d, another after 7 d, and the final replicate was extracted after 21 d incubation.

Data analyses

We used ANOVA with a factorial randomized block design to test difference mong treatments, forest sites, and sampling dates (Proc GLM, SAS, Cary NC). Treatments were blocked within sites. When significant effects were observed, differences between specific treatments were tested with Tukey's hsd. All differences reported were significant at $P \leq 0.05$.

Results

Nutrient flux in leaf litterfall responded to fertilization but not to litter removal (depleted treatment). Total N and P in annual litterfall were higher in fertilized than control or depleted plots (Table 1). Litterfall mass did not differ among treatments and so differences in nutrient flux were due primarily to differences in litter nutrient concentrations.

Forest floor depth was significantly less in depleted than fertilized or control plots (Table 1). Mass of the Oie horizon was significantly less in depleted than control or fertilized plots, but no differences were detected in Oa horizon mass. Most of forest floor in these stands was in the Oa (humus) horizon; Oie averaged only 7% of total forest floor mass. No differences were detected in mass or C for the total forest floor (Oie + Oa; Table 1). Treatments

Table 2. Results (F-values and significance) of ANOVA testing effects of forest treatment (fertilized, control, depleted) and sampling date on microbial biomass and respiration (per g organic matter basis). Treatments are blocked within sites, error degrees of freedom (DF) = 44.

Source	DF	F-values			
		Microbial C	Microbial N	Respiration	qCO_2
Treatment	2	7.85***	7.53**	9.56***	3.79*
Site	5	1.72	4.42**	16.92***	4.39**
Date	3	2.81*	9.32***	3.79*	5.19**
Treatment *Date	6	0.60	1.15	1.19	0.67

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

did not affect forest floor pH, which averaged 4.0 in each treatment (Table 1). Forest floor organic matter concentration averaged 57% and did not differ among treatments. Forest floor C:N averaged 16 and also did not differ among treatments.

Most microbial variables exhibited significant treatment effects in the ANOVA model. Microbial biomass C and N, respiration, qCO_2, and growth efficiency all differed among treatments (Table 2). Although gross N mineralization and N immobilization did not differ among treatments, significant effects were found for net N mineralization, gross nitrification, and NO_3^- immobilization (Table 3). Treatments were blocked within replicate forest sites and these blocks were a large component of variance in the ANOVA (Tables 2 and 3). All variables except microbial C and nitrification processes exhibited significant site effects. For microbial N and respiration, variation due to sites was greater than that due to significant treatment effects (Table 2).

Effects of sampling date were significant for all microbial variables, and sampling date was a larger source of variation than treatment or site (Tables 2 and 3). It was not our intent to explain temporal variation, but rather to compare treatment responses at representative times (May, July, August, October). The pattern among treatments did not differ among sampling times, and treatment by date interactions were not significant, with the exception of microbial growth efficiency.

Table 3. Results (F-values and significance) of ANOVA testing effects of forest treatment (fertilized, control, depleted) and sampling date on N transformations (per g organic matter basis) and microbial growth efficiency. Treatments are blocked within sites, error degrees of freedom (DF) = 44.

Source	DF	F-values						
		gross N min.	gross nitr.	N immob.	NO_3 immob.	net N min.	net nitr.	growth efficiency
Treatment	2	1.47	4.52*	0.55	4.58*	4.80*	2.08	8.72**
Site	5	2.89*	2.37	2.72*	1.66	2.64*	1.18	3.35*
Date	3	8.92***	6.92***	5.06**	11.07***	8.88***	10.81***	131.38***
Treatment *Date	6	0.81	0.50	1.43	2.20	1.14	0.45	5.58***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 4. Microbial biomass and respiration in fertilized, control, and litter-depleted plots in six northern hardwood forest stands. Values are expressed on a per g soil basis and on a per g organic matter basis and are the means of four sample collection dates (May, July, August, October). Standard errors of the mean are in parentheses, n = 24. See Table 2 for ANOVA results.

