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INFLUENCE OF RED ALDER ON SOIL NITROGEN TRANSFORMATIONS IN TWO CONIFER FORESTS OF CONTRASTING PRODUCTIVITY

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Summary—We conducted laboratory studies to determine the effects of red alder (*Alnus rubra* Bong.) on soil N transformations and N availability indices at two conifer forest sites of contrasting productivity. The inclusion of red alder in conifer forests significantly increased gross rates of N mineralization, N immobilization, nitrification and NO_3^- immobilization, and the effects of alder were generally similar for soils from low- and high-productivity sites. However, the addition of alder to the conifer stand at the high productivity site increased gross N mineralization and immobilization processes more than at the low productivity site. At both sites, gross N and NO_3^- production were enhanced by alder more than gross N immobilization processes, leading to higher rates of net N mineralization and nitrification. At the fertile site, most microbial N assimilation occurred from the NO_3^- pool, compared with less than half at the infertile site (none as NO_3^- in the less productive pure conifer stand). Heterotrophic nitrification (as indicated by a lack of C_2H_2 inhibition) accounted for 65–72% of the gross nitrification in all stands that exhibited nitrification (no nitrification was detected in the pure conifer stand at the infertile site). The inclusion of red alder had no effect on the proportion of total nitrification that was heterotrophic, despite the lower soil pH in mixed alder–conifer stands compared to conifer stands. Gross rates of N mineralization correlated well with both autotrophic and heterotrophic nitrification across all soils. Gross N mineralization may be a good index of NH_4^+ availability to autotrophic nitrifiers, as well as the quality of organic N as a substrate for heterotrophic nitrification. Most estimates of microbial biomass and activity, N availability and N transformation rates were significantly correlated with each other. In general, gross N transformations were better correlated with other indices of N availability and microbial activity than estimates of net N transformations. Similar N cycling rates and microbial biomass N pool sizes in pure alder and adjacent alder–conifer stands at the fertile site suggest that continued inputs of N via symbiotic N-fixation by red alder in coniferous forest stands can lead to the elimination of N-limitation to forest ecosystem production. © 1997 Elsevier Science Ltd

INTRODUCTION

The inclusion of red alder (*Alnus rubra* Bong.) in conifer forests of the Pacific Northwest increases soil total N capital and N availability. On N-limited sites, ecosystem production increases, but more fertile sites may show no change in net primary production, and soil pH may decrease with increased NO_3^- leaching (Binkley and Sollins, 1990; Binkley *et al.*, 1992b). The mechanism by which alder enhances N availability in infertile, coniferous forest sites is linked ultimately to the capacity of alder to symbiotically fix atmospheric N. Reported N-fixation rates for red alder in the Pacific Northwest range from about 50 to 200 kg ha^{-1} y^{-1} (Franklin *et al.*, 1968; Cole *et al.*, 1978; DeBell and Radwan, 1979; Binkley, 1981; Binkley *et al.*, 1992b). Nitrogen fixation increases soil N capital typically by 20–50%, but rates of N turnover increase by several-fold (Binkley *et al.*, 1992a, b). Alder leaves and roots have higher N and lower lignin concentrations than conifer litter (Edmonds, 1980; Harmon *et al.*, 1990), which improves the substrate quality of the soil organic matter (Van Miegroet *et al.*, 1992) and leads to greater rates of N turnover (Clein and Schimel, 1995).

In previous investigations of the effect of red alder on soil N status, N availability has usually been assessed using net changes in soil total inorganic-N pool size (net N mineralization) and soil NO_3^- pool size (net nitrification) during laboratory or *in-situ* incubations. These net measurements confound two or more N cycling processes that occur concurrently (Hart *et al.*, 1994a,b). For instance, higher net rates of N mineralization and nitrification may be due to higher gross (actual) N mineralization and nitrification rates, lower rates of microbial assimilation (immobilization) of NH_4^+

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and NO_3^-, or both. The degree to which alder affects specific N transformations and how these effects differ with site fertility are unknown.

High rates of net nitrification in sites containing red alder with soil pH below 5.0 are surprising, given the substantial negative effect that low pH has on autotrophic nitrifiers in non-forest soils (Schmidt and Belser, 1982). High net nitrification rates in low pH soils have been suggested as potential evidence of heterotrophic nitrification, which is less affected by low pH (Focht and Verstraete, 1977; Schmidt, 1982).

The use of selective biochemical blocks (such as acetylene, chlorate and nitrapyrin, which inhibit autotrophic nitrification) has provided a means of separating autotrophic from heterotrophic nitrification pathways. Several investigators of acid forest soils utilizing this approach have concluded that heterotrophic nitrification was the dominant pathway of NO_3^- production (Schimel *et al.*, 1984; Killham, 1986; Duggin *et al.*, 1991; Klingensmith and Van Cleve, 1993). However, other researchers have also found that the heterotrophic nitrification pathway was insignificant in other acidic forest soils (De Boer *et al.*, 1989, 1991, 1992; Tietema *et al.*, 1992; Pennington and Ellis, 1993). Investigators involved in these latter studies hypothesized that acid-tolerant or even acidophilic autotrophic bacteria oxidize NH_4^+ at low soil pH.

Barraclough and Puri (1995) developed a ^{15}N pool dilution–enrichment approach to separate autotrophic and heterotrophic nitrification pathways directly without relying on biochemical blocks. Using this technique, they found that heterotrophic nitrification contributed ≤8% of the total gross nitrification rate in an acid woodland soil. This was also the first study to assess the relative gross rather than net rates of nitrification via the two pathways. The assessment of gross nitrification rates is especially important when utilizing selective biochemical blocks in order to separate changes in NO_3^- production after the addition of a selective biochemical block from changes in NO_3^- consumption (Hart *et al.*, 1994a).

We conducted laboratory studies to determine the effects of red alder on soil N transformations and N availability indices in soils from two conifer forest sites of contrasting productivity. Our objectives were: (1) to assess the effect of red alder on net rates of N mineralization and nitrification and other measures of N availability; (2) to determine which N transformation process is responsible for any observed differences in net rates; (3) to estimate heterotrophic nitrification rates using measured rates of gross nitrification in the presence of a selective biochemical block of the autotrophic nitrification pathway; and (4) to assess the effects of red alder and inherent site fertility on gross rates of heterotrophic and autotrophic nitrification.

