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DYNAMICS OF GROSS NITROGEN TRANSFORMATIONS IN AN OLD-GROWTH FOREST: THE CARBON CONNECTION[1]

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Abstract. We conducted a 456-d laboratory incubation of an old-growth coniferous forest soil to aid in the elucidation of C controls on N cycling processes in forest soils. Gross rates of N mineralization, immobilization, and nitrification were measured by ^{15}N isotope dilution, and net rates of N mineralization and nitrification were calculated from changes in KCl-extractable inorganic N and NO_3^--N pool sizes, respectively. Changes in the availability of C were assessed by monitoring rates of CO_2 evolution and the sizes of extractable organic C and microbial biomass pools. Net and gross rates of N mineralization ($r^2 = 0.038$, $P = .676$) and nitrification ($r^2 = 0.403$, $P = .125$) were not significantly correlated over the course of the incubation, suggesting that the factors controlling N consumptive and productive processes do not equally affect these processes. A significant increase in the NO_3^- pool size (net nitrification) only occurred after 140 d, when the NO_3^- pool size increased suddenly and massively. However, gross nitrification rates were substantial throughout the entire incubation and were poorly correlated with these changes in NO_3^- pool sizes. Concurrent decreases in the microbial biomass suggest that large increases in NO_3^- pool sizes after prolonged incubation of coniferous forest soil may arise from reductions in the rate of microbial immobilization of NO_3^-, rather than from one of the mechanisms proposed previously (e.g., sequestering of NH_4^+ by microbial heterotrophs, the deactivation of allelopathic compounds, or large increases in autotrophic nitrifier populations). Strong correlations were found between rates of CO_2 evolution and gross N mineralization ($r^2 = 0.974$, $P < .0001$) and immobilization ($r^2 = 0.980$, $P < .0001$), but not between CO_2 evolution and net N mineralization rates. Microbial growth efficiency, determined by combining estimates of gross N immobilization, CO_2 evolution, and microbial biomass C and N pool sizes, declined exponentially over the incubation. These results suggest the utilization of lower quality substrates as C availability declined during incubation. Results from this research indicate the measurement of gross rates of N transformations in soil provides a powerful tool for assessing C and N cycling relationships in forests.

Key words: carbon availability; coniferous forest; immobilization; microbial biomass; microbial growth efficiency; microbial respiration; mineralization; ^{15}N; nitrification; nitrogen cycle; Oregon.

INTRODUCTION

Although numerous measurements have been made of net rates of internal nitrogen (N) cycling processes in forest soils, strikingly few studies have measured gross or actual N transformations rates (Binkley and Hart 1989). Net rates of N mineralization and nitrification are determined from the changes in soil inorganic N and NO_3^- pool sizes, respectively, during laboratory or field incubation. Although these measurements are conducted in the absence of some confounding processes (i.e., plant uptake and leaching) and under conditions that minimize others (i.e., denitrification), pool-size based estimates still represent the sum of competing consumptive and productive processes (Fig. 1). Therefore, using measurements of net rates to elucidate the controls on individual process rates is only possible if the controlling factors affect the individual processes in a similar manner.

Davidson et al. (1992) have recently shown that additional insights may be gained into N cycling processes by direct measurement of gross rates of N mineralization

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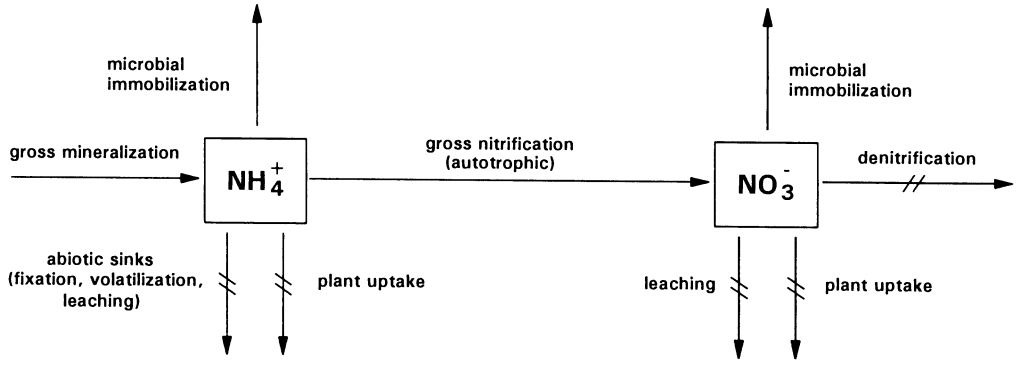

net mineralization = gross mineralization - microbial immobilization of $NH_4^+ + NO_3^-$

net nitrification = gross nitrification - microbial immobilization of NO_3^-

FIG. 1. Schematic representations of the major processes affecting NH_4^+ and NO_3^- pool sizes in forest soils. Net and gross rates of N mineralization and nitrification are measured in the absence of plant uptake and leaching and under conditions that minimize denitrification. Under these conditions net rate estimates are still influenced by both productive and consumptive processes. For the NH_4^+ pool, gross mineralization is the productive process; consumptive processes include abiotic sinks, gross nitrification, microbial immobilization, and plant uptake. For the NO_3^- pool, gross nitrification is the productive process; consumptive processes include denitrification, leaching, microbial immobilization, and plant uptake.

and nitrification. They found that in situ buried-bag estimates of net rates suggested that rates of internal N cycling were more rapid in a young forest plantation than in a nearby old forest and that nitrification was insignificant in the old forest. In contrast, in situ measurements of gross rates showed that rates of N mineralization were greater in the old forest and that rates of nitrification were similar. Their results suggested that a substantial amount of NO_3^- was being assimilated by microorganisms in both forests. Similarly, Zou et al. (1992) found contrasting results when comparing in situ buried-bag estimates of net N mineralization and nitrification with gross rates of these processes in soils from early successional and old-growth lowland tropical forests in Costa Rica. Although buried-bag estimates showed higher net rates of N mineralization and nitrification in the early successional forest, gross rates were higher in the old-growth forest. Their results, like those of Davidson et al. (1992), suggest that gross rates and net rates of N processes are not always correlated, and therefore the factors controlling N consumption and production do not equally affect these processes.