	Microbial C (mg/g)	Microbial N (mg/g)	Respiration (μg CO_2-C g^{-1} d^{-1})	qCO_2 (μg CO_2-C mg C^{-1} d^{-1})
			per g soil	
Fertilized	3.83 (0.225)	0.38 (0.46)	200 (12)	56 (3.2)
Control	5.79 (0.342)	0.60 (0.75)	270 (14)	48 (2.4)
Depleted	5.54 (0.220)	0.62 (0.60)	240 (15)	45 (2.5)
			per g organic matter	
Fertilized	7.07 (0.416)	0.71 (0.084)	390 (26)	
Control	10.00 (0.616)	1.08 (0.132)	490 (40)	
Depleted	9.63 (0.480)	1.10 (0.114)	430 (30)	

Microbial Biomass and Respiration

Fertilization reduced microbial C, N, and respiration by 25 to 35% (Table 4). Microbial C and N did not respond to litter removal, whereas respiration in 2 d incubations was significantly reduced (Table 4). In the 21 d incubations microbial respiration was linear over time and also was lower in fertilized and depleted than control plots (Figure 1). Microbial respiration quotients (qCO_2; respiration per unit biomass) were higher in fertilized than control or depleted plots (Table 4). The relative responses of microbial biomass and respiration to

Table 5. Microbial biomass, respiration, and gross N transformations in six northern hardwood forest sites. Values are means of fertilized, control, and depleted plots and four sampling dates, and are expressed on a per g soil basis and on a per g organic matter basis. Standard errors of the mean are in parentheses, n = 12. See Table 3 for ANOVA results.

Site	Microbial C (μg/g)	Microbial N (μg/g)	Respiration (μg CO_2-C g^{-1} d^{-1})	gross N min. (μg N g^{-1} d^{-1})	N immobilization (μg N g^{-1} d^{-1})
			per g soil		
1	4.3 (0.55)	0.35 (0.051)	210 (18)	11.3 (2.68)	10.4 (2.31)
2	5.3 (0.55)	0.48 (0.058)	200 (12)	16.5 (2.45)	10.1 (1.83)
3	5.4 (0.42)	0.57 (0.051)	270 (18)	14.0 (1.88)	10.3 (1.64)
4	5.1 (0.52)	0.74 (0.014)	280 (20)	17.3 (2.34)	11.2 (2.33)
5	4.8 (0.44)	0.61 (0.012)	280 (26)	20.2 (4.47)	14.1 (3.08)
6	5.3 (0.38)	0.42 (0.031)	210 (17)	13.4 (1.78)	6.9 (1.80)
			per g organic matter		
1	9.0 (0.63)	0.75 (0.048)	471 (28)	26.9 (7.38)	24.9 (6.24)
2	8.4 (0.83)	0.78 (0.088)	320 (16)	26.8 (4.11)	17.5 (3.45)
3	8.5 (0.65)	0.88 (0.086)	430 (23)	22.2 (3.03)	16.6 (2.85)
4	10.3 (1.03)	1.43 (0.217)	580 (52)	35.8 (4.99)	23.1 (4.63)
5	10.6 (1.01)	1.28 (0.206)	600 (45)	42.6 (10.11)	29.9 (6.89)
6	7.3 (0.48)	0.57 (0.045)	290 (24)	19.0 (2.81)	10.2 (2.85)

treatments were the same on each sampling date (data not shown) and were the same whether results were expressed per g soil or per g organic matter (Table 4).

Microbial N and respiration were consistently high in two of the sites (4 and 5) and low in site 6 (Table 5). The same pattern was found on a per g soil basis as on a per g organic matter basis. This variation was not related to stand age as both the lowest and the highest values occurred in the older stands. Nor was it related to ANPP or to other forest or soil characteristics that we measured (litterfall chemistry, soil pH, soil organic matter).

