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# DENITRIFICATION ENZYME ACTIVITY OF DOUGLAS-FIR AND RED ALDER FOREST SOILS OF THE PACIFIC NORTHWEST

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**Summary**—Nitrogen-fixing red alder (*Alnus rubra* Bong.) increases soil organic matter and N content of forest soils. This study compared denitrification enzyme activity (DEA) to related N-cycling and microbial indicators in adjacent stands of alder and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) in two Pacific Northwest U.S.A. research forests over 16 months. Laboratory denitrification rates were measured in non-amended soils and soils amended with combinations of water, $NO_3^-$, and glucose. The $NO_3^-$-and glucose-amended soils provided estimates of DEA. DEA in alder soils was greater than or equal to that in corresponding Douglas-fir soils. Denitrification in alder soils was occasionally limited by energy source (glucose) but not by $NO_3^-$, whereas in Douglas-fir soils, it was frequently limited by both $NO_3^-$ and glucose. For a given soil, DEA was generally not well related to respiration potential, anaerobic mineralizable N, or exchangeable ammonium over time, but it was well related to nitrification potential across different soils and over time within two soils. © 1998 Elsevier Science Ltd. All rights reserved

## INTRODUCTION

Denitrification may be an important process influencing N balance in forest ecosystems wherein N loss from soils via nitrous oxide and dinitrogen production is of similar magnitude to N input in wet deposition and N fixation (Davidson *et al.*, 1990). The proximal controls of denitrification are anaerobic (low oxygen) environment, energy source, $NO_3^-$, suitable conditions for microbial and enzymatic activity (temperature, pH, soil water content, and nutrients), and the presence of denitrifying enzymes (Robertson and Tiedje, 1984; Davidson *et al.*, 1990). Denitrifying enzymes are often quantified by the denitrifying enzyme activity (DEA) technique, in which denitrification is measured under anaerobic conditions when energy and $NO_3^-$ are not limiting (Smith and Tiedje, 1979).

Strong relationships between denitrification and proximal controls exist across a range of sites or over time at individual sites. For grassland and arable soils, $NO_3^-$-amended anaerobic denitrification was highly correlated with water soluble C, a possible indicator of energy source available to microorganisms (Katz *et al.*, 1985; Bijay-Singh *et al.*, 1988; Davidson *et al.*, 1990). Carbon availability has been highly correlated with denitrification capacity (Burford and Bremner, 1975; Davidson and Swank, 1987) and field denitrification rates (Robertson and Tiedje, 1984; Myrold, 1988; Bergstrom *et al.*, 1994). For cultivated soils, unamended anaerobic denitrification was highly correlated with soil organic C and microbial biomass (Drury *et al.*, 1991). In a grass field, increases in DEA often followed increases in soil water content (Parsons *et al.*, 1991). Across a range of forest sites, *in situ* denitrification was poorly correlated with $NO_3^-$ concentrations and soil water content, but DEA was highly correlated with soil water content over several seasons (Vermes and Myrold, 1992).

Denitrification may be quantitatively related to other N cycling processes because of its dependence on a supply of $NO_3^-$ (Fig. 1). Over a range of tropical soils, nitrous oxide flux was positively correlated with net nitrogen mineralization (Matson and Vitousek, 1987). Denitrification rates in soils have also been shown to correlate with $NO_3^-$ concentrations (Davidson and Swank, 1986; Vermes and Myrold, 1992).

Because most denitrifying bacteria are heterotrophic and aerobic (Tiedje, 1982), denitrification indicators may be quantitatively related to the heterotrophic activity of the microbial community. In a grass field, increases in DEA were commonly associated with increases in soil respiration (Parsons *et al.*, 1991). Across a range of forest sites, DEA was positively correlated with soil respiration over all seasons, but *in situ* denitrification rates were

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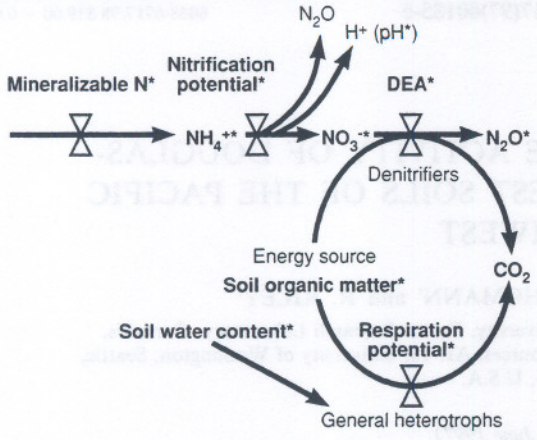


*Variables measured in this study

Fig. 1. Conceptual relation of DEA to microbial indicators and soil properties.

poorly correlated with soil respiration (Vermes and Myrold, 1992).