The significance of microbial immobilization of NO_3^- as a factor controlling NO_3^- pool sizes has been overlooked in many N cycling studies, which have considered net nitrification to be equal to gross nitrification (e.g., Johnson and Edwards 1979, Vitousek et al. 1982, Van Miegroet et al. 1990). This conclusion may be due in part to some early studies demonstrating that in sieved agricultural soils, microbial immobilization of NO_3^- did not occur unless high carbon to nitrogen ratio substrates were added (Winsor and Pollard 1956). Other studies showed, either directly (Jansson et al. 1955, Jansson 1958, Broadbent and Tyler 1962) or indirectly (Jones and Richards 1977), that the dominant populations in certain sieved soils preferred NH_4^+ to NO_3^- as a N source. In addition, investigations using pure cultures of microorganisms showed that NH_4^+ can repress synthesis of assimilatory NO_3^- reductase (Van't Riet et al. 1968, Sias and Ingraham 1979) or inhibit transport of NO_3^- into cells (Betlach et al. 1981). Hence, because measurable quantities of NH_4^+ are almost always present in salt extracts of bulk soils, most researchers have assumed that NH_4^+ will be the source of N utilized by microbes rather than NO_3^- and that microbial immobilization of NO_3^- will be minimal (Tiedje et al. 1981, Myrold and Tiedje 1986).

In undisturbed coniferous forest soils, NO_3^- concentrations are usually low and NO_3^- generally fails to accumulate in these soils during 1-mo laboratory or in situ incubations (Robertson 1982, Aber et al. 1989, Myrold et al. 1989). However, longer incubations of soil from these ecosystems generally result in a dramatic increase in NO_3^- pools (Johnson et al. 1980, Vitousek et al. 1982, Gosz and White 1986, Van Miegroet et al. 1990). Such large and rapid increases in NO_3^- pools following a long lag period of small and relatively stable NO_3^- pools have been interpreted as resulting from increases in nitrifier population sizes that were small initially (Sabey et al. 1959, Johnson et al. 1980, Vitousek and Matson 1985, Van Miegroet et al. 1990) or the inactivation of allelopathic compounds (Vitousek and Matson 1985, White 1986). Another

plausible explanation of this phenomenon is a change in the relative rates of gross nitrification by autotrophic nitrifiers and NO_3^- immobilization by microbial heterotrophs as NH_4^+ availability increases. The availability of NH_4^+ would be predicted to increase over time as carbon (C) availability to microbial heterotrophs decreases in response to the utilization of labile C substrates and the prevention of new C inputs. Increased NH_4^+ availability may result in greater net nitrification rates both by increasing gross nitrification rates and by reducing NO_3^- immobilization because of the apparent preference of the dominant soil microbial populations for NH_4^+ over NO_3^- as a N source (Jansson 1958, Jones and Richards 1977).

The objective of the present study was to compare changes in net and gross rates of N mineralization and nitrification rates during a long-term laboratory incubation of an old-growth coniferous forest soil that consistently shows very low soil NO_3^--N concentrations in the field (<1.0 mg/kg). Prolonged incubation of soil that receives no new C inputs from plants should induce C deficiency in the heterotrophic microorganisms. In turn, these changes in C availability should result in concurrent changes in the balance between N consumptive and productive processes. By comparing the changes in C availability and gross N cycling processes resulting from long-term laboratory incubation, we hoped to achieve a better understanding of C controls on N cycling in forest soils.

Materials and Methods

Soil characteristics and incubation procedure

Surface mineral soil (0–15 cm) was collected in early April 1990, soon after snowmelt, from an old-growth stand (>450 yr old) located on the H. J. Andrews Experimental Forest, in the central Oregon Cascade mountains (44°14′ N, 122°11′ W) at an elevation of 950 m. The climate in this region is quasi-Mediterranean with mild, wet winters and warm, dry summers. Annual precipitation is ≈250 cm, January mean air temperature is 2°C, and July mean air temperature is 20°C. This forest is dominated by Douglas-fir (*Pseudotsuga menziesii*), western hemlock (*Tsuga heterophylla*), and Pacific silver fir (*Abies amabilis*). The soil is classified as a loamy-skeletal, mixed frigid Typic Dystrochrept. Selected soil properties of the surface mineral soil (0–15 cm) are: 5.4 pH (saturated paste); 87 g/kg total C and 3.3 g/kg total N (dry combustion using a Europa Scientific CN Analyzer); 26.8 C:N ratio; and 1.9 g/kg total P (Kjeldahl digest). Soil was sampled systematically from five soil pits (each ≈0.25 m^2 in area) and was composited. Soil pits were located at the intersection and in the middle of each of four legs of two perpendicular 100-m transects. The upper 15 cm of mineral soil was used because previous soil research in this stand has indicated that this soil layer shows the greatest microbial activity (D. A. Perry, *unpublished data*). Upon returning to the laboratory, the soil was sieved (4 mm) and mixed thoroughly in a cement mixer. A 4-mm mesh sieve was used instead of the more standard 2-mm mesh sieve because soils were sieved under a moist rather than air-dry condition and as a compromise between obtaining a large homogeneous sample and minimizing soil handling effects on soil aggregates. The soil was allowed to dry slightly in the open air to bring the soil water content to 0.46 kg/kg soil. A preliminary laboratory incubation showed that maximum rates of net N mineralization and CO_2 evolution occurred at this water content (data not shown). The soil was then stored in polyethylene bags in a cold room (5°C) for ≈2 wk prior to the study to reduce the effect of sample mixing on microbial processes.

Experimental units consisted of ≈15-g samples of moist soil weighed into 20-mL glass scintillation vials. Two vials were placed into a 0.95-L glass Mason jar that was sealed with a lid containing a butyl rubber septum. Each Mason jar also contained 30 mL of deionized water to reduce water loss from the soil during incubation.

A randomly selected subset of six Mason jars was used for monitoring CO_2 evolution frequently (every few days early into the incubation to every few weeks during the latter part of the incubation). Carbon dioxide concentrations in the headspace were determined by sampling the headspace gas and injecting this sample into a gas chromatograph equipped with a thermal conductivity detector. After measuring CO_2 concentrations in these six jars, all Mason jars containing soil were opened and the soil vials weighed. Deionized water was added if necessary to replace any water loss, and the Mason jars were flushed with ambient air. All Mason jars were flushed (following headspace gas sampling) so that the CO_2 concentration in the headspace never exceeded 3% by volume. Soil vials were replaced and Mason jars were again closed and incubated in the dark at 25° ± 2°C.