MATERIALS AND METHODS

Study sites

Both study sites are U.S. Department of Agriculture Forest Service Experimental Forests, and are described in detail in Binkley *et al.* (1992b). The low productivity site at the Wind River Experimental Forest in southwestern Washington (45°49′ N) is at 625 m elevation with 2500 mm y^{-1} of precipitation (about 75% falling as snow between November and March). The mean annual air temperature is about 9°C. The soil at this site is an unclassified Andic Haplumbrept with silty clay loam surface texture. The stand was established by planting 2-y-old Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings in 1929 at a density of 1700 trees ha^{-1} after successive wildfires in 1902, 1922 and 1927. In 1933, a 22-m wide strip was interplanted with 2-y-old red alder seedlings at a density of 3000 trees ha^{-1}, to provide a fuelbreak across the plantation. The site index (expected height of canopy-dominant tree species) for Douglas-fir in the absence of red alder is 25 m at 50 y.

The high productivity site is the Cascade Head Experimental Forest, near the coast of Oregon (45°03′N) at an elevation of 180 m. Precipitation averages 2400 mm y^{-1} with little in the form of snow. The mean annual air temperature is 15°C. The soil is classified as a member of the well-drained Astoria silty clay loam series (Typic Dystrandept). The land was farmed and then abandoned in 1925. By 1935, an 8-y-old naturally regenerated mixed stand contained Douglas-fir, western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), Sitka spruce (*Picea sitchensis* (Bong.) Carr.) and red alder. At this time, conifer density was about 4500 trees ha^{-1} and alder density 3000 trees ha^{-1}. Between 1935 and 1936, three plots were established: a 0.4-ha alder–conifer unthinned plot; a 0.2-ha pure conifer plot, where the stand density was reduced to about 2800 trees ha^{-1} by removing all the alders and many of the smaller conifers; and a 0.2-ha pure alder plot where the stand density was reduced to about 1800 trees ha^{-1} by removing all conifers and some of the alder to create a 2.4 × 2.4 m spacing between remaining alder trees (Berntsen, 1961). The site index at Cascade Head for Douglas-fir in the absence of red alder is 40 m at 50 y.

Soil sampling and incubation

Within each stand (i.e. conifer and alder–conifer mixture at Wind River; conifer, alder–conifer mixture, and alder at Cascade Head) a 40-m transect was located using a randomly selected direction and starting point. Mineral soil was sampled (0–15 cm depth) every 10 m along these transects, giving five replicate samples per stand. Soil was sampled on

the 17th (Wind River) and 18th (Cascade Head) of November 1990 at both sites, stored in sealed polyethylene bags to maintain field wetness, and transported in a cooler on ice back to Oregon State University. Soil samples were kept in a cold room at 2°C for 1 week, then sieved (4-mm mesh), mixed, and returned to the cold room for 3 days before initiating the experiment. The 3-day period in cold storage was intended to reduce the effect of soil mixing on N transformation and availability assays (Hart *et al.*, 1994a).

From each soil sample, eight subsamples (approximately 10-g oven-dry weight equivalent) were weighed into 20-ml scintillation vials: five were used for measuring gross N transformation rates (see below); one was incubated for 10 days at 20 ± 1°C (aerobic incubation subsample); one was fumigated with ethanol-free $CHCl_3$ for 24 h and then incubated for 10 days at 20 ± 1°C after removing the $CHCl_3$ vapor (chloroform fumigation–incubation subsample); and one was extracted immediately with 50 ml of 2 *M* KCl (initial inorganic-N pool size subsample). One additional subsample was placed in a 120-ml specimen container, waterlogged by adding 25 ml deionized water, and incubated at 40 ± 1°C for 7 days (anaerobic incubation subsample). Subsamples used for aerobic incubation, chloroform fumigation–incubation and initial inorganic-N pool sizes received 0.6 ml of deionized water via a needle and syringe immediately after weighing to provide the subsamples with the same water content as those receiving ^{15}N solutions for gross N transformation rate measurements (see below).

Vials containing soils used for aerobic incubation and chloroform fumigation–incubation were placed within 975-ml Mason jars and sealed with air-tight lids fitted with a butyl rubber septum. About 30 ml of deionized water were placed in a 120-ml specimen container within each Mason jar to maintain soil wetness (Hart *et al.*, 1994a).

Carbon dioxide concentrations were determined initially and after 10 days in the headspace of Mason jars containing the aerobic incubation and chloroform fumigation–incubation subsamples by sampling the headspace gas with a 1-ml syringe. The headspace of each Mason jar was mixed repeatedly using a 60-ml syringe prior to taking gas samples. Headspace gas subsamples were introduced into a Carle AGC Series 100 isothermal gas chromatograph fitted with a thermal conductivity detector (EG&G Chandler Engineering, Broken Arrow, OK, U.S.A.). Carbon dioxide evolution from soil (microbial respiration) was calculated from increases in headspace CO_2 concentrations during the 10-day incubation.

After sampling the headspace, aerobic incubation and chloroform fumigation–incubation subsamples were extracted with 50 ml of 2 *M* KCl. Net N mineralization rates were calculated for each soil by subtracting initial total inorganic-N pool sizes from total inorganic-N pool sizes determined after 10 days of aerobic incubation. Net nitrification rates were calculated by subtracting initial NO_3^- pool sizes from post-incubation NO_3^- pool sizes (Binkley and Hart, 1989).

Microbial biomass C was calculated by dividing the CO_2-C evolved from the chloroform fumigated incubation subsample (C_F) by 0.41 (Voroney and Paul, 1984). Microbial biomass N was calculated by dividing the net accumulation of NH_4^+-N in the fumigated sample during the incubation (N_F) by a value k_N, determined using the equation (Paul and Clark, 1989):

$$k_N = 0.8 \times (C_F/N_F)^{-0.43}.$$

Anaerobic incubation subsamples were extracted with 25 ml of 4 *M* KCl after the 7-day incubation. Anaerobically-mineralizable N was calculated by subtracting the initial NH_4^+ pool size from the post-incubation NH_4^+ pool size (Binkley and Hart, 1989).

Soil total C and N concentrations had been determined in the conifer and alder–conifer stands at both sites (Binkley and Sollins, 1990; Binkley *et al.*, 1992b). We measured soil total C and N concentrations in the Cascade Head alder stand using a LECO 12 C analyzer (LECO Corp., St. Joseph, MI, U.S.A.) and a microKjeldahl digestion (Bremner and Mulvaney, 1982) followed by NH_4^+ analysis (see below), respectively.