Nitrogen Transformations

In short-term (2 d) incubations gross N mineralization was greater in control plots than in fertilized plots on all sampling dates; however, because of high variation the differences were not significant (Figure 2, Table 3). Total nitrogen immobilization showed no clear pattern and also did not differ among treatments (Figure 2, Table 3). In contrast, net N mineralization in 2 d incubations was significantly lower in fertilized than control plots (Table 6). Gross nitrification and NO_3^- immobilization both were significantly higher in

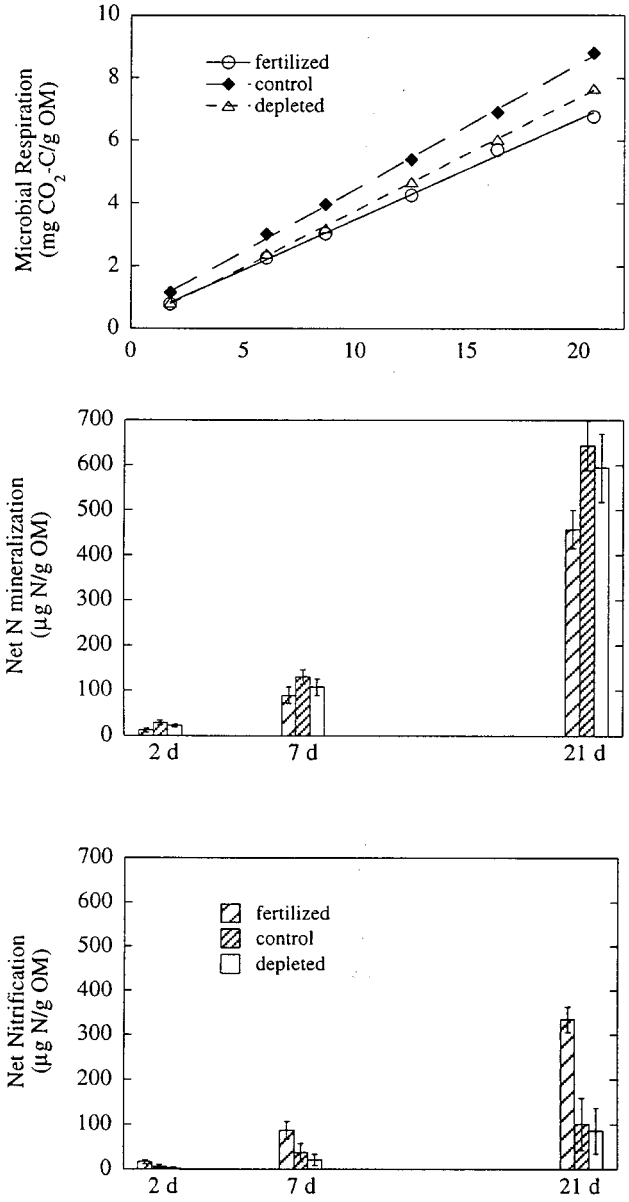

Figure 1. Microbial respiration, net N mineralization, and net nitrification in 21-d laboratory incubations (22 °C) of forest floor of fertilized, control, and litter-depleted plots. Bars are standard errors of the mean; n = 6 sites.