Carbon dioxide evolution (microbial respiration) rates were calculated from the net changes in CO_2 concentrations in the Mason jars over time. Carbon dioxide concentrations determined on the sampling dates immediately prior to and after the sampling dates for measurement of gross N cycling processes were used for calculating CO_2 evolution rates on the day gross rates of N cycling processes were measured.

Periodically, over a 456-d period, soil vials were destructively sampled for K_2SO_4-extractable NH_4^+, NO_3^-, and organic C and N, microbial C and N, and estimates of rates of gross N mineralization and nitrification by ^{15}N isotope dilution. All K_2SO_4 soil extracts were shaken on a mechanical shaker for 1 h and then filtered through Whatman number 40 filter paper. The filter paper had been preleached with ≈50 mL of 0.5 mol/L K_2SO_4 to remove any NH_4^+, NO_3^-, or soluble C that was initially present. Ammonium (salicylate/nitro-

prusside) and NO_3^- (diazotization following cadmium-reduction) were determined using an Alpkem RFA 300 Rapid Flow Analyzer.

^{15}N isotope dilution for estimating gross rates

Gross rates of N mineralization were determined by $^{15}NH_4^+$ isotope dilution, and gross rates of nitrification were determined by $^{15}NO_3^-$ isotope dilution (Davidson et al. 1992, Hart et al. 1994). On each sampling date that gross rates were measured, six Mason jars were randomly selected: three for $^{15}NH_4^+$ isotope dilution and three for $^{15}NO_3^-$ isotope dilution. For each Mason jar, the two soil vials were labeled with ^{15}N by numerous small-volume injections with a needle and syringe. A total of 0.6 mL of solution was added to each vial. Mixing of the soil was kept to a minimum to reduce the effect of soil disturbance on rate estimates. Solution N concentrations added were 10 or 20 mg/L with ^{15}N enrichments of 99%. Higher concentrations were added when a given N pool was relatively large (early in the incubation for NH_4^+ and late in the incubation for NO_3^-), and lower concentrations were added when a given N pool was relatively small (late in the incubation for NH_4^+ and early in the incubation for NO_3^-). This was done so that the relative increases in the N pool size from ^{15}N addition were more uniform during the different assay periods over the course of the incubation. Nitrogen additions increased N pool sizes by ≈0.6–1.2 mg/kg. The corresponding relative increases in N pool sizes from ^{15}N additions ranged from ≈10 to 5% for the NH_4^+ pool, and from ≈180 to 1% for the NO_3^- pool during the course of the incubation. Within 15 min after ^{15}N addition, one vial from each jar was extracted with 50 mL of 0.5 mol/L K_2SO_4 (time 0 samples). The other was incubated in the sealed Mason jar in the dark for 24 h, and was then extracted with 50 mL of 0.5 mol/L K_2SO_4 (time t samples).

Gross rates were determined from changes in atom percentage of ^{15}N excesses (APE) above background values determined using soils unenriched in ^{15}N, and N pool sizes of pre- and post-incubated soils using the following equation developed by Kirkham and Bartholomew (1954):

$$m = \frac{[NH_4^+]_0 - [NH_4^+]_t}{t} \times \frac{\log(APE_0/APE_t)}{\log([NH_4^+]_0/[NH_4^+]_t)}$$

$$c_i = m - \frac{[NH_4^+]_t - [NH_4^+]_0}{t},$$

where m = gross N mineralization rate of the soil ($mg \cdot kg^{-1} \cdot d^{-1}$); c_i = NH_4^+-N consumption rate of the soil ($mg \cdot kg^{-1} \cdot d^{-1}$); t = time (1 day); APE_0 = atom percent ^{15}N excess of NH_4^+ pool at time 0; APE_t = atom percent ^{15}N excess of NH_4^+ pool at time t; $[NH_4^+]_0$ = total NH_4^+ concentration (N, mg/kg) at time 0; $[NH_4^+]_t$ = total NH_4^+ concentration (N, mg/kg) at time t. Gross rates of nitrification and NO_3^- consumption are calculated in a similar manner by substituting NO_3^- concentrations and atom percent ^{15}N excesses in the above equations. The NH_4^+ immobilization rate is then determined by subtracting the gross nitrification rate from the gross NH_4^+ consumption rate. The gross NO_3^- consumption rate is equivalent to the gross rate of NO_3^- immobilization (see Fig. 1).

Extracts of soil were prepared for ^{15}N isotopic analysis using the diffusion procedure described in Brooks et al. (1989). Atom percent ^{15}N enrichments were determined on a Europa Scientific Automated Nitrogen Carbon Analyzer.

Calculation of net rates and gross rates of immobilization

Net rates of N mineralization and nitrification were calculated from the changes in (NH_4^+ + NO_3^-)-N and NO_3^--N pool sizes, respectively, over the course of the incubation. Soil N pool sizes determined on the sampling dates most recently prior to and after the sampling dates for gross N rate measurements were used for calculating net rates on the days gross rates were measured.

Although gross rates of NH_4^+ and NO_3^- immobilization can be calculated from ^{15}N isotope dilution data (Davidson et al. 1991, Hart et al. 1994), these rates may be overestimates because it is necessary to add the substrates of these processes (i.e., NH_4^+ and NO_3^-) in order to estimate their rates. Therefore, gross N immobilization was also calculated from the difference between gross N mineralization determined by ^{15}N isotope dilution and net N mineralization determined by changes in inorganic N pool sizes (Fig. 1). Similarly, gross NO_3^- immobilization rates were also calculated from the difference between gross nitrification rates determined by ^{15}N isotope dilution and net nitrification rates determined by changes in NO_3^- pool sizes. Immobilization rates determined by ^{15}N isotope dilution data alone and by combining ^{15}N isotope dilution data with pool size data from soils unamended with ^{15}N were then compared.