Estimation of gross rates of soil N transformations

Gross rates of N mineralization and nitrification were determined using ^{15}N isotope dilution methodology (Hart *et al.*, 1994a,b). Two soil subsamples were labeled with ^{15}N by adding 0.6 ml of a solution containing 10 mg NH_4^+-N l^{-1} (^{15}N enrichment of 99%). Three soil subsamples were labeled in a similar manner with 0.6 ml of a solution containing 10 mg NO_3^-N l^{-1} (also 99% ^{15}N enrichment). Solutions were added to the soil by numerous small-volume injections with a needle and syringe (Hart *et al.*, 1994a). Immediately after ^{15}N labeling (within 0.25 h), one $^{15}NH_4^+$-amended and one $^{15}NO_3^-$-amended subsample were extracted with 50 ml of 2 *M* KCl. These samples were used to determine the initial quantities of ^{15}N and ^{14}N in NH_4^+ and NO_3^- pools, respectively. The other two samples were each placed in a Mason jar similar to those used for the aerobic and chloroform-fumigation incubations. Acetone-free tank C_2H_2 was added through a septum in the lid to one of the Mason jars containing a $^{15}NO_3^-$-amended sample using a needle and a 60-ml syringe. Enough C_2H_2 was added to create a 10 kPa C_2H_2 atmosphere in the headspace of the incubation chamber. This partial pressure of C_2H_2 is over 1000 times the amount

that has been shown to completely inhibit autotrophic nitrification even when amended with NH_4^+ (Berg *et al.*, 1982). The C_2H_2 was mixed within the Mason jar by repeated withdrawals and injections of headspace gas using the needle and syringe. The Mason jars were then kept in the dark at 20°C. After 24 h, the soil subsamples were removed and extracted with 50 ml of 2 *M* KCl.

Gross N mineralization was calculated from changes in the $^{15}NH_4^+$ and $^{14+15}NH_4^+$ pool sizes in $^{15}NH_4^+$-amended soils during a 1-day incubation using the equations of Kirkham and Bartholomew (1954). Gross (autotrophic + heterotrophic) nitrification was calculated with the same equation except using changes in the $^{15}NO_3^-$ and $^{14+15}NO_3^-$ pool sizes in $^{15}NO_3^-$-amended soils that did not receive C_2H_2. Gross heterotrophic nitrification was calculated in a similar manner except that $^{15}NO_3^-$-amended soils that received C_2H_2 were used.

Gross rates of total inorganic N and NO_3^- immobilization were determined by subtracting the respective average daily net N mineralization and nitrification rates, determined from the 10-day aerobic incubation subsamples from the gross rates determined during a 1-day incubation. This difference method was used (Hart *et al.*, 1994a) because the isotope dilution method requires the addition of inorganic N in order to estimate gross rates of N immobilization, which may enhance N assimilation (Davidson *et al.*, 1991; Hart *et al.*, 1994b).

Inorganic N and ^{15}N analyses

All KCl-soil suspensions were shaken for 1 h on a mechanical shaker, and then filtered through a Whatman No. 40 filter paper that had been leached with approximately 50 ml of 2 *M* KCl to remove any NH_4^+ and NO_3^-. Ammonium (phenolate-nitroprusside; Keeney and Nelson, 1982) and NO_3^- (diaotization following cadmium reduction; Keeney and Nelson, 1982) were determined using an Alpkem RFA 300 Rapid Flow Analyzer (Clackamas, OR, U.S.A.).

Filtered extracts of ^{15}N-amended soils were prepared for ^{15}N isotopic analysis using the diffusion procedure described in Brooks *et al.* (1989). Atom percentage ^{15}N enrichments were determined on a Europa Scientific Automated Nitrogen–Carbon Analyzer–Mass Spectrometer (Cincinnati, OH, U.S.A.). In addition, initial KCl extracts from unamended soil subsamples were prepared and analyzed for their ^{15}N enrichments to determine background $^{15}NH_4^+$ and $^{15}NO_3^-$ pools in these soils; background ^{15}N enrichments are required for determining ^{15}N excesses of N pools used in the isotope dilution equations of Kirkham and Bartholomew (1954).

Gravimetric soil water contents of each soil were determined from a separate subsample (approximately 30-g fresh weight) oven-dried at 105°C for 48 h. All soil data are expressed on an oven-dry weight basis.

Statistical analyses

Pearson product-moment correlation coefficients were used to assess relationships among N transformation rate and N availability assays across stands. Least-squared linear regression was used to test for a relationship between gross N mineralization and gross nitrification across stands.

Fixed effects, two-factor analyses of variance (ANOVAs) were used to test for significant differences in N transformation rate and N availability assays between conifer and conifer–alder stands at high- (Cascade Head) and low-productivity (Wind River) sites. Factors in the ANOVA were standtype, site and their interaction. The alder stand at Cascade Head was not included in these analyses because no comparable stand was present at the Wind River site. All data were log-transformed to meet the normality and homogeneity of variances criteria for ANOVA.

A one-factor ANOVA was performed on the ratios of gross nitrification rate with C_2H_2 to gross nitrification rate without C_2H_2 to test for differences in the relative proportion of heterotrophic to total nitrification among stands. These proportionate data were transformed using the arcsine function to normalize the data prior to statistical analysis.

All presented means and standard errors are arithmetic, not back-transformed means and standard errors. Statistical analyses were performed using Statgraphics software (STSC, Rockville, MD, U.S.A.).

RESULTS

Soils from all stands were acidic (Table 1). Soil pH_{water} decreased with the inclusion of alder at both sites, but the relative decrease was much greater at Cascade Head. The alder soil at Cascade Head had the lowest pH_{water} (3.9). Differences in soil pH measured in 10 m*M* $CaCl_2$ among stands were less pronounced, but showed a pattern similar to pH measured in water (Table 1). The alder–conifer and alder soils at Cascade Head had the lowest pH_{salt} (3.7).

Total N in forest soils increased in the order: Wind River conifer, Wind River alder–conifer, Cascade Head conifer, Cascade Head alder–conifer and Cascade Head alder (Table 1). Soil total C increased across stands in a similar order, except that total C values in conifer and alder–conifer stands at Cascade Head were similar. Soil C-to-N ratios increased across stands in the reverse order as total N. Soil C-to-N ratios ranged from 47.6 in the Wind River conifer stand to 16.4 in the Cascade Head alder stand.