Vegetation can influence soil properties and microbial transformation rates (Vitousek and Walker, 1989; Van Miegroet *et al.*, 1990, 1992; Griffiths *et al.*, 1993; Klingensmith and Van Cleve, 1993). For example, red alder (*Alnus rubra* (Bong.)) is known to fix large quantities of nitrogen, resulting in an enrichment of soil nitrogen (Binkley *et al.*, 1992b). This may be an important mechanism by which nitrogen availability is maintained in conifer forests of the Pacific Northwest (Tarrant and Miller, 1963; Van Miegroet and Cole, 1985; Binkley and Sollins, 1990; Hart *et al.*, 1991; Binkley *et al.*, 1992a). Most forms of nitrogen and denitrification rates are higher in red alder than in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) soils (Binkley *et al.*, 1992a; Vermes and Myrold, 1992). Compared with coniferous trees, alder increases soil organic matter, total nitrogen, and soluble $NO_3^-$, and reduces pH (Tarrant and Miller, 1963; Cole *et al.*, 1978; Bormann and DeBell, 1981; Binkley and Sollins, 1990; Van Miegroet *et al.*, 1990, 1992; Binkley *et al.*, 1992a). *In situ* denitrification rates were found to be higher in red alder than in coniferous soils (Binkley *et al.*, 1992a; Vermes and Myrold, 1992), but the importance of the various proximal controls on denitrification rates has not been fully examined.

The objective of our laboratory study was to determine whether soil DEA was limited by $NO_3^-$ or energy availability in red alder and coniferous soils collected at two sites in the Pacific Northwest and to examine how DEA is related to other microbial indicators. We hypothesized that (i) higher DEA exists in alder than in conifer soils, (ii) denitrification is limited by $NO_3^-$ in conifer soils and by energy source in alder soils, and (iii) there are seasonal differences in the responses of denitrifier populations in alder and Douglas-fir soils to the addition of nitrate and glucose.

## MATERIALS AND METHODS

### *Site descriptions and sampling*

The Cascade Head Experimental Forest, located on the Pacific coast of Oregon (45°02′N; 123°55′W) and the Allen E. Thompson Research Center, located in the Cedar River watershed east of Seattle, Washington (47°24′N, 122°15′W) were chosen as study sites. Each site has been studied extensively and contains plots with essentially pure stands of second-growth Douglas-fir and red alder that were established at about the same time. The two sites differ in climate, tree productivity and soil type.

The Cascade Head site was described by Tarrant and Miller (1963) and more recently by Binkley *et al.* (1992a). This well-drained site had been farmed until about 1925, when it was abandoned and stands regenerated naturally. In 1935, two 0.2 ha plots were thinned to produce one alder-free conifer plot and one conifer-free alder plot. The average annual rainfall at this site is 240 cm; the average monthly temperatures range from about 20°C in July to 10°C in January. The soil type is Typic Dystrandept. The site is considered to be very productive for tree growth, with high concentrations of soil nitrogen and phosphorus (Cole *et al.*, 1978).

The original forest at the Thompson Research Center was harvested between 1910 and 1920 (Cole and Gessel, 1968; Turner *et al.*, 1976; Cole *et al.*, 1978). After a series of wildfires, part of the area was planted with Douglas-fir in 1931, and the rest was invaded by red alder in subsequent years, resulting in adjacent stands of nearly pure Douglas-fir and red alder. The glacial soils at the site are gravelly sandy loam of the Alderwood series, which was previously classified as Dystric Entic Durochrept, but was recently reclassified as Aquic Haplorthod. The soil is poorly drained because of a compacted basal till at approximately 1 m depth. The average July temperature is 16.8°C and the average January temperature is 2.8°C. This site is considered to be relatively poor for both alder and Douglas-fir production (Turner *et al.*, 1976).

From 1991 to 1993, soil samples were collected periodically from the 0–10 cm depth of mineral soil with a trowel and transported to the laboratory in plastic bags within an ice chest. Five samples were collected from each of the two forest types at both research sites at each of four or six sampling times. With one exception, all soils were stored at 15°C until the initiation of the analyses, which was within 16 h of their receipt into the laboratory. The exception was the August 1992 soils, which were stored for 48 h at 5°C prior to analysis.