Microbial C and N measurements

Microbial C and N were determined by chloroform ($CHCl_3$) fumigation-extraction (Brookes et al. 1985, Vance et al. 1987, Davidson et al. 1989). At each sampling date 2–4 Mason jars were selected at random. One soil vial from each Mason jar was extracted with 50 mL 0.5 mol/L K_2SO_4, and the other was fumigated for 1 d with ethanol-free chloroform in a glass desiccator. Chloroform was removed from the soil by repeated evacuations of the desiccator, and the soils were then immediately extracted with 50 mL 0.5 mol/L

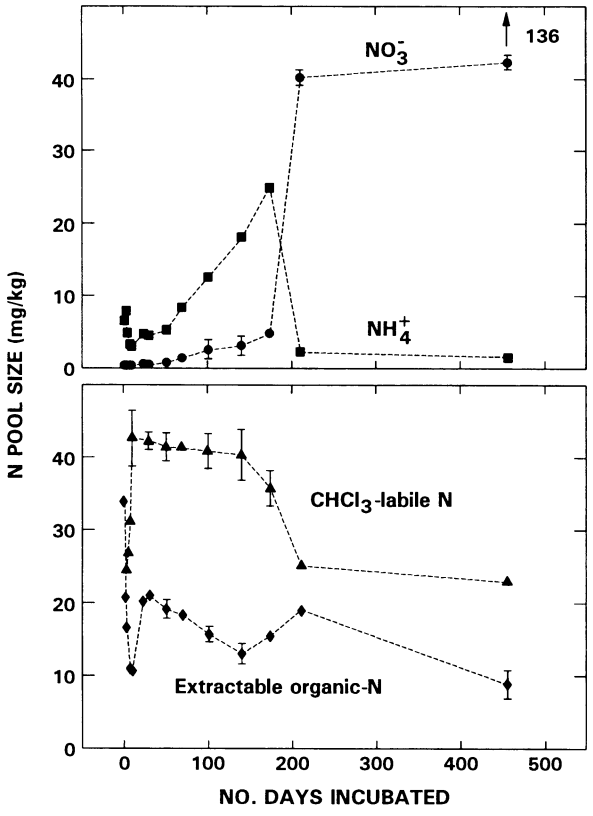

FIG. 2. Changes in mean K_2SO_4-extractable NH_4^+ (■), NO_3^- (●), organic N (◆), and $CHCl_3$-labile N (▲) pool sizes during laboratory incubation of an old-growth forest soil. For this soil, microbial biomass N is equivalent to 5 times the $CHCl_3$-labile N values. Data are means ± 1 SE (n = 2–4). Some SE bars have been eliminated for clarity where extractable organic and $CHCl_3$-labile N pool sizes were similar early in the incubation. Where no SE bars are shown for these pools later in the incubation, or for NH_4^+ and NO_3^- pools, the SE is smaller than the symbol.

K_2SO_4. A time-series study of the effect of fumigation time on extractable C and N pools using this soil showed that a 1-d fumigation released all the C and N that could be released by fumigation (data not shown). Total N extracts was determined using a modified Kjeldahl procedure that excludes NO_2^- and NO_3^- (Davidson et al. 1989). Organic carbon was analyzed on acidified samples by UV [ultraviolet]-enhanced persulfate oxidation using a Dohrmann DC-80 Carbon Analyzer with an infrared detector.

In displaying the data, we have not converted our $CHCl_3$-labile C and N values to microbial biomass equivalents so that these values fall within the same range of magnitude as the other measured C and N pools. A separate experiment using this same soil, which compared microbial biomass C and N determined by the fumigation-incubation method (Jenkinson and Powlson 1976, Davidson et al. 1989) with the direct extraction method used in this study, indicated that the microbial biomass C and N were 10 and 5 times the $CHCl_3$-labile C and N pools, respectively (i.e., k_{EC} = 0.10, k_{EN} = 0.20; data not shown).

Results and Discussion

Dynamics of C and N in soil pools

During the first seven days of the incubation, N was rapidly immobilized by a growing microbial biomass, as indicated by the rapid increase in $CHCl_3$-labile N (Fig. 2). Concurrently, extractable organic N declined markedly, NH_4^+ declined slightly, and NO_3^- pool sizes remained unchanged (Fig. 2). Microbial respiration rates were high during this period (Fig. 3), also suggesting an increase in microbial activity. Such rapid microbial growth is not surprising given that the soil was sampled at the end of the winter period when low soil temperatures (near freezing) probably limited the size of the microbial biomass. Incubating the soil at the elevated temperature of 25°C, coupled with the previous mixing of the soil (which would reduce spatial effects that may also limit microbial activity; Schimel et al. 1989), were

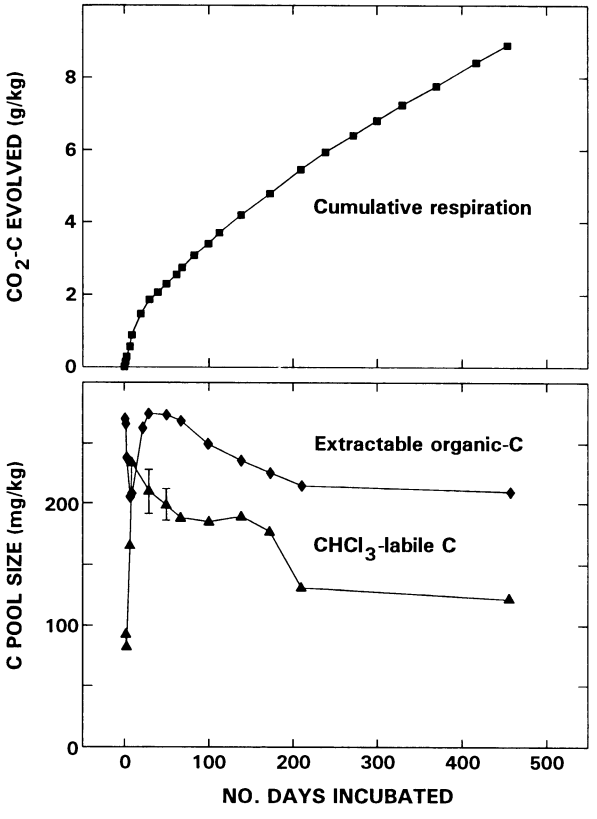

FIG. 3. Cumulative microbial respiration (■, n = 6) and changes in mean K_2SO_4-extractable organic C (◆, n = 2–4) and $CHCl_3$-labile C (▲, n = 2–4) pool sizes during laboratory incubation of an old-growth forest soil. For this soil, microbial biomass C is equivalent to 10 times the $CHCl_3$-labile C values. Data are means ± 1 SE. Some SE bars have been eliminated for clarity where extractable organic and $CHCl_3$-labile C pool sizes were similar early in the incubation. Where no SE bars are shown for these pools later in the incubation, or for cumulative respiration, the SE is smaller than the symbol.