Table 1. Soil pH, total and microbial C and N, CO_2 evolution and anaerobically mineralizable N in the upper 15 cm of mineral soil from adjacent alder, conifer and alder–conifer forest stands[a]

Site/stand[b]	pH		Total			Microbial			
	H_2O[c]	Salt[c]	C[c]	N[d]	C:N	C	N	CO_2-C evolved[e]	Anaerobically mineralizable N
			g kg^{-1} soil					mg kg^{-1} soil	
WC	5.4	4.3	43.8	0.92	47.6	752	47.9	26.9	12.3
	(0.1)	(0.1)	(3.6)	(0.08)	(5.7)	(72)	(9.9)	(3.4)	(1.5)
WM	5.1	4.3	67.0	2.33	28.7	1500	235	86.6	64.7
	(0.3)	(0.2)	(9.0)	(0.23)	(4.8)	(74)	(17)	(21.3)	(11.7)
CC	5.4	4.4	118	4.33	27.1	1681	332	36.3	109
	(0.4)	(0.4)	(9)	(0.28)	(2.7)	(222)	(50)	(3.9)	(22)
CM	4.3	3.7	118	6.70	17.6	2264	444	93.3	107
	(0.4)	(0.3)	(8)	(0.88)	(2.6)	(324)	(62)	(28.1)	(21)
CA	3.9	3.7	160	9.74	16.4	2172	419	57.7	96.8
	(0.1)	(0.1)	(13)	(0.87)	(2.0)	(244)	(43)	(9.6)	(9.6)

[a]Mean and (standard error); $n = 5$ except where noted.
[b]WC = Wind River conifer; WM = Wind River alder–conifer mixture; CC = Cascade Head conifer; CM = Cascade Head alder–conifer mixture; CA = Cascade Head alder.
[c]Data from Binkley and Sollins (1990) ($n = 10$), except for CA, which were determined in this study.
[d]Data from Binkley *et al.* (1992b) ($n = 10$), except for CA, which were determined in this study.
[e]CO_2-C evolved during a 10-day aerobic laboratory incubation (see text).

Microbial C and N generally increased with soil total C and N (Table 1). An exception to this pattern occurred at Cascade Head, where microbial C and N values were similar between alder and alder–conifer soils, but total C and N were both substantially higher in the alder soil.

Within a given site, CO_2 evolution during the 10-day aerobic incubation was higher from alder–conifer soils than conifer soils (Table 1). However, there was little difference in CO_2 evolution between high and low productivity sites for a given stand-type. The alder soil at Cascade Head had lower CO_2 evolution than the alder–conifer soil.

At the Wind River site, anaerobically mineralizable N was substantially higher in the alder–conifer soil than in the conifer soil (Table 1). In contrast, soils from all stands at Cascade Head had similar anaerobically-mineralizable N values, and the values were greater than at Wind River.

Gross N mineralization and nitrification (in the absence of C_2H_2) rates were lowest in the Wind River conifer soil, intermediate in the Wind River alder–conifer and Cascade Head conifer soils, and highest in the Cascade Head alder–conifer and alder soils [Fig. 1(a)]. Gross nitrification did not differ from zero in the Wind River conifer soil (one-tailed t-test, $P > 0.10$).

Gross rates NO_3^- and total ($NH_4^+ + NO_3^-$) N immobilization rates followed a pattern across stands similar to gross N mineralization and nitrification, except that gross N immobilization was lower at the Cascade Head alder soil compared to the alder–conifer soil [Fig. 1(b)]. Gross NO_3^- immobilization did not differ from zero in the Wind River conifer forest. Nitrogen was immobilized primarily from the NH_4^+ pool in both stands at Wind River; however, N immobilization in all stands at Cascade Head was almost exclusively from the NO_3^- pool.

In general, net rates of N mineralization and nitrification followed a pattern among stands similar to gross rates [Fig. 1(c)]. However, net N mineralization and nitrification rates were substantially higher in the alder soil than in the alder–conifer soil at Cascade Head, whereas there were no differences between these two soils in gross N transformation rates [Fig. 1(a)]. Net N mineralization and net nitrification were not significantly different from zero in the Wind River conifer soil, and net nitrification was not significantly different from zero in the Wind River alder–conifer soil [Fig. 1(c)].

After combining the results from all soil samples ($n = 25$), we found several significant correlations among soil N transformations and N availability assays (Table 2). Microbial biomass C and N correlated highly with anaerobically mineralizable N ($r = 0.84$ and 0.90, respectively). Gross N mineralization and nitrification correlated with their respective net rates, but the correlations were weaker ($r = 0.67$ for N mineralization and $r = 0.44$ for nitrification). Carbon dioxide evolution correlated with gross rates of N mineralization and immobilization, and with microbial biomass C and N; however, CO_2 evolution did not correlate with net N transformations, nor with gross transformations involving NO_3^-.

We found a strong linear relationship between gross N mineralization and gross nitrification ($r = 0.92$, $P < 0.01$; Fig. 2). A similar strong correlation ($r = 0.85$, $P < 0.01$) was found between gross N mineralization and gross nitrification in the presence of C_2H_2 (gross heterotrophic nitrification; data not shown).

The inclusion of red alder significantly increased ($P < 0.10$) all measured gross and net N transformations (Table 3). These increases in N transformations were similar for both the high (Cascade Head) and low (Wind River) productivity sites, except for rates of gross N mineralization and im-

