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The relative importance of autotrophic and heterotrophic nitrification in a conifer forest soil as measured by ^{15}N tracer and pool dilution techniques

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Abstract. The importance of heterotrophic nitrification was studied in soil from a mixed-conifer forest. Three sites in the forest were sampled: a clear cut area, a young stand and a mature stand. In the mature stand, the mineral soil (0–10 cm) and the organic layer were sampled separately. Gross rates of N mineralization and nitrification were measured by $^{15}NH_4^+$ and $^{15}NO_3^-$ isotopic pool dilution, respectively. The rates of autotrophic and heterotrophic nitrification were distinguished by use of acetylene as a specific inhibitor of autotrophic nitrification. In samples supplemented with $^{15}NH_4^+$ and treated with acetylene, no $^{15}NO_3^-$ was detectable showing that the acetylene treatment effectively blocked the autotrophic nitrification, and that NH_4^+ was not a substrate for heterotrophic nitrification. In the clear cut area, autotrophic nitrification was the most important NO_3^- generating process with total nitrification (45 ug N kg^{-1} h^{-1}) accounting for about one-third of gross N mineralization (140 ug N kg^{-1} h^{-1}). In the young and mature forested sites, gross nitrification rates were largely unaffected by acetylene treatment indicating that heterotrophic nitrification dominated the NO_3^- generating process in these areas. In the mature forest mineral and organic soil, nitrification (heterotrophic) was equal to only about 5% of gross mineralization (gross mineralization rates of 90 ug N kg^{-1} h^{-1} mineral; 550 ug N kg^{-1} h^{-1} organic). The gross nitrification rate decreased from the clear cut area to the young forest area to the mineral soil of the mature forest (45; 17; 4.5 ug kg^{-1} h^{-1} respectively). The ^{15}N isotope pool dilution method, combined with acetylene as an inhibitor of autotrophic nitrification provided an effective technique for assessing the importance of heterotrophic nitrification in the N-cycle of this mixed-conifer ecosystem.

Introduction

Nitrification in forest soils has been one of the most intensively studied reactions in the nitrogen cycle. Nitrification in soil is generally thought to be performed by a suite of autotrophic soil bacteria. Heterotrophic microorganisms are also known to produce NO_3^- *in vitro*, although the significance of this process in N-cycling is largely unknown (Killham 1986). Few studies have directly addressed the relative quantitative importance of autotrophic and heterotrophic nitrification in forest ecosystems (Hart et al. 1997).

It has been suggested, that acidic forest soil might be a place where heterotrophic nitrification could be important (Stroo et al. 1986). However, other work indicates that acidophilic autotrophs are also involved in the production of NO_3^- in acid forest soils (Duggin et al. 1991; Pennington & Elli 1993). Heterotrophic nitrification was measured using ^{15}N techniques and N-serve with an acid woodland soil in a laboratory experiment (Barraclough & Puri 1995) and their results indicated that a maximum of 8% of the observed nitrification at that site came from heterotrophic nitrification. Killham (1986) suggests that the character and availability of substrates are more important than soil pH in controlling the significance of heterotrophic nitrification in forest soil.

In experiments using soil slurries from a mixed-conifer forest (Blodgett Forest Research Station, CA), Schimel et al. (1984) found that NO_3^- production was unaffected by specific inhibitors of autotrophic nitrification indicating that the potential for heterotrophic nitrification was greater than that for autotrophic nitrification. Nitrogen-15 labeling of the NH_4^+ pool demonstrated that NH_4^+-N was not the substrate for NO_3^- production in the presence of acetylene. Further studies at Blodgett Forest showed that soil from a 10-yr-old plantation contained measurable NO_3^- pools and accumulated NO_3^- in buried bags, whereas a nearby old-growth mixed-conifer preserve soil contained predominately NH_4^+ (Hart & Firestone 1989). Davidson et al. (1992) used ^{15}N isotope pool dilution to measure gross and net nitrification rates at this site. The NO_3^- pools were smaller and net nitrification rates were lower in the old-growth stand than the young plantation; however, the gross nitrification rates were similar in the plantation and old-growth stands. Microbial assimilation of NO_3^- was observed in both sites. While the potential for heterotrophic nitrification has been demonstrated in some forest soils, and the importance of measuring gross rates is clear, only one previous study (Hart et al. 1997) has attempted to determine rates of heterotrophic nitrification and autotrophic nitrification simultaneous to gross rates of mineralization in order to provide the context necessary for assessing the quantitative significance of heterotrophic and autotrophic nitrification in the N-cycle of a forest system.