*Analytical procedures*

All soils were sieved (2 mm) before analysis. Soil pH was measured in 1:10 (soil:water) slurries of oven-dried (100°C) soil with distilled water. These slurries were shaken for 1 h before pH values were read with a Sigma model E4753 electrode. After oven drying at 100°C, soil organic matter (SOM) was measured by loss-on-ignition at 550°C.

*Denitrification and DEA*

Microbiological methods were as described by Griffiths *et al.* (1990) except that denitrification and respiration were measured at 25°C. Time course experiments conducted on selected soils from the test sites showed an initial lag in the rate of $N_2O$ production, which typically lasted up to 1 h under conditions used in these experiments. The rate was then linear for up to 4 h. For this reason, denitrification was measured as $N_2O$ released over a 2-h period starting 1 h after the reaction vessel (25 ml Erlenmeyer flask) was sealed with a serum bottle stopper. Each reaction vessel contained 5 g of sieved field-moist soil incubated under an Ar atmosphere to provide anaerobic conditions.

The method used to measure DEA was similar to that reported by Groffman and Tiedje (1989); 2 ml of a 1-mM solution of glucose and $NO_3^-$ was added to each flask containing 5 g of field moist-sieved soil. Measurements of $N_2O$ production were also made after samples were amended with water, 1 mM glucose, or 1 mM $NO_3^-$. Nonamended denitrification rates were measured with the same technique except that no water, glucose, or $NO_3^-$ was added to the soils prior to incubation. In our experiments, water was added to measure the response of the population to soil water content rather than as a means of reducing oxygen, since the samples were already in an Ar atmosphere. $N_2O$ concentrations were determined by injecting 0.5 ml of flask headspace gas into a gas chromatograph fitted with an electron capture detector. Checks of selected samples showed that the $N_2O$ production rate was constant over the incubation period (data not shown).

Ten percent of the samples were randomly chosen to determine whether a 10% atmospheric concentration of acetylene increased the rate of $N_2O$ production in the headspace of the reaction vessels. We did not see a significant difference in the rates observed in reaction vessels with or without acetylene in any of the treatments. This is the same approach taken by Christensen *et al.* (1990), who found that in soils of low pH, most of the end-product of denitrification was $N_2O$; therefore, the acetylene block was not necessary to estimate denitrification rates by $N_2O$ production rates.

*Other soil properties*

*Nitrification potential and* $NO_3^-$ *analyses* Nitrification potentials were measured using essentially the same method described by Hart *et al.* (1994). Field-moist soil (15 g) was added to a 250-ml Erlenmeyer flask, followed by an $NO_3^-$-free solution (100 ml) containing $(NH_4)_2SO_4$ (500 μM), $KH_2PO_4$ (280 μM), and $K_2HPO_4$ (720 μM) adjusted to pH 7.2 (Hart *et al.*, 1994). The flasks were continuously shaken; after 2, 4, 22 and 24 h, 5 ml of the slurry solution was pipetted from each flask, filtered, then frozen. Cadmium reduction to $NO_2^-$ followed by colorimetric analysis for $NO_2^-$ was used to analyze the samples for $NO_2^-$. The rate of nitrification in the slurries was determined from the best-fit linear regression slope through the four time points.

*Exchangeable ammonium and mineralizable N* Exchangeable $NH_4^+$ concentration was determined by shaking 10 g field-moist soil with 50 ml of 2 M KCl for 1 h (Keeney and Nelson, 1982). After adding 300 μL of 10 M NaOH to the slurry, $NH_4^+$ concentration was measured with a Orion model 95-12 ammonium electrode (Orion Research Inc., Boston, MA). The waterlogged technique of Keeney and Bremner (1966) was used to measure mineralizable N. Field-moist soil (10 g) was added to 53 ml of distilled water in a 20 × 125 mm screw-cap test tube and incubated at 40°C. After 7 d, 53 ml of 4 M KCl was added to the slurry and $NH_4^+$ concentration was determined with the ammonium electrode. Mineralizable N was calculated from the difference between initial and final $NH_4^+$ concentrations.