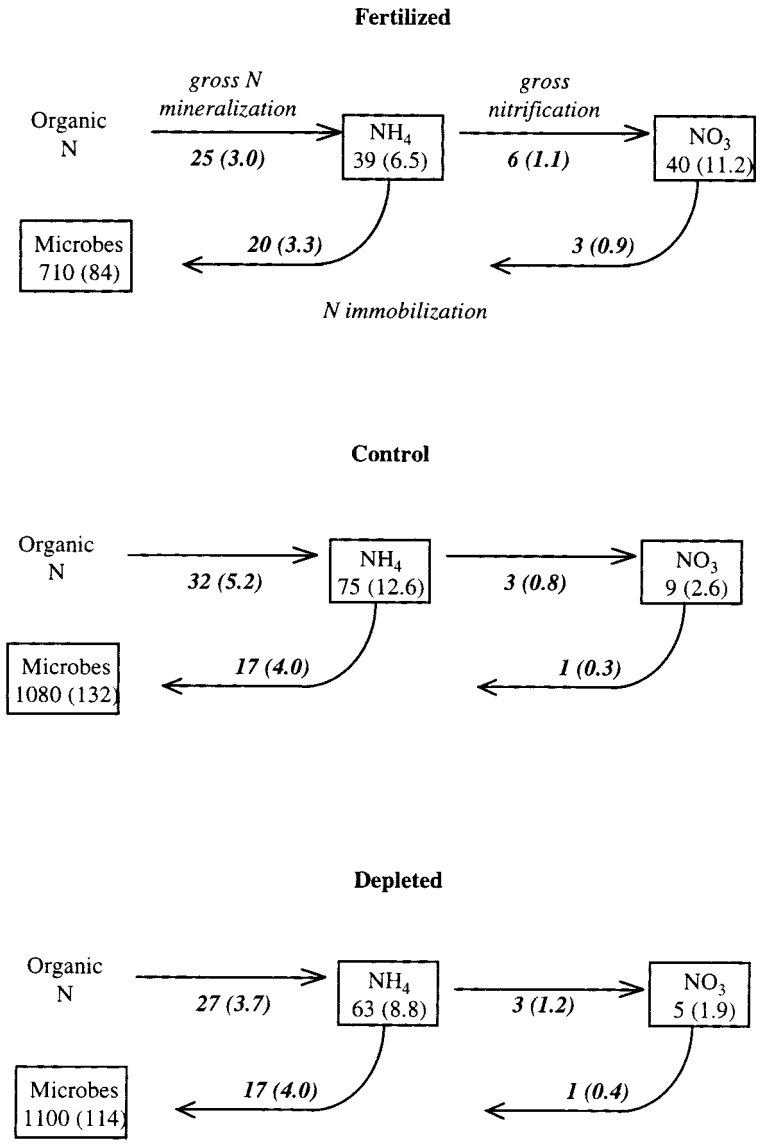

Figure 2. Microbial nitrogen cycling processes in forests floor of fertilized, control, and litter depleted plots in six northern hardwood forest sites. Microbial N is μg N/g organic matter and N transformations are μg N g OM^{-1} d^{-1}. Standard errors of the mean are in parentheses, n = 24. See Table 3 for ANOVA results.

Table 6. N transformations and microbial growth efficiency in fertilized, control, and litter-depleted northern hardwood forest sites. Standard errors of the mean are in parentheses, n = 24. See Table 3 for ANOVA results.

	% of gross min.		net N mineralization (μg N g OM^{-1} d^{-1})	net nitrification (μg N g OM^{-1} d^{-1})	growth efficiency
	immob.	nitrified			
fertilized	94 (8.3)	25 (3.7)	7.2 (2.59)	12.0 (3.50)	0.34 (0.033)
control	58 (6.2)	10 (2.6)	17.1 (3.61)	7.1 (2.87)	0.23 (0.028)
depleted	73 (6.4)	12 (3.7)	10.7 (2.36)	5.3 (1.85)	0.26 (0.028)

fertilized than control or depleted plots (Figure 2, Table 3). Finally, treatment effects on net nitrification were not significant in 2 d incubations.

Patterns of net N mineralization in long-tern (21 d) incubations generally correspond to those in short-term incubations, with significantly lower values in fertilized than control plots (Figure 1). The pattern among treatments was the same for each time interval and treatment differences were significant. Fertilization clearly increased net nitrification in the 21 d incubation (Figure 1).

Gross N mineralization, N immobilization, and net N mineralization differed by as much as two-fold among replicate forest sites (Table 5). Like microbial biomass and respiration, these processes were highest in sites 4 and 5 and lowest in site 6. Site effects were not significant for nitrification processes (Table 3).

Although neither gross N mineralization nor immobilization responded to fertilization, the comparison of N transformations is useful for illustrating both the relative importance of immobilization and nitrification in the N cycles of these forests, and the more subtle differences in N transformations that affected net N availability. Total N immobilization was a high proportion of gross N mineralization, varying among samples from 40 to 100%. Immobilized N was a significantly higher proportion of gross N mineralization in fertilized (94%) than control (58%) plots (Figure 2, Table 6); this higher proportion was consistent enough across sites to result in lower net N mineralization in fertilized plots (Table 6). The proportion of gross N mineralization that was immobilized in depleted plots (73%) was intermediate to fertilized and control plots.