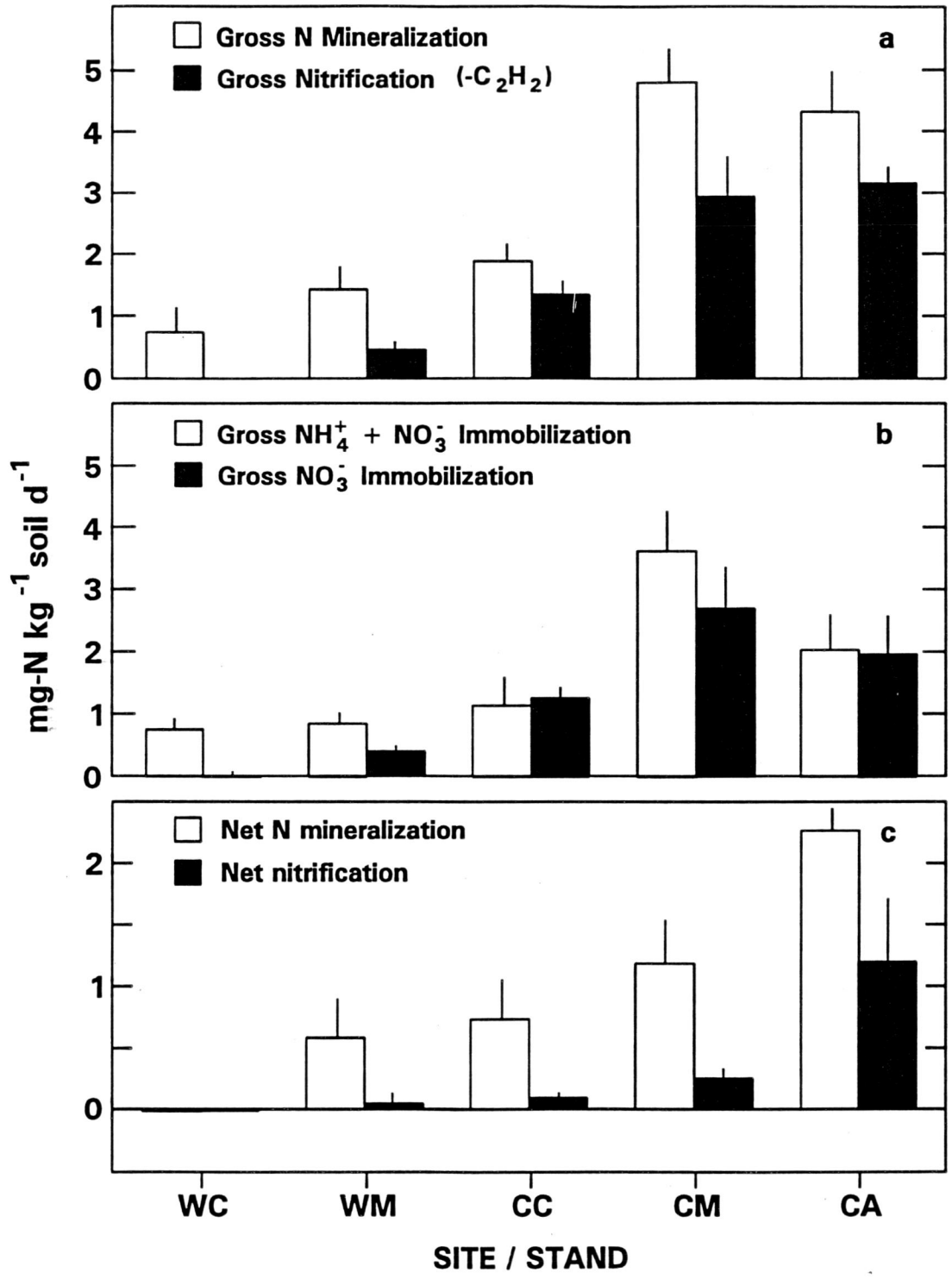

Fig. 1. Calculated rates of gross N mineralization and nitrification (a), gross total N (NH_4^+ + NO_3^-) and NO_3^- immobilization (b), and net N mineralization and nitrification (c) in soils from low (Wind River) and high productivity (Cascade Head) coniferous forest sites. Note that the gross NH_4^+ immobilization rate is the difference between total N immobilization and NO_3^- immobilization rates shown in (b). Legend: WC = Wind River conifer stand; WM = Wind River alder–conifer mixed stand; CC = Cascade Head conifer stand; CM = Cascade Head alder–conifer mixed stand; CA = Cascade Head alder stand. Vertical bar is one standard error of the mean (n = 5).

Table 2. Pearson product-moment correlation coefficients (r) among soil N transformations, CO_2 evolution, microbial biomass and anaerobically mineralizable N

	GNM	GN	GHN	GNI	GNITI	NNM	NN	MBN	MBC	CO_2E
GN	0.92***									
GHN	0.89***	0.91***								
GNI	0.87***	0.74***	0.70***							
GNITI	0.87***	0.89***	0.47*	0.84***						
NNM	0.67***	0.69***	0.71***	0.21	0.46**					
NN	0.30	0.44**	0.28	−0.02	−0.02	0.61**				
MBN	0.81***	0.80***	0.79***	0.70***	0.71***	0.54**	0.34			
MBC	0.82***	0.78***	0.78***	0.73***	0.74***	0.51**	0.27	0.97***		
CO_2E	0.40**	0.31	0.24	0.47**	0.34	0.08	0.01	0.53**	0.61**	
AMN	0.57**	0.55**	0.57**	0.45*	0.48*	0.44*	0.27	0.90***	0.84***	0.43*

The correlation matrix was generated using individual soil samples taken from all forest stands combined (n = 25). GNM = gross N mineralization; GN = gross nitrification; GHN = gross heterotrophic nitrification; GNI = gross N immobilization; GNITI = gross NO_3^- immobilization; NNM = net N mineralization; NN = net nitrification; MBN = microbial biomass N; MBC = microbial biomass C; CO_2E = CO_2 evolution; AMN = anaerobically mineralizable N.
*, ** and *** indicate significance at the 0.05, 0.01 and 0.001 probability levels, respectively.

mobilization, which were greater when alder was included with conifers at the high productivity site (significant stand × site interactions, P = 0.0069 and 0.0107, respectively; Table 3). All gross and net N transformation rates were significantly higher ($P < 0.05$) at Cascade Head compared to Wind River (Table 3).

From 65 to 72% of total gross nitrification occurring in all soils (except the Wind River conifer forest, which exhibited no detectable nitrification) was