*Respiration potential* Respiration potentials were measured by a technique similar to glucose-amended denitrification, except the headspace was not purged with Ar. $CO_2$ concentrations in the headspace were measured with a g.c. fitted with a thermal conductivity detector using the same methods described by Griffiths *et al.* (1990). These methods are similar to glucose-induced respiration that has been used to measure microbial activity in soils (Smith *et al.*, 1985; Wardle and Parkinson, 1990).

*Statistical analyses*

Except where indicated, all values were expressed in $g^{-1}$ dry mass of soil. Because of different sampling times and different qualitative temporal trends, sites were analyzed separately in two-way (forest type × sampling time) ANOVA. Denitrification, denitrification potential and nitrification potential values were not normally distributed; therefore, these data were log transformed before ANOVA analyses. Fisher's protected least significant difference test was used to determine whether differences between mean values were significant ($P < 0.05$). Statistics were calculated with Statgraphics (Statistical Graphics Corporation, Rockville, MD).

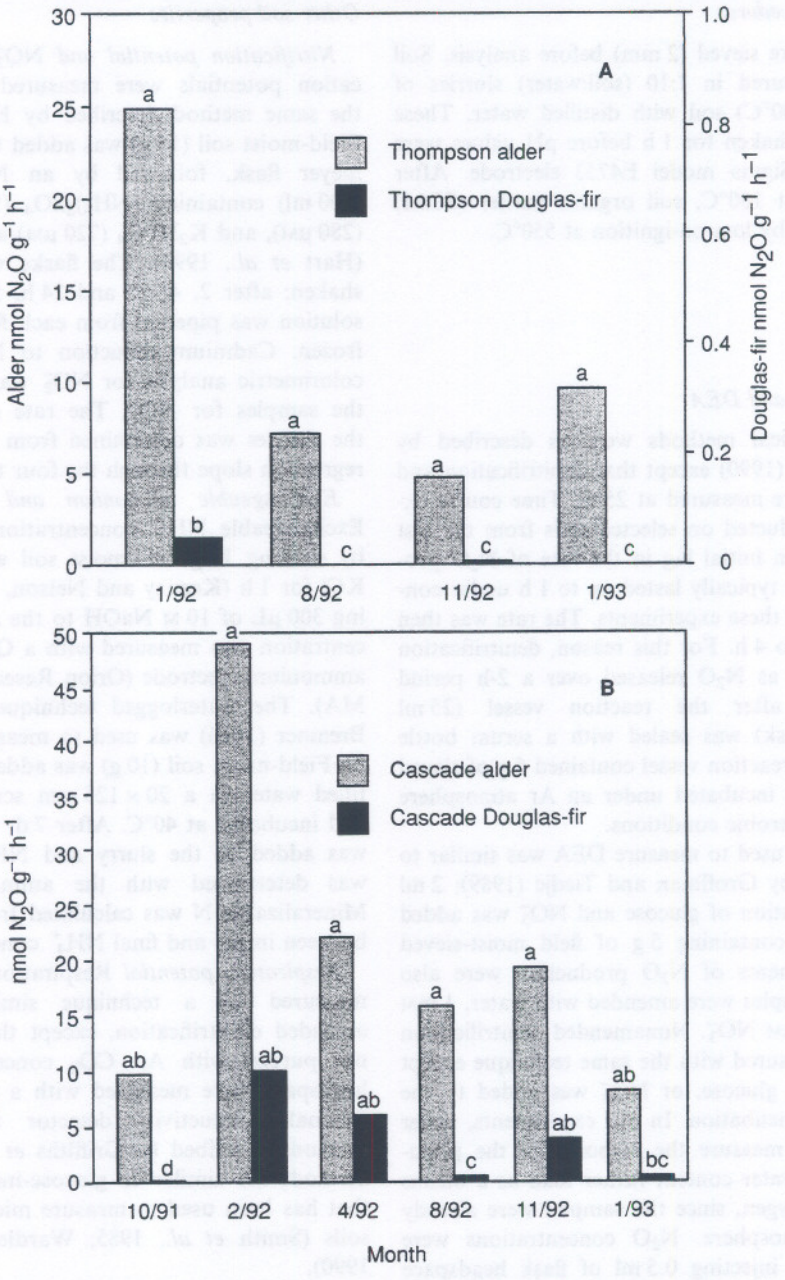


Fig. 2. Laboratory denitrification rates for non-amended Douglas-fir and red alder soils from (a) Thompson and (b) Cascade Head sites. Within each graph, values identified by the same letter are not different at $P < 0.05$.