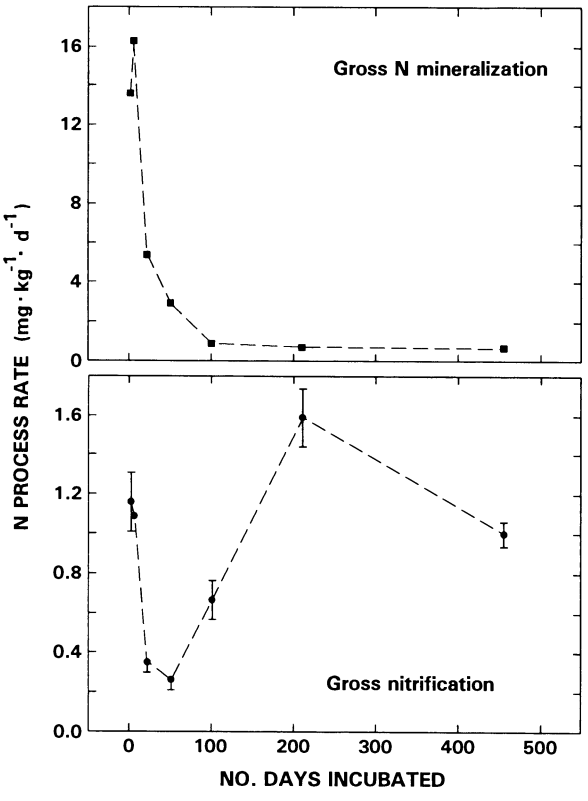

FIG. 4. Changes in gross rates of N mineralization (■) and nitrification (●) during laboratory incubation of an old-growth forest soil. Data are means ± 1 SE (n = 3). Where no SE bars are shown, the SE is smaller than the symbol.

likely responsible for this rapid increase in microbial biomass.

During this early period, and typically over the entire course of the incubation, an inverse relationship existed between the size of the $CHCl_3$-labile N and extractable organic N pools (Fig. 2). This was also generally the case for $CHCl_3$-labile C and extractable organic C (Fig. 3). Hart and Firestone (1991) found a similar inverse relationship between $CHCl_3$-labile and extractable organic N and ^{15}N pools after $^{15}NH_4^+$ addition to an old-growth forest soil in California. These results suggest that extractable organic pools are major sources of C and N for forest soil microorganisms.

Between 7 and 140 d of incubation, $CHCl_3$-labile C and N pool sizes reached a maximum, and then slowly declined (Figs. 2 and 3). During this period, NH_4^+ pool sizes began increasing rapidly, NO_3^- increased slightly, and microbial respiration rates declined. After 140 d of incubation, the $CHCl_3$-labile N pool began declining markedly, followed by a large decrease in the NH_4^+ pool and a large increase in NO_3^- (Fig. 2). These results suggest that as the microbial demand for N declined, presumably as C availability also declined, more NH_4^+ became available to autotrophic nitrifiers leading to most of the NH_4^+ pool being converted to NO_3^-.

Other studies that have shown similar dynamics of NH_4^+ and NO_3^- pool sizes over the course of a forest soil incubation have concluded from their results that autotrophic nitrifiers are weak competitors for NH_4^+ relative to microbial heterotrophs (Johnson and Edwards 1979, Vitousek et al. 1982). However, in the present study gross rates of nitrification were significant throughout the entire incubation (Fig. 4), and ranged from 9 to 230% of the gross N mineralized (Table 1). Gross nitrification expressed as a proportion of gross N mineralization was lowest during the period when the heterotrophic microbial biomass was increasing rapidly (Table 1, Fig. 2). These results suggest that both heterotrophs and autotrophic nitrifiers compete for NH_4^+, and that heterotrophs assimilate most of the available NH_4^+ during periods when heterotrophic demand for NH_4^+ is high because of a rapidly growing microbial population. Much of the time the microbial biomass is not growing or is declining in size however; under these circumstances, as suggested by higher gross nitrification to gross N mineralization rates during the latter part of the incubation (Table 1), autotrophic nitrifiers are very competitive for NH_4^+.

There still may be a critical NH_4^+ pool size that needs to be reached before gross nitrification rates and gross nitrification : gross N mineralization ratios increase substantially. This conclusion is supported by the results showing that gross nitrification rates were low and <10% of the gross N mineralization rate during periods when microbial N pools were stable (days 22–51; Table 1, Figs. 2 and 4). Under field situations where plant roots and mycorrhizae are probably competing for NH_4^+ and supplying C to microbial hetero-

TABLE 1. Ratios of gross and net N mineralization and nitrification rates during the course of laboratory incubation of an old-growth forest soil. n = 3 for each sampling date.

No. days incubated	Net min./gross min.		Net nit./gross nit.		Gross nitr./gross min.		Net nitr./net min.	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	0.06	0.02	−0.05	0.07	0.09	0.02	−0.07	0.11
5	−0.02	0.00	−0.03	0.02	0.07	0.01	0.10	0.06
22	0.02	0.00	0.01	0.02	0.06	0.02	0.03	0.07
51	0.04	0.00	0.10	0.04	0.09	0.03	0.21	0.05
101	0.20	0.02	0.04	0.02	0.76	0.10	0.15	0.05
211	0.50	0.06	0.60	0.11	2.30	0.43	2.80	0.30
456	0.60	0.08	0.39	0.05	1.51	0.26	1.00	0.02

trophs during much of the growing season, gross nitrification rates and gross nitrification : gross mineralization ratios are probably better represented by the results of these first 50 d of laboratory incubation.