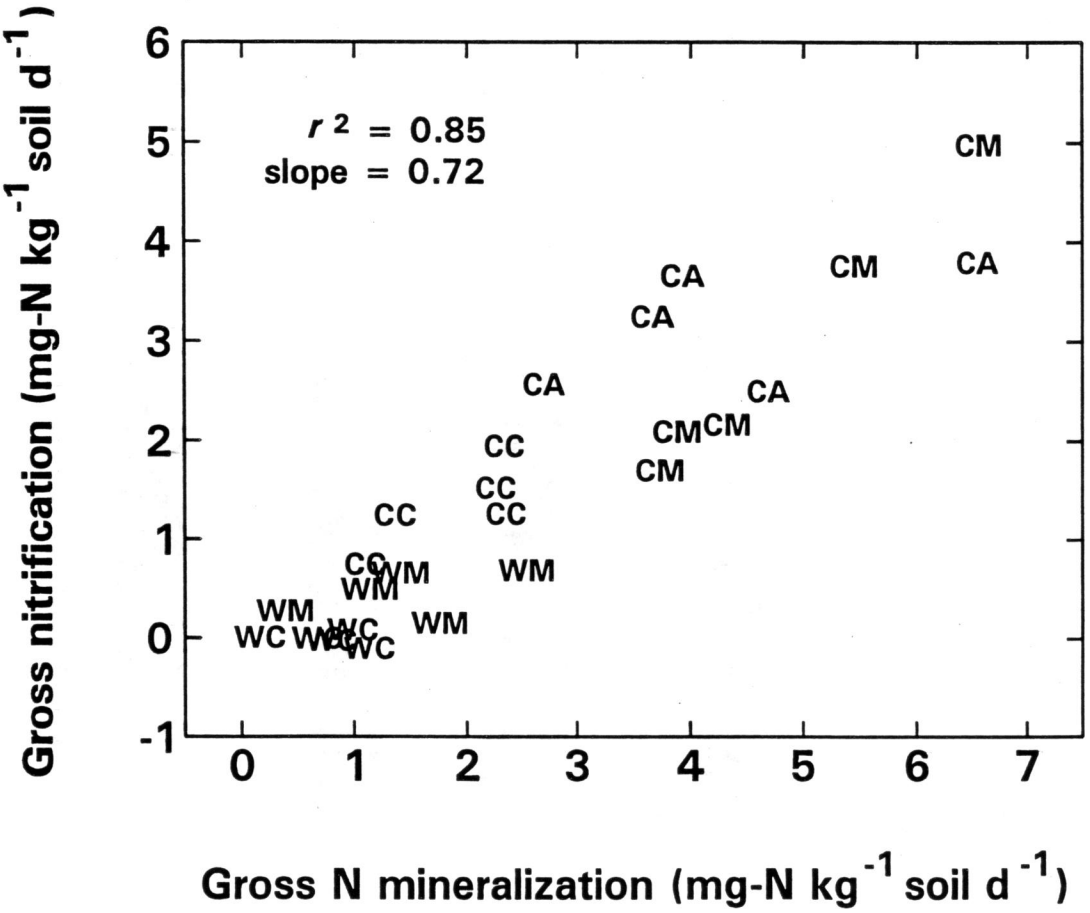

Fig. 2. Relationship between gross N mineralization and gross nitrification rates determined in soil samples taken from low (Wind River) and high (Cascade Head) productivity coniferous forest sites. Plotted symbols denote the stand origin of the assayed soils (see Fig. 1 for key to symbols).

Table 3. Probability values for the effect of stand-type (conifer or alder–conifer) and site (low or high productivity) on soil N transformations determined by fixed effects, two-factor analyses of variance (ANOVAs)[a]

Effect	df	Gross N mineralization	Net N mineralization	Gross N immobilization	Gross nitrification	Net nitrification	Nitrate immobilization	Gross heterotrophic nitrification
Stand	1	0.0001	0.0863	0.0067	0.0004	0.0913	0.0170	0.0016
Site	1	< 0.0001	0.0306	0.0015	< 0.0001	0.0058	0.0001	< 0.0001
Stand × site	3	0.0069	0.7923	0.0107	0.5590	0.4718	0.1522	0.6043

[a]The alder site at Cascade Head (CA) was not included in the analysis; all data were log-transformed prior to conducting the ANOVAs.

unaffected by C_2H_2 (Fig. 3). The proportion of the total nitrification rate unaffected by C_2H_2 did not differ among soils ($P = 0.57$).

DISCUSSION

Relationships among gross and net N transformations and N availability assays

Most estimates of microbial biomass C and N, microbial activity, N availability and N transformations correlated with each other. Many other forest studies have found similar high correlations ($r = 0.6$–0.8) between anaerobic and aerobic incubation assays of N availability conducted under laboratory conditions (Binkley and Hart, 1989). Myrold (1987) and Myrold *et al.* (1989) also found strong correlations between anaerobically-mineralizable N and microbial C and N determined using the chloroform fumigation–incubation method for soils across a wide range of forest types in Oregon ($r = 0.78$ and 0.85, respectively). Similar correlations between microbial biomass C and N and tests of N availability have been found for agricultural soils (Carter and Rennie, 1982) and other forest soils (Adams, 1986b).

In general, we found that gross rates of N transformations correlated better with other indices of N availability and microbial activity than net N transformation rates. Hart *et al.* (1994a) also found no significant correlation between net N mineralization

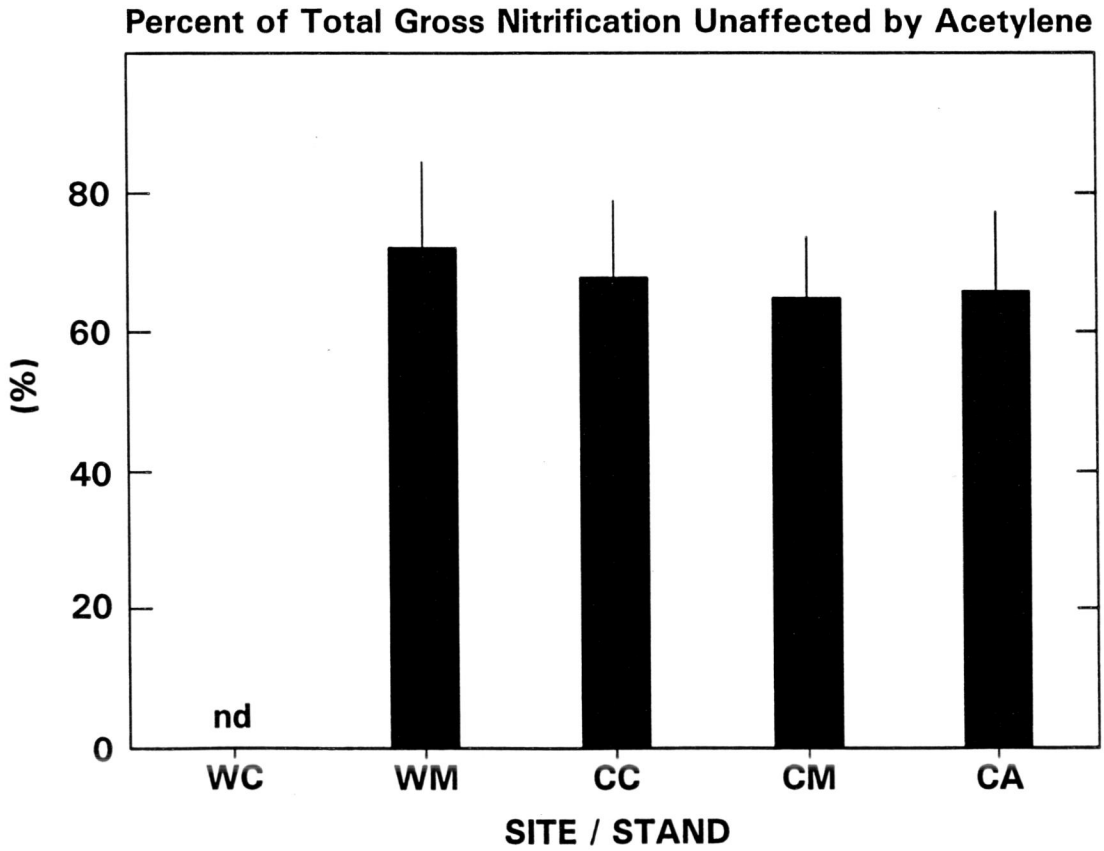

Fig. 3. Percentage of total gross nitrification that was unaffected by acetylene in soils from low (Wind River) and high (Cascade Head) productivity coniferous forest sites (see Fig. 1 for key to legend). Vertical bar is one standard error of the mean ($n = 5$). No value is shown for the Wind River conifer stand because gross nitrification was not detected.