Three different areas of a mid-elevation, mixed conifer forest (Blodgett Forest Research Station, CA) were chosen for this study in order to determine the rates and proportion of heterotrophic and autotrophic nitrification under conditions of varying disturbance and stand age. Use of these sites also allowed comparisons to previous work. In the experiments reported here, we have used short-term incubation with $^{15}NO_3^-$, $^{15}NH_4^+$ and acetylene in laboratory studies to determine gross rates of autotrophic nitrification, heterotrophic nitrification, and mineralization of organic-N to NH_4^+. With these data, we can begin to assess the importance of heterotrophic as well as autotrophic nitrification in the nitrogen dynamics of this conifer forest soil.

Material and methods

Site description and sampling

Samples were collected at Blodgett Forest Research Station of the University of California located in the foothills of the west slope of the northern Sierra Nevada Mountains (38°52′ N, 120°40′ W) at 1400 m elevation. The forest represents a major ecotype in California and an important timber growing area. Seasonally dry, the area receives 1680 mm of precipitation as snow and rain (about 2:1) from November through May. The mixed-conifer forest type is dominated by *Abies concolor*, *Calocedrus decurrens*, *Pseudotsuga menziesii*, *Pinus ponderosa*, *Pinus lambertiana*, and *Quercus kelloggii*.

The three sites used all have eastern aspects, similar slopes and are on well-drained, sandy-loam soil of the Holland series (Ultic Haploxeralf). A mature stand (mature forest area) in a reserve area was chosen which closely matched the characteristics of the mature stand used in Davidson et al. (1992) and in Schimel et al. (1984). The 14-yr old mixed-conifer plantation (young forest area) is the site of a 1980 clear-cut. The third site had been clear-cut in 1991 (clear cut area).

In April of 1994, three evenly-spaced, parallel, replicate transects approximately 20 m apart and 100 m long, were made in each area. In order to avoid edge effects a buffer area of approximately 5 m within the perimeter of each area was not sampled. Samples were taken every 3 m along each transect from 0–10 cm in the mineral layer (A horizon) and composited in order to capture the natural variation of the area. Thus each of the three replicate samples resulted from compositing of 33 subsamples taken along the transect. The organic layer of the forest floor (O horizon) in the mature forest was also sampled. This organic layer was from 6 to 10 cm in depth and comprised mainly of conifer foliage in various stages of decomposition. Soils

were transported on ice to Berkeley, CA, USA for analysis. All experiments were performed within 1 week after sampling.

Experimental set-up

The three mineral soil samples from each plot were mixed by hand removing rocks greater than approximately 3 mm in diameter, sticks greater than 1 cm and large roots. Each sample was divided into two 600 g subsamples. One subsample was sprayed with 10 ml of a 25 atom % enriched ^{15}N-NH_4^+ solution (233 mg/l $(NH_4)_2SO_4$), the other with 10 ml of a 25 atom% enriched ^{15}N-NO_3^- solution (356 mg/l KNO_3). The enrichment equaled 0.8 mg-N kg^{-1} mineral or organic soil. Spraying the soil while mixing facilitated an even distribution of the tracer solution. Immediately after spraying, the soil was divided into 50 g subsamples in 225 ml screw-cap glass Mason jars with butyl rubber septa installed in the lids. To half of the subsamples, 3 ml of acetylene gas was injected through the septa. The second half received 3 ml of 100% N_2. The final gas phase concentration of acetylene was 8 KPa.

The mature forest organic material received comparable treatment to the mineral soils. The ^{15}N solution was added as 20 ml of a 1:1 dilution of the starting solution. Subsamples (30 g) were placed in 225 ml Mason jars, half received acetylene and half received N_2.

Samples were incubated in the lab at 25 °C. At 6, 13, 18, 24 and 30 h after start of the experiment, one sample from each treatment (+ and − acetylene) was extracted with 150 ml of 4 °C 2N KCl solution. The samples were shaken (150 rotations/min) for 1 hour at 4 °C. Extraction and filtration was performed at 4 °C to minimize continued microbial transformation. Filtrate was collected by gravity filtration through Whatman No. 1 filters (pre-rinsed 3 times with 2N KCl). Filtrate was immediately frozen for later analysis.