^{15}N isotope dilution estimates of gross N immobilization

Previous work using ^{15}N isotope dilution to measure gross rates of NH_4^+ and NO_3^- immobilization has suggested that this method may produce overestimates because the substrates of the processes are added in order to estimate their rates (Davidson et al. 1991). Evidence in support of this potential problem includes results from studies using intact soil cores that show rates of NH_4^+ consumption (immobilization plus autotrophic nitrification) and NO_3^- consumption (immobilization) nearly always exceed gross rates of NH_4^+ production (gross N mineralization) and NO_3^- production (gross nitrification), respectively (Davidson et al. 1990, 1992). Therefore, we also independently determined gross immobilization rates of NH_4^+ and NO_3^- by combining ^{15}N isotope dilution values of gross N mineralization and nitrification with net N mineralization and nitrification values determined by changes in inorganic N pool sizes in soil subsamples that did not receive a ^{15}N amendment. Estimation of gross N mineralization and nitrification should be unaffected by ^{15}N amendments because the pools increased by ^{15}N injections are the products of these processes not the substrates (Davidson et al. 1991). We call this latter technique of calculating immobilization rates the "difference method" to distinguish it from the "isotope dilution method" that strictly relies on isotope dilution data from ^{15}N-amended soil.

There was a very close agreement between the two methods of calculating NH_4^+ immobilization (r^2 = 0.850, P = .003) and NO_3^- immobilization (r^2 = 0.757, P = .011) in this soil (Fig. 5). Additionally, the slopes of the lines representing these relationships where not significantly different from one (mean ± 1 SE = 0.79 ± 0.15 for NH_4^+ immobilization and 1.22 ± 0.31 for NO_3^- immobilization). These results suggest that the low-level additions of ^{15}N required for the estimation of N immobilization by the isotope dilution method may not always result in the stimulation of ambient rates. However, this result may in part be an artifact of the mixing of the soils prior to assessing N immobilization rates because mixing might generally increase N availability to microorganisms, thus reducing a substrate-limited response.

Net vs. gross rates

Net and gross rates of N mineralization (r^2 = 0.038, P = .676) and nitrification (r^2 = 0.403, P = .125) were not well correlated over the course of the incubation. As noted above, two recent in situ studies of N cycling in forest soils also found this same lack of correspondence between net and gross rates (Davidson et al. 1992, Zou et al. 1992). We attribute this poor correlation between net and gross rates to the confounding of two or more microbial processes when net rates are measured (Davidson et al. 1992).

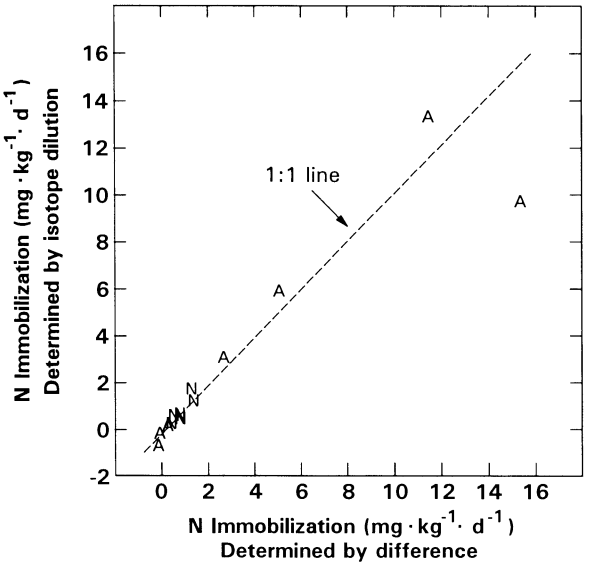

FIG. 5. Relationship between gross N immobilization estimates determined using two different calculation methods. The "difference method" uses data from both ^{15}N-amended and unamended soils, while the "isotope dilution" method uses data from only ^{15}N-amended soil. Ammonium immobilization results are denoted by "A" and nitrate immobilization by "N." Each data point is the mean value determined for a given sampling date (n = 3).

Burke et al. (1989) found strong and significant (r^2 > 0.90, P < .05) positive correlations between net and gross rates of N mineralization for soil from a sagebrush steppe. This relationship held true for soils incubated in the laboratory for 5 and 15 d, although net N mineralization became a greater proportion of gross N mineralization from day 5 to day 15 for all soils assessed. Increasing ratios of net to gross N mineralization as the incubation length increases is consistent with the results of the present study (Table 1), and suggests that microbial demand for N declines over the course of soil incubation in concert with declining C availability. However, this trend is not apparent until much later in the incubation for the old-growth forest soil (after 50 d). Differences between net and gross N cycling rates in these sagebrush steppe soils and the forest soils may be due in part to the substantially higher total and available C in the forest soils. Nevertheless, the work of Burke et al. (1989) indicates that given certain conditions and for some soils, similar factors may control both gross N mineralization and immobilization. Under these circumstances, net rates adequately reflect gross rate dynamics.

Net nitrification rates varied from −5 to 60% of the gross nitrification rates over the course of the incu-

TABLE 2. Residence time of N (in days) in various pools during the course of incubation of an old-growth forest soil. $n = 3$ for each sampling date.

No. days incubated	NH_4^+*		NO_3^- †		Microbial biomass‡	
	Mean	SE	Mean	SE	Mean	SE
1	0.60	0.02	0.30	0.11	11	0.2
5	0.30	0.01	0.60	0.08	7.3	0.2
22	0.90	0.12	1.7	0.5	40	5
51	1.8	0.2	2.9	1.1	75	7
101	14	1	3.8	1.1	286	24
211	3.2	0.6	25	5	360	53
456	2.5	0.3	136	16	425	138

* NH_4^+ pool size divided by the gross N mineralization rate.
† NO_3^- pool size divided by the gross nitrification rate.
‡ Microbial biomass N pool size divided by the gross N immobilization rate.

bation (Table 1). As with N mineralization, net to gross nitrification rates generally increased over time. This result, along with the decline in microbial demand for total inorganic N noted above, suggests that microbial demand for NH_4^+ and NO_3^- declined concurrently.

Frequently the ratio of net nitrification to net N mineralization is used to express the relative activity of autotrophic nitrifiers in forest soils (Pastor et al. 1984, Harris and Riha 1991). Table 1 illustrates that such a comparison of net rates can lead to an inaccurate conclusion regarding the actual proportion of the NH_4^+ produced that is being converted to NO_3^-. Nevertheless, both net and gross rate ratios provided similar general patterns of changes in nitrifier activity during the incubation.