and microbial respiration for an old-growth forest soil in the Oregon Cascades during long-term laboratory incubation, but did find a strong correlation between gross N mineralization rates and microbial respiration ($r = 0.99$). Other investigators have shown that microbial respiration is typically not well correlated with net N mineralization during laboratory incubations of soil for ≤2 months (Johnson and Edwards, 1979; Johnson *et al.*, 1980; Burke *et al.*, 1989). Higher correlations between estimates of microbial biomass and activity and gross compared to net N transformations are not surprising because net N transformation rates are measures of the end result of two or more concurrent processes. If the factors controlling individual N transformations are different, simple relationships between the controlling factors (i.e. microbial activity) and N process rates likely will be obscured (Hart *et al.*, 1994a).

In our study, gross and net soil N transformation rates across stands generally increased as the soil C-to-N ratio declined. In soils with a C-to-N ratio >29, net N mineralization did not occur during a 10-day aerobic incubation, and gross nitrification and NO_3^- immobilization rates were insignificant. These results are in contrast with some previous studies that have shown the soil total C-to-N to be a poor indicator of N cycling rates (Robertson, 1982; Van Miegroet *et al.*, 1990). However, our results are consistent with those of Heilman (1974), who found that unfertilized soils with total C-to-N ratios >27 showed little net nitrification.

Effect of red alder on soil N transformations

Our results are similar to previous studies showing that the inclusion of red alder in conifer forests increases net N transformation rates in soil (Binkley *et al.*, 1992a). However, our application of ^{15}N isotope dilution allowed us to determine the individual N transformation processes altered by the inclusion of red alder resulting in greater net N production. The assessments of gross N transformation rates clearly indicate that the inclusion of red alder causes a general increase in all soil N transformations. Higher rates of net N mineralization and nitrification in sites containing alder resulted from the gross production of N and NO_3^- being enhanced slightly more than gross N and NO_3^- immobilization processes, respectively. Binkley *et al.* (1992b) also showed that other N transformations, such as N leaching and denitrification, increase with the inclusion of alder in conifer stands at these sites.

In general, all N transformation rates were enhanced in sites of both low and high productivity. However, the addition of alder to the conifer stand at the high productivity site increased gross N mineralization and immobilization processes more than at the low productivity site. This suggests that factors other than N, such as phosphorus availability, may limit the response of the soil N cycle to alder addition at the low productivity site. This conclusion would not have been reached if only net rates of N transformations were assessed.

Our estimates of N availability are similar to those found for these sites by Binkley *et al.* (1992a) who sampled soils 5 y before our study. Anaerobic incubations conducted by Binkley *et al.* (1992a) also showed a substantial increase in available N in the alder–conifer stand compared to the conifer stand at Wind River, but very little effect of alder inclusion on N availability at Cascade Head. Furthermore, 30-day aerobic incubations conducted by Binkley *et al.* (1992a) showed a similar pattern among sites to our 10-day aerobic incubation. In both studies, little net N mineralization and net nitrification occurred in the Wind River conifer soil, but a substantial increase in both net N transformations occurred with the inclusion of alder. At Cascade Head, both studies also showed that the inclusion of alder had a much smaller effect on net N transformations in conifer soil than at Wind River.

Even though soil total N was substantially greater in the alder stand compared to the adjacent alder–conifer stand at the fertile Cascade Head site, most measures of gross N cycling rates, N availability and microbial activity were similar between the two stands. Furthermore, rates of microbial respiration and N immobilization were lower in the alder stand than in the alder–conifer stand, but rates of gross N mineralization were similar, resulting in greater rates of net N production in the alder stand. Based on these observations, we hypothesize that the trees and soil microflora within the Cascade Head alder stand are not limited by N. Unfortunately, previous N cycling work at Cascade Head has not studied all three stand-types; we expect that rates of N loss via leaching and denitrification from the alder stand greatly exceed rates found for the conifer and alder–conifer stands.

The substantial rates of microbial NO_3^- immobilization for stands (except the extremely infertile Wind River conifer stand) are consistent with research showing high rates of microbial NO_3^- assimilation in conifer forests (Davidson *et al.*, 1992; Hart *et al.*, 1994a; Stark and Hart, 1997). Nitrate immobilization correlated highly with NO_3^- production ($r = 0.89$), as found in these previous studies. Across the wide range in soil fertility in this study, gross NO_3^- immobilization correlated with gross N mineralization ($r = 0.87$). These results suggest that the rate of N turnover in forest soils partially controls the rate of gross nitrification, which, in turn, limits microbial assimilation of NO_3^-. Because the inclusion of alder increased all soil N cycling rates together, the occurrence of alder in conifer stands increased the amount of NO_3^- assimilated by the soil

microflora. The lack of measurable soil NO_3^- pools and gross NO_3^- production at the Wind River conifer stand resulted in no microbial assimilation of NO_3^-. This lack of gross N flux through the NO_3^- pool is indicative of an extremely nutrient-poor soil.

Autotrophic vs. heterotrophic nitrification

Most of the nitrification occurring in soil is generally thought to be carried out by autotrophic (chemolithotrophic) bacteria that oxidize NH_4^+ to NO_3^- (Paul and Clark, 1989). Acetylene is a particularly useful selective biochemical block of the autotrophic pathway (Berg *et al.*, 1982; Hynes and Knowles, 1982), which does not inhibit the heterotrophic pathway (Hynes and Knowles, 1982; Schimel *et al.*, 1984). Heterotrophic (chemoorganotrophic) nitrification, carried out by fungi using organic substrates, is believed to be the dominant source for C_2H_2-insensitive NO_3^- production (Stroo *et al.*, 1986).