A subset of soil was analyzed for gravimetric water content. Measurements of soil pH were made in water-saturated pastes (n = 6 for each). For each site, bulk density was determined for mineral soils by weighing 5 oven-dried soil samples of known volume. For the organic horizon, the surface organic material within a 0.25 m^2 template was removed down to the mineral horizon. The depth to mineral soil varied between 6 to 10 cm. The collected organic materials were oven dried to constant weight.

Analysis

Soil NH_4^+ and NO_3^- plus NO_2^- concentrations were measured on a Lachat autoanalyser using Lachat standard methods. Sixty ml of soil extract from samples supplemented with $^{15}NH_4^+$ were prepared for mass spectrometry according to Brooks et al. (1989). After the NH_4^+ had been extracted from

Table 1. Net mineralization and nitrification rates with or without acetylene addition.

	NH_4^+ production – mg kg^{-1} h^{-1}		NO_3^- production – mg kg^{-1} h^{-1}	
	+Acetylene	−Acetylene	+Acetylene	−Acetylene
Clear cut area	0.055**	0	0	0.067***
Young forest	0	0	0.015**	0.017**
Mature forest mineral soil	0	0	0	0
Mature forest organic horizon	0	0	0.017*	0.024***

Certainty that rate is not zero: * $p = 0.10$, ** $p = 0.05$, *** $p = 0.005$
Values shown are generated via linear regression analysis using all data from each of three sampling times.

these samples, Devarda's alloy was added to reduce NO_3^- to NH_4^+ and the NH_4^+-N extraction procedure was repeated (Brooks et al. 1989). This step included the addition of a surfactant to the soil extract (Herman et al. 1995). The ^{15}N enrichment of the samples were determined using an automated nitrogen and carbon analyzer coupled to an isotope mass spectrometer (ANCA-IRMS, Europa Scientific, Ltd. Crewe, England). From samples supplemented with $^{15}NO_3^-$, NH_4^+ was removed by MgO treatment of 60 ml of the extract in an open container. Forty eight hours after MgO addition, Devarda's alloy was added and the NH_4^+ was collected as described for the $^{15}NH_4^+$ samples.

The rates of gross mineralization and gross nitrification were calculated from the experiments with ^{15}N labeled NH_4^+ or NO_3^- (Blackburn 1979; Lund & Blackburn 1989; equations shown in appendix). We distinguish between two situations; (case A) where the net N pool size (defined as gross production (d) − consumption (i)) changes linearly with time and consumption does not equal gross production ($d \neq i$); and (case B) where gross production rate is balanced by consumption causing the pool size to remain constant during the incubation ($d = i$). Both situations, where $d \neq i$ and where $d = i$, were encountered in our experiment. When the slopes of the lines describing the net change in pool sizes with time ($d - i$) were significantly different from zero ($P \leq 0.05$) the $d \neq i$ model was used. Otherwise, rates were calculated by the $d = i$ model.

Statistical analyses

Net mineralization rates were calculated by regression analysis on all soil replicates ($n = 3$) using JMPIN (SAS Institute Inc. 1995). Rates reported in Table 1 are those significantly different than zero ($p \leq 0.10$). We used analysis of variance to detect differences in mean gross nitrification rates among sites (JMPING).

Results

Bulk density and pH values

Mean values (± standard error) for pH measurements from the clear-cut soil were 5.7 (±0.13), young forest soil, 5.9 (±0.17), mature forest mineral soil, 5.8 (±0.08), and the organic horizon material, 5.2 (±0.07). Density values for the mineral horizons were determined to be 0.83 g cm^{-3} (±0.04) for the clear cut site, 0.94 (±0.05) g cm^{-3} for the young forest and 0.82 (±0.03) g cm^{-3} for the mature forest. For the organic horizon from the mature forest site, the mass per area ($n = 3$) was 3.9 (±0.26) kg m^{-2}.