Pool turnover and the significance of NO_3^- in the soil N cycle of old-growth forests

Nitrogen mean residence times (MRT) indicate the average length of time an N atom resides in a given soil pool. MRT is inversely related to the pool turnover rate; low MRTs indicate pools that turn over rapidly, whereas high MRTs indicate a slowly turning over N pool. Table 2 shows calculated MRTs of NH_4^+, NO_3^-, and microbial biomass pools during the course of the incubation. Initially, these pools were turning over extremely fast, with MRTs of <1 d for NH_4^+ and NO_3^- pools, and ≤ 11 d for N contained within the microbial biomass. These MRTs are of a similar magnitude to those determined by Davidson et al. (1992) under field conditions for an old-growth mixed-conifer forest in California. In their study, MRTs in the mineral soil ranged seasonally from 0.6 to 1.1 d for NH_4^+, 2.6 to 4.2 d for NO_3^-, and 37 to 52 d for microbial biomass pools.

Mean residence times of N in all three soil pools generally increased over time, probably indicating slower N pool turnover in response to decreasing C availability to drive the internal N cycle. After 100 d of incubation, MRT of N in the NH_4^+ pool began to decline as the size of this pool decreased, and an increasing amount of N accumulated in the NO_3^- pool.

Davidson et al. (1992) emphasized that neither the relative size of the NO_3^- pool nor net changes in NO_3^- pool sizes over time are good indices of the importance of NO_3^- in the internal N cycle of an ecosystem. The old-growth coniferous forest used in the present study, as well as the old-growth coniferous forest in Davidson et al.'s (1992) study, fit the classical paradigm of a nonnitrifying ecosystem where NO_3^- pools are small and net rates of nitrification are low over short-term incubations in the laboratory or in the field. In both of these forests, however, rates of gross nitrification were significant and NO_3^- pools turned over rapidly despite the appearance of a stable NO_3^- soil pool. Results from these and other studies (Schimel 1986, Davidson et al. 1990) indicate the microbial assimilation of NO_3^- can obscure the significance that NO_3^- plays in microbial N dynamics when only net rates are measured. However, gross N flow into a soil pool may also be a misleading index of the dynamics of that N pool if its size is changing concurrently (such as the case of the NO_3^- pool towards the end of the incubation).

We suggest that the best measure of the relative dynamics of NH_4^+ and NO_3^- pools in the internal N cycle is the MRT of that pool, where a lower MRT (faster pool turnover rate) indicates a more dynamic pool. This index integrates both pool size information and process rate information into one value. Using this criterion, the NO_3^- pool was as dynamic as the NH_4^+ pool in the internal N cycle of this forest soil (Table 2). Only after extended incubation did the NO_3^- pool begin to act like a large sink for N and not actively participate in the internal N cycle. This result is in contrast to the paradigm proposed by Jansson (1958) for agricultural soils, which states that NO_3^- will only actively participate in the internal N cycle after substantial additions of C. However, our results do support Jansson's conclusion that microbial immobilization of NO_3^- will only be significant when sufficient available C is present in the soil. We cannot determine from this study if the relative importance of NH_4^+ and NO_3^- as sources of N for plant uptake may be correlated to the MRTs of these pools. However, we suspect that other factors such as a given plant's physiological capabilities

and soil water content may confound any simple relationships.

The mechanisms that result in microbial assimilation of NO_3^- must be related to the relative spatial compartmentalization of NH_4^+, NO_3^-, and C availability in soil (Schimel et al. 1989, Davidson et al. 1990, Drury et al. 1991). As noted previously, soil slurry and pure culture studies clearly indicate that NH_4^+ is the preferred N source by microbial heterotrophs when both NH_4^+ and NO_3^- are available (Sias and Ingraham 1979, Betlach et al. 1981, Rice and Tiedje 1989). Ammonium-depleted microsites may occur at sites that have high C availability; the greater diffusion rate of NO_3^- relative to NH_4^+ in soil (Clarke and Barley 1968) may then lead to significant NO_3^- assimilation. Such spatial factors are not apparent when conducting routine salt extractions of NH_4^+ and NO_3^- of bulk soils that average N concentrations across all microsites of the soil sample, and thus may suggest ample NH_4^+ concentrations to inhibit NO_3^- assimilation.

C control of N cycling

The microbial respiration rate was not correlated with the net N mineralization rate ($r^2 = 0.078$, $P = .544$). Other studies have shown that microbial respiration and net N mineralization rates are generally not well correlated during laboratory incubations of soil for ≤2 mo (Johnson and Edwards 1979, Johnson et al. 1980, Burke et al. 1989).

Both the rate of microbial respiration and gross N mineralization declined exponentially over time (Figs. 3 and 4), and were well correlated (Fig. 6). An equally strong correlation ($r^2 = 0.980$, $P < .0001$) existed between microbial respiration and gross N immobilization and had a similar slope (data not shown). Schimel (1986) found that during laboratory incubation microbial respiration and gross N mineralization were not significantly correlated among various grassland and cropland soils derived from different parent materials; microbial respiration was well correlated with gross N immobilization, but the slope of the relationship was 0.05. The strong relationships between microbial respiration and gross rates of N mineralization and immobilization suggest that C availability is an important control on N cycling rates in soil and that measurements of CO_2 may be useful as an index of soil N cycling. However, a unique relationship may exist between microbial respiration and gross N process rates for each soil type, as suggested by the very different slopes of the lines describing the relationships between these processes found by Schimel (1986) and in the present study. Furthermore, these relationships may change over time in the field with changing soil water content, soil temperature, availabilities of other limiting nutrients, or with periodic inputs of C from plant turnover. More comparisons need to be made between microbial respiration and gross N transformation rates for a variety of soils to test the generality of these relationships and under what conditions they are valid.

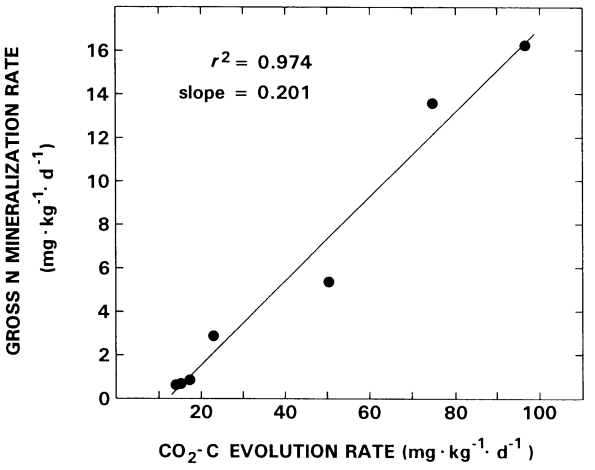

FIG. 6. Relationship between microbial respiration (CO_2 evolution, $n = 6$ per sampling date) and gross N mineralization rates ($n = 3$ per sampling date) during laboratory incubation of an old-growth forest soil ($P < .0001$). The sampling date means ($n = 7$) were used in the least squares regression analysis because rates were determined on different soil samples.