## RESULTS AND DISCUSSION

### *Denitrification and DEA*

At both the Thompson and Cascade Head sites, denitrification in non-amended soils was greater in red alder than in Douglas-fir soils throughout the study (Fig. 2). Vermes and Myrold (1992) found similar results during their seasonal study of *in situ* denitrification at three disturbed Douglas-fir, four red alder, and eight old-growth sites in Oregon. Denitrification was highest in the red alder soils, intermediate in the disturbed soils (roughly equivalent to our Douglas-fir soils but the stands were younger), and not measurable in the old-growth soils.

At both the Thompson and Cascade Head sites, there were statistically significant temporal differences in denitrification in Douglas-fir soil but not in alder soil (Fig. 2). However, temporal trends in Douglas-fir soils were not related to season. For

example, high denitrification occurred at both sites in winter 1992, whereas lower rates occurred in winter 1993.

Thompson Douglas-fir soil showed considerable and consistent increases in denitrification in response to the addition of $NO_3^-$-plus-glucose (Fig. 3), as indicated by significantly greater DEA than water-amended denitrification (WAD) on all dates. In contrast, DEA and WAD were equal in Thompson alder soil (Fig. 3). DEA was greater than WAD in Cascade Head Douglas-fir soils for all sample dates, but these differences were only

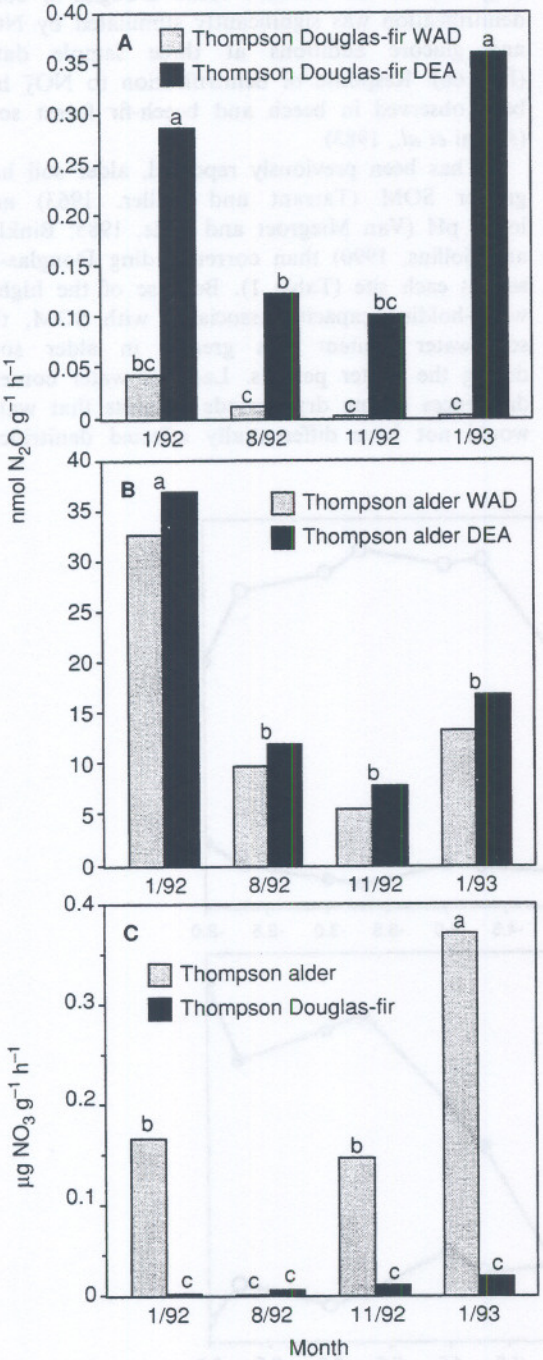


Fig. 3. Water-amended denitrification (WAD); water, nitrate, and glucose-amended denitrification (DEA) for (a) Douglas-fir and (b) red alder soils and (c) nitrification potential from the Thompson site. Within each graph, values identified by the same letter are not different at $P < 0.05$.