NH_4^+ and NO_3^- pools

The patterns of extractable soil NH_4^+ and NO_3^- were different between the sites sampled over the 30 h incubations (Figure 1). All pool sizes shown in Figure 1 include the added label (0.8 mg-N kg^{-1}). In the clear cut area, the NO_3^- concentration was twice as high as the NH_4^+ concentration and acetylene had a significant effect on the pool size development during the incubation (Figure 1). Soil samples without acetylene additions accumulated NO_3^- at a rate of 67 ug-N kg^{-1} h^{-1} while the NH_4^+ concentration remained unchanged during the incubation. In samples with acetylene, NH_4^+ accumulated at a rate of 55 ug-N kg^{-1} h^{-1} while NO_3^- remained unchanged (Table 1).

In the young forest area, the NO_3^- accumulated at the same rate in the presence and absence of acetylene, at rates of 15 and 17 ug-N kg^{-1} h^{-1}, respectively (Table 1). The NH_4^+ concentration showed no significant increase during the incubation period in either treatments.

In the samples from the mature forest (mineral soil and organic material), the NH_4^+ concentration remained unchanged during the incubation. For the mature forest mineral soil, the rate of net NO_3^- accumulation was not significantly different from zero. In the mature forest organic material samples, NO_3^- accumulated at rates of 17 and 24 ug-N kg^{-1} h^{-1} in the presence and absence of acetylene, respectively (Table 1).

Autotrophic and heterotrophic nitrification

Gross rates of nitrification were measurable in all soils except the mineral soil from the mature forest (Figure 2). The gross nitrification rate (−acetylene) was highest in the clear cut area decreasing through the young forest area to the mature forest sample set ($p > F < 0.1$). When rates were calculated on a mass basis, the rate of gross nitrification in the mature forest organic horizon was one of the highest measured (Figure 2). However, when rates were

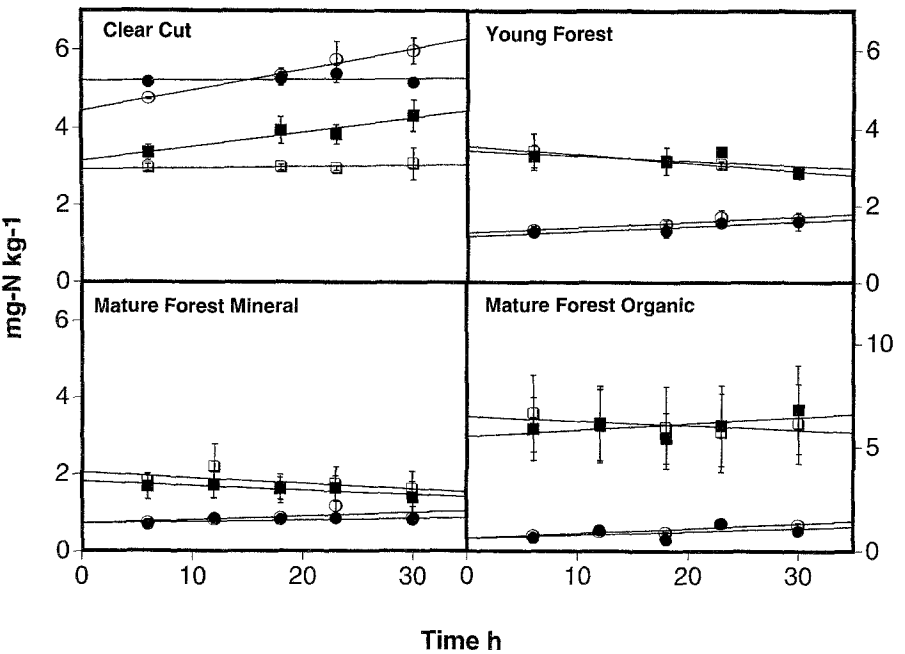

Figure 1. Extractable NH_4^+ (squares) and NO_3^- (circles) in soil over time, with (filled symbols) or without (open symbols) acetylene addition (n = 3; error bars represent one standard error).

calculated on an areal basis, the rate in the organic horizon was the lowest (Table 2). Soil samples from the clear cut showed a significantly lower gross nitrification rate when the samples were treated with acetylene (P = 0.05, Students t-test), indicating the importance of autotrophic nitrification (Figure 2; Table 2). In the clear-cut area, the autotrophic nitrification accounted for approximately 80% of the total gross nitrification rate; the remainder apparently originated from heterotrophic nitrification. Heterotrophic nitrification appeared to be the process responsible for NO_3^- formation in the forested sites since the rates of gross nitrification were not different in the presence and in the absence of acetylene (Figure 2, Table 2). The rate of heterotrophic nitrification in the clear cut area was within the same range as the rates in the forested sites (Figure 2, Table 2).