Gross nitrification and NO_3^- immobilization were low relative to gross mineralization and NH_4^+ immobilization (Figure 2). Nevertheless, fertilization affected the importance of NO_3^- in the forest floor N cycle. One-fourth of gross mineralized N was nitrified in fertilized plots, compared to only 10%

in controls, and gross nitrification and NO_3^- immobilization both were higher in fertilized than control plots (Figure 2, Table 6). Although total extractable inorganic N did not differ among treatments, extractable NO_3^- was significantly higher in fertilized than control plots (Figure 2). Depleted plots did not differ from controls in NO_3^- pools or transformations.

The ratio of microbial biomass N to N immobilization gives the N residence time in microbial biomass, and provides a relative index of the rate of N turnover through microbial biomass. Microbial N content was consistently lower in fertilized than control plots, whereas N immobilization tended to be higher (Figure 2). As a result, N residence time was almost twice as high in control (60 d) compared to fertilized (32 d) plots, indicating a much higher relative rate of N turnover in fertilized plots.

Growth efficiency of 0.34 in fertilized plots was significantly greater than control (0.23) or depleted (0.26) treatments (Tables 3 and 6). This pattern was not consistent over time; growth efficiency was greater in depleted than control or fertilized plots in August and there was a significant treatment by date interaction (Table 3).

Discussion

Feedbacks between plant nutrient use and microbially-mediated mineralization of plant detritus have been suggested and supported for a variety of ecosystems (Vitousek 1982; Flanagan & Van Cleve 1983; Nadelhoffer et al. 1983; Pastor et al. 1984; Hart et al. 1997). We examined the response of microbial biomass and activity to long-term fertilization and litter removal to test hypotheses concerning the mechanisms whereby microbially-mediated processes interact with soil resource availability to regulate nutrient supply in forest ecosystems. Our results did not support the hypothesis that fertilization and enhanced plant productivity increase microbial activity and N cycling rates. Instead, microbial biomass and net N mineralization were significantly reduced by long-term fertilization. The mechanisms that underlie these responses, which have been observed in other forest fertilization studies (Söderström et al. 1983; Smolander et al. 1994; Arnebrandt et al. 1996; Magill et al. 1997; Aber et al. 1998; Scott et al. 1998), were suggested by our concurrent measurements of gross N transformations and microbial respiration. We found that soil microorganisms immobilized a higher proportion of mineralized N in fertilized plots, and this was likely due to greater specific activity and turnover of microbial biomass in fertilized plots.

Our experimental treatments included litter removal (depletion), which did not consistently affect microbial biomass or activity. Although litter removal was intended to reduce fertility, Fahey et al. (1998) did not find any significant

aboveground responses to this treatment and suggested that large soil pools buffered the effect. Our results support this interpretation for N availability. Nevertheless, this treatment changed the nature of resource supply to soil microorganisms via the elimination of leaf litter inputs of C and nutrients, and it is surprising that this did not have a more marked effect on microbial biomass and N cycling. A small decrease in respiration was observed, but there was no significant effect on gross N transformations. These results indicate that belowground C supply exerts greater control of forest floor microbial processes than leaf litter inputs, at least over the 8-yr time scale of this study.

Microbial biomass and respiration

Fertilization suppressed microbial biomass and respiration in the forest floor of the young northern hardwood forests that we studied. These results are consistent with other studies that fertilized with N alone, for several years or more (Söderström et al. 1983; Nohrstedt et al. 1989; Smolander et al. 1994; Scott et al. 1998). Fahey et al. (1998) reported the same response in our study sites after 5 years of fertilization. Comparable measurements in mature northeastern hardwood forests are lacking, however. In contrast, microbial biomass has been found to increase in response to shorter-term fertilization (Gallardo & Schlesinger 1994; Hart & Stark 1997). Whereas the immediate increase in microbial biomass following N addition suggests an N-limitation, there is not an obvious explanation for the decrease in microbial biomass over longer periods of treatment. Hart et al. (1994) demonstrated that the size of the microbial pool does not always reflect its importance in C or N cycling, and the same appears true in our study. Higher specific respiratory activity (qCO_2) and faster N turnover through the microbial pool were observed in the fertilized plots. Turnover processes thus may be as important as C or nutrient limitations in regulating the size of the microbial biomass pool.