The occurrence of net NO_3^- production in soils with a pH below that which has completely inhibited autotrophic nitrification in laboratory pure culture studies (about 5.8) has led to speculation that heterotrophic nitrification may be the dominant pathway of NO_3^- production in acidic forest soils (Killham, 1986). Based on this premise, we hypothesized that some of the gross nitrification observed in soils from all stands would be heterotrophic because of the moderate to strong acidity of these soils ($pH_{water} \leq 5.4$). In addition, we hypothesized that the inclusion of alder in conifer stands from fertile but not infertile sites would result in a greater proportion of heterotrophic than autotrophic nitrification, because inclusion of alder reduced soil pH at the fertile site (Binkley and Sollins, 1990). The former, but not the latter, hypothesis was supported by our results; heterotrophic nitrification was a constant proportion of total gross nitrification among all stands where nitrification was observed. Heterotrophic nitrification rates increased in direct proportion to total nitrification. Killham (1986) speculated that the N form and N mineralization rate may play a role in determining the relative dominance of nitrification pathways in soil. However, we found that gross and net N mineralization rates did not correlate with the relative amount of autotrophic vs. heterotrophic nitrification. Our results are consistent with the conclusion of Killham (1986) that pH does not control the relative extent of the two nitrification pathways in acidic soil.

Because we exposed the soil to C_2H_2 after adding the $^{15}NO_3^-$ used to assess gross nitrification rates, it is possible that some autotrophic nitrification had occurred before the C_2H_2 completely inhibited the autotrophic pathway. Any autotrophic nitrification occurring prior to the C_2H_2 block becoming effective would cause overestimation of the fraction of total gross nitrification that was heterotrophic. However, Berg *et al.* (1982) found that C_2H_2 concentrations 1000 times lower than ours completely inhibited net nitrite and NO_3^- production in an agricultural soil during the first day following C_2H_2 addition. Their result suggests that the inhibitory effect of C_2H_2 on autotrophic nitrification is essentially instantaneous in sieved, well-aerated soils such as those that we used.

Our gross rates of N mineralization correlated well with both total and heterotrophic nitrification, suggesting that gross N mineralization may be a good index of both NH_4^+ availability to autotrophic nitrifiers and the quality of organic N as a substrate for heterotrophic nitrification. Strong correlations between rates of N mineralization and nitrification alone should not be used as evidence that the nitrification is primarily autotrophic.

Our results clearly indicate that heterotrophic nitrification may be the dominant pathway of nitrification in many acidic forest soils. Several other investigators have found significant rates of presumably heterotrophic nitrification in acidic soils using different techniques. Schimel *et al.* (1984) used the appearance of $^{15}NO_3^-$ after the addition of $^{15}NH_4^+$ to soil slurries to partition autotrophic and heterotrophic nitrification in moderately acidic forest soils (pH = 5.8) in California. Using this technique, they found that 95% and 67% of the total nitrification was heterotrophic, using an organic-N source in mature and recently clearcut coniferous forest soils, respectively. Further evidence supporting heterotrophic nitrification in these soils came from studies demonstrating that net NO_3^- production: was almost entirely uninhibited by C_2H_2; was inhibited substantially by the addition of a fungicide (cycloheximide), but insignificantly by the addition of a bactericide (streptomycin); and was stimulated by the addition of an organic-N source (peptone). The study by Schimel *et al.* (1984) is still the most complete to date, rigorously indicating the dominance of the heterotrophic pathway in some forest soils.

Numerous other investigators have used one or more of these techniques to demonstrate the occurrence or dominance of the heterotrophic nitrification pathway (Remacle, 1977a,b; Tate, 1977; Johnsrud, 1978; Adams, 1986a,b; Killham, 1986, 1987; Duggin *et al.*, 1991; Klingensmith and Van Cleve, 1993). Several other investigations of acid soils, however, have shown the dominance of the autotrophic nitrification pathway (Killham, 1986; De Boer *et al.*, 1989, 1992; Stams *et al.*, 1990; Tietema *et al.*, 1992; Pennington and Ellis, 1993). Studies showing that C_2H_2 inhibited nitrification in acidic soils indicate that nitrification in these soils is being carried out by acid-tolerant or even acidophilic, autotrophic nitrifiers (De Boer *et al.*, 1991, 1992).

However, in all of these previous studies, only net nitrification rates (net change in NO_3^- pool size) were measured. Any selective effect of the experimental conditions on microbial NO_3^- immobilization rates (i.e. addition of process substrates or selective biochemical blocks) may alter both the total gross nitrification rate and the relative degree of autotrophic vs. heterotrophic nitrification. Because microbial NO_3^- immobilization can be significant in many forest soils (Davidson *et al.*, 1992; Hart *et al.*, 1994a; Stark and Hart, 1997), we recommend measuring gross nitrification rates without substrate additions when making comparative assessments of these two nitrification pathways. Alternatively, the coupled ^{15}N pool dilution–enrichment approach, proposed by Barraclough and Puri (1995), can be used to measure the relative autotrophic and heterotrophic nitrification pathways directly. However, the ^{15}N pool dilution–enrichment approach requires NH_4^+ addition, which may alter the relative rates of the two nitrification pathways.

Factors unique to coniferous forest soils may regulate the relative dominance of the two nitrification pathways. This hypothesis is supported by evidence showing that nitrification in acidic soils from grasslands and agricultural fields, but not conifer forests, is predominantly autotrophic (i.e. affected by C_2H_2; Killham, 1986). However, studies of some acidic, coniferous forest soils have shown little heterotrophic nitrification (De Boer *et al.*, 1989, 1992). We suggest that the rate of N turnover is not a factor regulating the relative rates of autotrophic and heterotrophic nitrification in acidic, coniferous forest soils.

In summary, we found that the inclusion of red alder in two conifer forests of contrasting productivity significantly increased gross rates of N production and consumption. However, alder increased gross N mineralization and immobilization more at the high productivity conifer site than at the low productivity conifer site. Net N transformations were enhanced in alder–conifer stands, compared to conifer stands, because N production processes were increased slightly more than N consumptive processes. Despite the lower soil pH of alder–conifer stands, compared to conifer stands, heterotrophic nitrification (as indicated by a lack of C_2H_2 inhibition) accounted for 65–72% of the gross nitrification in all stands that exhibited nitrification. This result suggests that soil pH alone may not greatly alter the relative dominance of autotrophic and heterotrophic nitrification pathways. Nevertheless, the inclusion of alder in conifer stands did increase the role of NO_3^- in the soil internal N-cycle. Our results suggest that the inclusion of red alder in both low and high productivity conifer sites has considerable effects on soil N cycling processes.

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