Soil from the clear cut area and the young forest area contained sufficient NO_3^- to allow mass spectrometry analysis for the $^{15}NO_3^-$ content in $^{15}NH_4^+$ supplemented samples. In the absence of acetylene, soils from the clear cut and the young forest accumulated $^{15}NO_3^-$. However, no $^{15}NO_3^-$ accumulated in these samples when treated with acetylene (Figure 3) indicating that acetylene completely blocked autotrophic nitrification. These data also indicate

Table 2. Gross mineralization and gross nitrification rates (on an areal basis) with or without acetylene addition.

	Gross mineralization mg-N m^{-2} h^{-1}		Gross nitrification mg-N m^{-2} h^{-1}	
	+Acetylene	−Acetylene	+Acetylene	−Acetylene
Clear cut area	17.0 ± 3.7	15.9 ± 4.4	0.96 ± 0.38	5.21 ± 2.08
Young forest	11.8 ± 3.7	9.9 ± 3.7	1.63 ± 0.13	2.08 ± 0.63
Mature forest mineral soil	9.3 ± 3.1	10.0 ± 3.6	0.46 ± 0.42	0.50 ± 0.50
Mature forest organic horizon	5.7 ± 2.5	5.8 ± 2.7	0.26 ± 0.15	0.39 ± 0.14

± Standard deviation of 3 field replicates

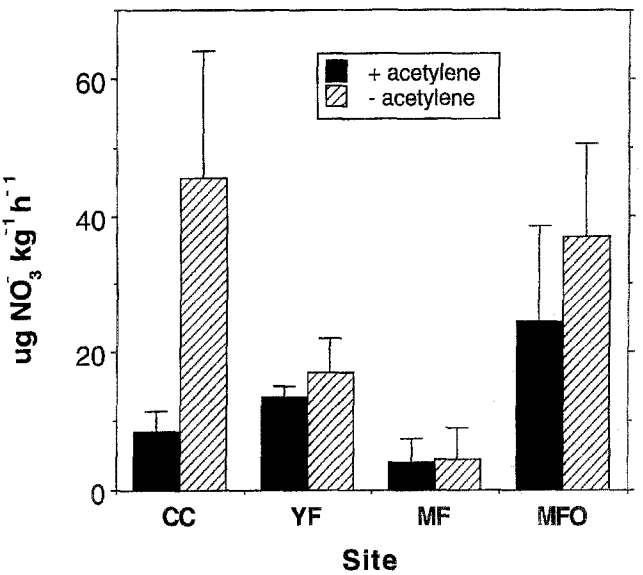

Figure 2. Gross nitrification rates in samples with and without acetylene additions. Sites for soil sampling: clear cut (CC), young forest (YF), mature forest mineral soil (MF), mature forest organic horizon (MFO) (n = 3; error bars represent one standard deviation).

that NH_4^+ was not the substrate for the heterotrophic nitrification found in these areas.

Gross mineralization and nitrification

Rates of gross mineralization were not significantly different between the clear cut, the young forest and the mature forest mineral soils, on a mass basis (Figure 4). When calculated on a mass basis, the gross mineralization rate appeared to be highest in the mature forest organic material; however