Higher qCO_2 in response to fertilization can be interpreted in two ways. One interpretation of qCO_2 is that it represents the efficiency of C use for microbial growth. Higher values of qCO_2 can result if microbial biomass assimilates carbon less efficiently and so respires a greater proportion of C (Anderson & Domsch 1985; Insam & Domsch 1988; Anderson 1994). In this case qCO_2 is proposed as an indicator of stresses on microbial metabolism and growth (Wardle & Ghani 1995). A second interpretation of qCO_2 is that it reflects variation in the proportion of microbial biomass that is metabolically active or growing. Not all microbial biomass is active in soil (Paul & Johnson 1977; Smith & Paul 1990), and a larger proportion of total biomass that is metabolically active would produce higher values for qCO_2.

In this study the latter explanation is more likely, and we interpret the higher qCO_2 in fertilized plots to indicate a more physiologically active microbial biomass than found in control plots. Because pH did not change and nutrients were in greater supply, it seems unlikely that fertilization would increase stress and decrease the efficiency of C use for growth by soil microorganisms. Furthermore, a smaller but physiologically more active biomass is consistent with more rapid turnover of microbial biomass that occurred in response to fertilization. This explanation might be unlikely for studies that determine qCO_2 with the substrate-induced respiration (SIR) method, because SIR specifically targets an 'active' fraction of microbial biomass (Wardle & Parkinson 1990). However it is consistent with the use of chloroform fumigation-extraction (CFE) in this study, because CFE includes both active and inactive microbial biomass.

Higher microbial growth efficiency measured in fertilized plots is also consistent with a more physiologically active biomass. We estimated growth efficiency based on N immobilization as an index of microbial growth, assuming that N immobilization is proportional to biomass growth (Schimel 1988). The most serious potential flaw in this assumption is that we do not know the C:N of growing biomass. Growing biomass C:N is likely to be less than that of standing biomass and could vary among treatments, but unfortunately this is a difficult assumption to test. We can also consider the growth efficiency calculation as a simple index of N uptake per C respired. Greater N uptake relative to respiratory activity indicates more growth-related respiration in fertilized plots.

Nitrogen transformations

Contrary to our expectation, negative feedback mechanisms appear to maintain conservative N cycling processes after long-term fertilization in these young aggrading forests. Lower net N mineralization in fertilized plots represents a negative feedback to N cycling rates, by limiting the availability of N for plant uptake or for nitrification. A trend towards lower gross N mineralization, combined with the microbial immobilization of a greater proportion of that mineralized N, were responsible for lower net N mineralization in fertilized plots. Greater physiological activity and turnover of microbial biomass likely drive this negative feedback to N availability by influencing microbial N demand. Other studies have reported differences in the balance between gross N mineralization and N immobilization that determine patterns of net N mineralization (Vitousek & Matson 1985; Schimel 1986; Davidson et al. 1992; Hart et al. 1994), and our results further emphasize the importance of microbial activity and N demand for understanding net N mineralization.

In northeastern hardwood and conifer forests, net N mineralization rates declined after initial increases in response to chronic N additions (Magill et al. 1996; McNulty et al. 1996; Magill et al. 1997). Similarly, in a sub-set of our sites, we found that shorter-term fertilization (3 yr) stimulated net N mineralization (M.C. Fisk, unpublished data). It is not clear how the use of a nutrient-balanced fertilizer on our study compares with N-fertilization, but it is possible that similar changes in microbial growth and N demand affect net N mineralization rates in the chronic N addition studies. Better understanding of these temporal trends awaits further study, but changes over time in the interactions between plant C supply and soil microbial activity are likely.

Net nitrification typically is a small proportion of net N mineralization in the forest floor horizon of mature northern hardwoods (Federer 1983; Bohlen et al. 2001) and in young northern hardwoods of the ages that we studied (Fisk & Fahey 1990). Nevertheless there is great interest in nitrification and its controls because of high NO_3^- mobility in soil (Vitousek et al. 1982) and because the increase in net nitrification can be an indicator of ecosystem N saturation (Aber et al. 1998). Our results support prior findings of relatively low net nitrification in northern hardwood forest floor. We also found relatively low rates for gross nitrification, from 10% of gross N mineralisation in controls to 25% in fertilized plots. In some ecosystems gross nitrification rates are high even when net rates are mineral (Davidson et al. 1992), but that does not appear to be the case in these young northern hardwood forests.