The C:N ratio of a substrate has often been used as an index of the quality of the substrate for microbial utilization (Paul and Clark 1989). High C:N ratio substrates are considered to be of relatively low quality, whereas low C:N ratio substrates are considered to be of higher quality. A linear relationship between microbial respiration and gross N mineralization rates (Fig. 6) would suggest that the C:N ratio of the substrate being utilized by soil microbial heterotrophs was constant (Schimel 1986). However, this is true only if the microbial growth efficiency (Y_C), which is equal to the amount of C assimilated into new microbial biomass divided by the amount of total C utilized, is constant over time. This may be a good assumption for a short-term incubation, but is likely not to hold true over longer term incubations, such as in the present study. Therefore, we used the method of Schimel (1988) to estimate the microbial growth efficiency over the course of the incubation, where:

$$Y_C = \frac{\left(\begin{array}{c}\text{C:N ratio}\\ \text{of microbial}\\ \text{biomass}\end{array}\right) \times \left(\begin{array}{c}\text{Gross N}\\ \text{immobilization}\\ \text{rate}\end{array}\right)}{\left[\left(\begin{array}{c}\text{C:N ratio}\\ \text{of microbial}\\ \text{biomass}\end{array}\right) \times \left(\begin{array}{c}\text{Gross N}\\ \text{immobiliza-}\\ \text{tion rate}\end{array}\right)\right] + \left(\begin{array}{c}\text{CO}_2\text{-C}\\ \text{evolution}\\ \text{rate}\end{array}\right)}.$$

The microbial biomass C:N ratio was calculated from the $CHCl_3$-labile C and N pools; the calculated C:N of the microbial biomass increased from ≈7 to 11 over the course of the incubation. Note that this equation assumes that the microbial C and N pool sizes determined by the direct extraction method reflect the C:N

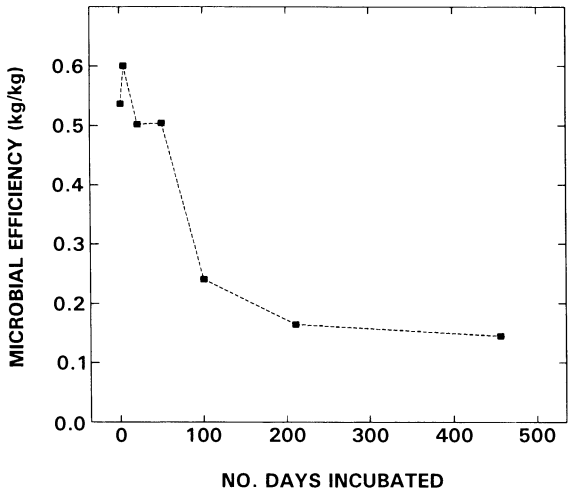

FIG. 7. Change in microbial growth efficiency during laboratory incubation of an old-growth forest soil. Microbial growth efficiency was calculated using the method proposed by Schimel (1988). Mean values of each variable (i.e., C:N ratio of microbial biomass, $n = 2$–4; CO_2-C evolution rate, $n = 6$; and gross N immobilization rate, $n = 3$) were used in the calculations.

ratio of the new biomass being produced during a given sampling period. In reality, these pools probably reflect both old and new biomass. Because the C:N ratio of the microbial biomass was increasing generally over time, Y_C values calculated in this manner are likely underestimates.

Fig. 7 shows that the estimated microbial growth efficiency decreased exponentially over time. This is exactly the pattern that would be expected if substrate quality was also decreasing over time. The C:N ratio of the substrate being utilized can now be determined by taking the reciprocal of the slope of the relationship between CO_2 evolution and gross N mineralization (Fig. 6) and dividing this value by $1 - Y_C$ ($1 -$ values in Fig. 7). Substrate C:N ratios determined in this way indicate that microbial heterotrophs were using substrates with C:N ratios between 10 and 12 early in the incubation. The C:N ratio of the extractable organic pool was similar to the estimated C:N ratio of the substrate being utilized early on in the incubation. This result corroborates the pool size data (Figs. 2 and 3) and further suggests that the extractable organic pool was a major pool being utilized by microbial heterotrophs during this period.

The increasing C:N ratio of the microbial biomass suggests that the microbial population was becoming dominated by fungi over time, which have higher C:N ratios than bacteria (Paul and Clark 1989). However, this result along with the reduction in Y_C appears to contradict results from pure culture studies that have shown fungi to have higher microbial growth efficiencies (30–70%) than bacteria (20–50%) in a constant, non-limiting growth environment (Holland and Coleman 1987). Either the method we used for calculating microbial growth efficiencies is invalid, or the microbial growth efficiencies determined in pure culture are not representative of natural microbial populations.

Conclusions

The results from this laboratory investigation support the general conclusion that measurement of gross rates of N transformations provides additional insights into N cycling in forests beyond those that can be made from traditional net rate estimates. Measurement of gross N transformation indicated that microbial heterotrophs and autotrophic nitrifiers compete for NH_4^+ in soil and that nitrifiers are successful competitors under conditions of stationary or declining populations of heterotrophs. Our results also clearly indicated that microbial immobilization of NO_3^- can be substantial in forest soils; therefore, lack of net increases in soil NO_3^- pool sizes during incubation is not unequivocal indication that the nitrification process is insignificant or absent. We suggest that the mean residence time of N in soil pools is a better indicator of the dynamics of N within a given pool than are changes in pool size or fluxes into or out of the pool alone. Finally, combining measurements of microbial biomass, respiration, and gross N immobilization indicated that growth efficiencies of microbial heterotrophs declined exponentially during the incubation. The work reported here illustrates that the integration of gross N rate measurements with measurements of C turnover in soil provides a powerful tool for elucidating substrate controls of N cycling processes in forest ecosystems.

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