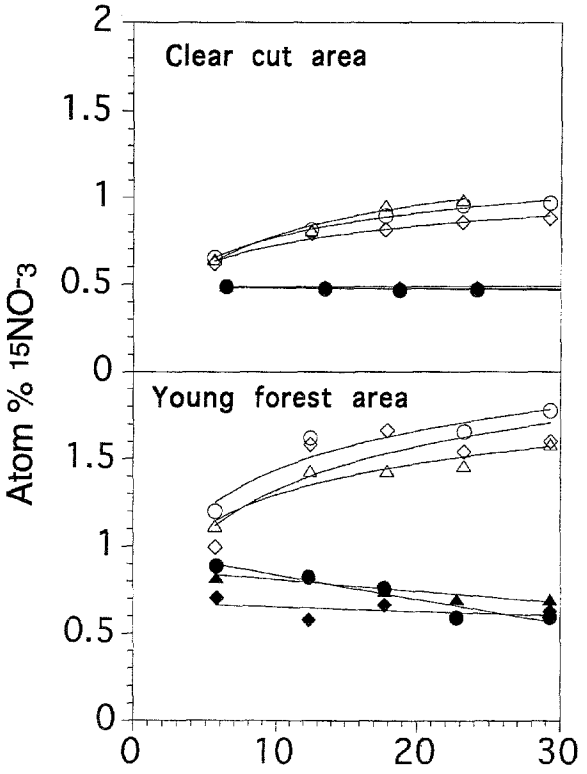

Figure 3. Atom % $^{15}NO_3^-$ in samples supplemented with $^{15}NH_4^+$ vs. time. A three replicate sample set is shown with (filled symbols) or without (open symbols) acetylene addition.

when calculated on an areal basis, it was the lowest of any horizon sampled. The acetylene treatment did not affect the gross mineralization rate in any of the sample areas (Figure 4). When no acetylene was added, nitrification accounted for 33% and 21% of gross mineralization in the clear cut soil and the young forest soil respectively (Table 3). Nitrification was of less importance in the mature forest accounting for less than 5% of the gross mineralization (Table 3).

The net accumulation rate of NO_3^- in the clear cut soil samples (67 ug-N kg^{-1} h^{-1}, Table 1) was not significantly different from the gross nitrification rate (45 ug-N kg^{-1} h^{-1}, Figure 2). A similar result was observed in the young forest area, where net nitrification rate was 17 ug-N kg^{-1} h^{-1}, and the gross nitrification rate was 17 ug-N kg^{-1} h^{-1}.

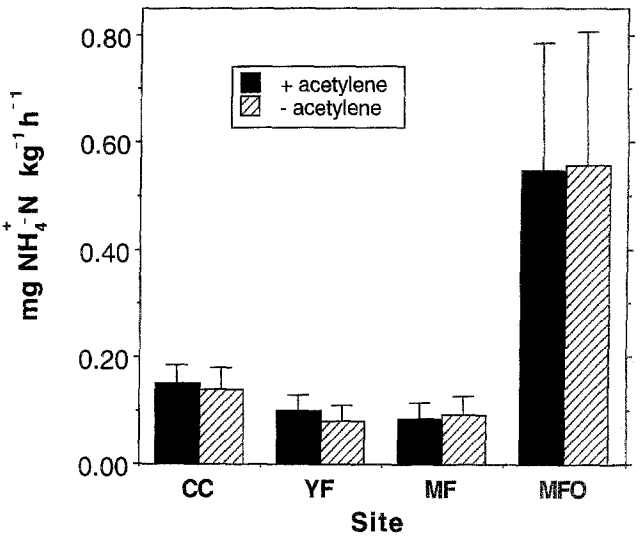

Figure 4. Gross mineralization rate in samples with or without acetylene addition. Sites for soil sampling: clear cut (CC), young forest (YF), mature forest mineral soil (MF), mature forest organic horizon (MFO) ($n = 3$; error bars represent one standard deviation).

Table 3. The relationship between gross nitrification and gross mineralization.

Sampling site	Gross nitrification/gross mineralization, %	
	−Acetylene	+Acetylene
Clear cut area	33 ± 13	6 ± 2
Young forest	21 ± 6	14 ± 1
Mature forest mineral soil	5 ± 5	5 ± 5
Mature forest organic horizon	7 ± 3	5 ± 3

± Standard deviation of 3 field replicates

Discussion

^{15}N pool dilution to measure gross rates of mineralization and nitrification

Most studies of nitrification in soil have measured NO_3^- pool sizes and net nitrification rates. Although in many cases appropriate, the results in such experiments can provide little information on the importance of the nitrification process in the N-cycling of the system. Since NO_3^- can be produced and consumed in soil, the pool size reflects the balance between production and consumption. Gross rates of NO_3^- production and consumption can be determined by use of the ^{15}N label. The results of this work demonstrate that the $^{15}NO_3^-$ pool dilution method, combined with the nitrification inhibitor acety-

lene, is an effective approach to determining the importance of heterotrophic and autotrophic nitrification in the N-cycle of terrestrial systems.

Several zero-order models have been used for calculation of rates from ^{15}N labeling experiments (Smith et al. 1994). In the present experiment, we used the steady state model developed by Blackburn (1979) which is an alternative expression of the model proposed by Kirkham and Bartholomew (1954), but has the advantages of using multiple data points instead of only t_{zero} and t_{final}.