Fertilization did not affect or even reduced organic N turnover in the forest floor of these stands. Despite long-term nutrient supplements that caused clear increases in leaf litter substrate quality and litter nutrient flux, gross N mineralization was not significantly affected. In fact, a non-significant but consistent trend toward decreased gross N mineralization was observed in fertilized plots. Better leaf litter quality often corresponds to more rapid microbial N cycling processes (Flanagan & VanCleve 1983; Pastor et al. 1984; Clein & Schimel 1995; Hart et al. 1997). In our study sites, Fahey et al. (1998) reported lower lignin, faster decay, and greater nutrient mineralization from fertilization plot leaf litter. We expected gross and net N mineralization measured in the underlying (Oe and Oa) forest floor horizons to be consistent with these decomposition processes in the litter layer. It appears, however, that nutrient cycling in the litter layer interacts little with that in the underlying forest floor layers. Similarly, Currie and Nadelhoffer (1999) found little transfer of organic N from litter to humus in northeastern pine and oak stands.

Our results highlight the need for further study of belowground C supply as a control of microbial physiological status and N cycling processes. Two aspects of our work point to the importance of belowground C supply: 1) the lack of clear microbial responses to litter removal, and 2) the decrease

in microbial biomass, respiratory activity, and net N mineralization despite higher ANPP and aboveground litterfall N flux. The Oa horizons that accounted for the majority of the forest floor (> 90%) consist primarily of humus, known to be very old and to have turnover times of hundreds of years or more (Kononova 1966; Stevenson 1982). This material provides an organic matter matrix that supports a high microbial biomass. However, sources of labile substrates that can be metabolized rapidly are needed to fuel microbial growth and activity. Given the importance of root biomass and turnover in the forest floor of northern hardwoods in this region (Fahey & Hughes 1994), it is likely that root inputs are the primary source of C and N substrates that drive microbial processes in the forest floor of these young forest stands. Decreased plant C allocation to support mycorrhizal activity and biomass could be one consequence of greater nutrient availability that contributes to observed patterns of N turnover. Fertilization decreased fine root biomass in five of the six sites that we studies (Fahey et al. 1998). The decreased rhizosphere environment associated with fine roots would impact biomass and activity of saprotrophic microorganisms (Newman 1985; Bottner et al. 1988), as well as mycorrhizal fungi.

The young northern hardwood forests that we studied varied widely in ANPP, but differences in microbial properties across forest sites were not related to differences in productivity or litter chemistry. Belowground processes again may contribute to the lack of relationships. In addition, ANPP is mostly wood in these young forests and is thus not representative of annual detritus inputs that support microbial activity. It is also likely that soil properties vary in an unpredictable manner among these recently harvested forest stands. The quality of organic matter in the forest floor is a legacy of the pre-cut forest and the degree of soil disturbance is unknown. While we cannot explain site variation in microbial characteristics in this study, our ability to detect significant responses to fertilization given the large initial differences among study sites strengthens our conclusion that soil microorganisms exert a negative feedback on N availability at higher fertility in young northern hardwood forests.

In summary, fertilization suppressed microbial biomass and respiration but stimulated microbial N uptake and turnover in these young northern hardwood forests. Results of this study emphasize the need to better understand microbial biomass as a dynamic pool that is controlled not only by substrate and nutrient availability, but also by physiological status and turnover rates. The response of plant-microbial interactions to fertilization influenced N availability through regulation of microbial physiological status and N demand, in a departure from the more common conceptual model of positive feedbacks between plant litter quality and microbial N cycling

processes. Contrary to this conceptual model, the litter layer and the underlying Oe and Oa layers of the forest floor did not appear to interact in the short-term cycling of C and N in our study. Belowground C supply is thus likely the primary control of microbial activity in the forest floor of these young northern hardwood forests.

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