Autotrophic and heterotrophic nitrification in forest systems

Nitrification was an important process in the nitrogen cycle of the clear cut area (Table 3). The rate of nitrification in the clear cut area was higher than in the forested sites. Duggin et al. (1991) used net NO_3^- production in lab incubations with and without nitrapyrin to assess the relative importance of heterotrophic and autotrophic nitrification in forest floor material from Hubbard Brook hardwood forests before and after clearcutting. They found autotrophic and heterotrophic nitrification to be of equal importance in the undisturbed forest and heterotrophic nitrification mainly responsible for NO_3^- formation in the clear cut area. In contrast, our experiments using a conifer forest soil showed heterotrophic nitrification was dominant in the forested sites, whereas the high NO_3^- production in the clear cut site was caused primarily by autotrophic nitrifying organisms. In our study, the rates of heterotrophic nitrification in soil from the clear cut area were similar to those in soil from the mature forest. The higher rates of nitrification in the clear cut area were not caused by higher pH values in the soil; there was no major differences in pH values between the clear cut and the forested site. Neither KCl-extractable NH_4^+ or gross rates of mineralization were higher in soils from the clear cut site than in mineral soil from the forest site. Thus NH_4^+ availability to autotrophic nitrifiers does not simply explain the high rates of autotrophic nitrification in soil from the clear cut site.

Barraclough and Puri (1995) used ^{15}N techniques and N-serve with an acid woodland soil to assess the relative importance of heterotrophic and autotrophic nitrification. They found that the rates of heterotrophic nitrification were low compared to autotrophic nitrification. The rates of heterotrophic nitrification measured in the English woodland soil were very similar to those we have measured in our California conifer soil (5 ug N kg^{-1} h^{-1} and 3 ug N kg^{-1} h^{-1}, respectively). Barraclough and Puri measured rates of total nitrification and found these rates to be about twice those found in our clear-cut conifer soil (0.1 mg kg^{-1} h^{-1} and 0.05 mg kg^{-1} h^{-1}, respectively).

Previous studies using Blodgett Forest soil have shown that the potential for heterotrophic nitrification in soil slurries was greater than that for autotrophic nitrification (Schimel et al. 1984). These findings support the results

of the present experiment showing that NO_3^- formation in the forested sites were primarily caused by the process of heterotrophic nitrification. Schimel et al. (1984), however found that NO_3^- formation in soil slurries from a clear cut area resulted largely from the process of heterotrophic nitrification. In contrast we found autotrophic nitrification to be the important process for NO_3^- formation in the clear cut area studied. As in the present experiment, Schimel et al. (1984) demonstrated that NH_4^+ was not an important substrate for heterotrophic nitrification, indicating that unidentified organic-N compounds were the substrates. Pure culture experiments have shown various nitrogen compounds act as substrates (Focht & Verstraete 1977; Castignetti & Hollocher 1984; Castignetti et al. 1985; Rho 1986; Stroo et al. 1986; Papen et al. 1989). There is no information on whether sufficient quantities of these compounds were present in the soil to support rates of heterotrophic nitrification.

In the lab studies reported here, net rates of nitrate accumulation were generally very similar to the gross rates of nitrate production. This indicates that immobilization of NO_3^- and denitrification were not important fates of NO_3^- in these samples. While the absence of detectable denitrification activity has been previously noted in these conifer soils (unpublished MS thesis), immobilization of NO_3^- has been previously reported as an important fate of NO_3^- in studies utilizing field assays of these forest soils (Davidson et al. 1992). We have however previously noted the absence of NO_3^- assimilation activity in samples from these conifer soils when soil samples were returned to the lab, stored for a short period of time, and mixed before assay (unpublished data). This apparent contradiction may reflect the transient nature of soil micosites with the high carbon availability and NH_4^+ depletion necessary for assimilatory NO_3^- consumption to occur.

Although measured using mixed soil samples in the laboratory, the rates of gross mineralization and nitrification reported here are comparable to the results of *in situ* experiments from the Blodgett Forest (Hart & Firestone 1989; Davidson et al. 1992). Schimel et al. (1989) reported that mixing soils slightly increased gross rates of NH_4^+ production over those measured using intact cores.

Heterotrophic nitrification – microbial ecology

While the group of microorganisms capable of performing the process of heterotrophic nitrification is diverse and includes both heterotrophic bacteria, fungi and actinomycetes (Eylar & Schmidt 1959; Hirsh et al. 1961; Castignetti & Hollocher 1984; Rho 1986; Stroo et al. 1986), fungi are generally considered to be the most numerous and efficient (Killham 1986). Studies of fungal and bacterial heterotrophs isolated from soil have found very low

percentages of the isolates capable of this process (Johnsrud 1978; Pennington & Elli 1993). These results indicate that the potential for heterotrophic nitrification among soil microorganisms is generally limited. However, in soil from the forested sites used in the present experiment, heterotrophic nitrification was the dominant process for NO_3^- formation.

It may well be that we do not know how or where to look for organisms capable of oxidizing organic-N. It has been suggested that the hydroxyl radical, an extremely potent oxidizing agent produced by white-rot fungi during lignin decomposition, may be a primary mechanism of heterotrophic nitrification particularly in forest soils (Wood 1986, 1990) This intriguing suggestion may partially explain the tremendous difficulties that soil microbiologists have encountered for the past 30 years in attempting to find the organisms involved and understand the biogeochemical significance of this phantom microbial process.

The mainly autotrophic nature of the nitrification process in many soils is generally accepted. However, since autotrophic nitrifying organisms seem to be sensitive to low pH values, it has been suggested that heterotrophic nitrification might be dominant in acid forest soils (Ishaque & Cornfield 1976; Adams 1986; Stroo et al. 1986; Duggin et al. 1991). Heterotrophic nitrification was observed in our conifer soils although the soils had pH values in the range commonly accepted for autotrophic nitrification (Alexander 1977). Heterotrophic nitrification is not simply restricted to acid soils.

Significance of heterotrophic nitrification in this conifer forest

In the mature forest mineral and organic soils, rates of heterotrophic nitrification were low, accounting for less than 5% of the gross rates of N-mineralization. While these rates of heterotrophic nitrification are low, they may be of significance over long time periods as the primary source of NO_3^- in the mature forest system. In soil from the clear cut area, nitrification rates were 33% of the rates of gross mineralization and were dominated by autotrophic processes. Thus the relative importance of autotrophic nitrification versus heterotrophic nitrification differed substantially between the clear cut area and the mature forest. The rates of heterotrophic nitrification were however, fairly constant in all the sites. Comparison of heterotrophic nitrification over time in different ecosystems under varying management regimes would allow the evaluation of the general importance of this process. The ^{15}N isotope pool dilution method, combined with the acetylene treatment will be a powerful tool for such studies.

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Appendix

Gross mineralization and gross nitrification were calculated as follows:

Case A, $d \neq i$

$$P(t) = P_0 + (d - i)t$$

$$\ln(R - {}^{15}n) = \ln(R_0 - {}^{15}n) - \left[\frac{d}{d-i}\right] \ln\left[\frac{(d-i)t + P_0}{P_0}\right]$$

where P_0 = initial $^{14+15}$N pool
$P(t)$ = $^{14+15}$N pool size at time t
d = gross N production
i = consumption
t = time
R = relative abundance of ^{15}N, $\frac{^{15}N}{(^{15}N + ^{14}N)}$
^{15}n = naturally occurring relative abundance of ^{15}N
R_0 = initial relative abundance of ^{15}N

where d was calculated from the slope $[d/(d - i)]$ of the plot of $\ln(R - {}^{15}n)$ against $\ln[((d - i)t + P_0)/P_0]$ and the net production $(d - i)$ which was estimated from the slope of the plot of $P(t)$ against t. The model is described in detail by Blackburn (1979).

Case B, $d = i$

$$H(t) = H_0 e^{-kt} \text{ then}$$

$$\%H(t) = 100e^{-kt} \text{ and } d = i = Pk$$

where H_0 = initial ^{15}N pool
$H(t)$ = ^{15}N pool at time t
$\%H(t)$ = % of H_0 remaining at time t
k = turnover rate constant
P = $^{14+15}$N pool

where values of d and i were estimated using the turnover rate constant $-k$ multiplied by the pool size (P). $-k$ is the slope of the semilogarithmic plot of percent remaining ^{15}N label versus time. The model is described in detail by Lund and Blackburn (1989).

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