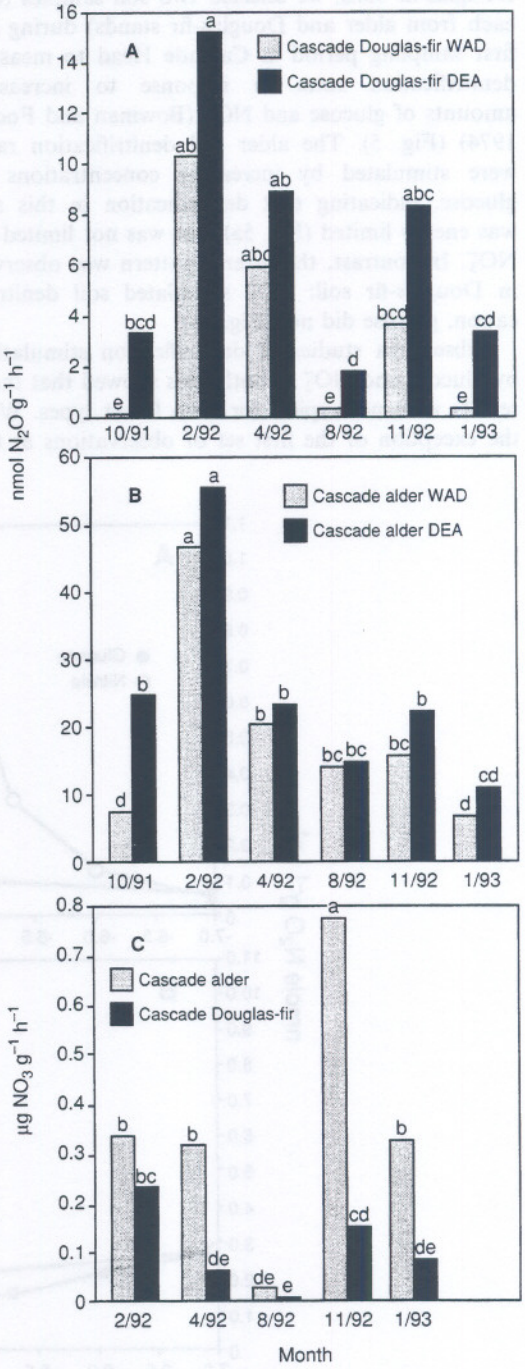


Fig. 4. Water-amended denitrification (WAD); water, nitrate and glucose-amended denitrification (DEA) for (a) Douglas-fir and (b) red alder soils and (c) nitrification potential from Cascade Head. Within each graph, values identified by the same letter are not different at $P < 0.05$.

statistically significant at the time where rates were low (Fig. 4). In the alder soils, DEA was also generally greater than WAD, but the difference was only significant for October, 1991. For all dates, DEA and WAD were greater in Cascade Head Douglas-fir soils than in Thompson Douglas-fir soils.

To test whether denitrification was limited by energy availability in alder soils and by $NO_3^-$ in Douglas-fir soils, we selected two soil samples (one each from alder and Douglas-fir stands) during the first sampling period at Cascade Head to measure denitrification rates in response to increasing amounts of glucose and $NO_3^-$ (Bowman and Focht, 1974) (Fig. 5). The alder soil denitrification rates were stimulated by increasing concentrations of glucose, indicating that denitrification in this soil was energy limited (Fig. 5a), but was not limited by $NO_3^-$. In contrast, the reverse pattern was observed in Douglas-fir soil: $NO_3^-$ stimulated soil denitrification, glucose did not (Fig. 5b).

Subsequent studies of denitrification stimulation by glucose and $NO_3^-$ at both sites showed that these results were not typical for both forest types. With the exception of the first set of observations at the Cascade Head site, none of the alder soils from either site showed a significant increase over WAD controls in denitrification when glucose and $NO_3^-$ were added (Fig. 3b and Fig. 4b). For the Thompson Douglas-fir soil, $NO_3^-$ was largely responsible for consistently higher DEA compared with WAD, although the soil was also responsive to glucose on three of the four measurement dates (Fig. 6a). In the Cascade Head Douglas-fir soils, denitrification was significantly stimulated by $NO_3^-$ and glucose additions at three sample dates (Fig. 6b). Response of denitrification to $NO_3^-$ has been observed in beech and beech-fir forest soils (Alfani *et al.*, 1983).

As has been previously reported, alder soil had greater SOM (Tarrant and Miller, 1963) and lower pH (Van Miegroet and Cole, 1985; Binkley and Sollins, 1990) than corresponding Douglas-fir soil at each site (Table 1). Because of the higher water-holding capacity associated with SOM, the soil water content was greater in alder soils during the wetter periods. Lack of water content differences during dry periods suggests that water would not have differentially affected denitrifica-

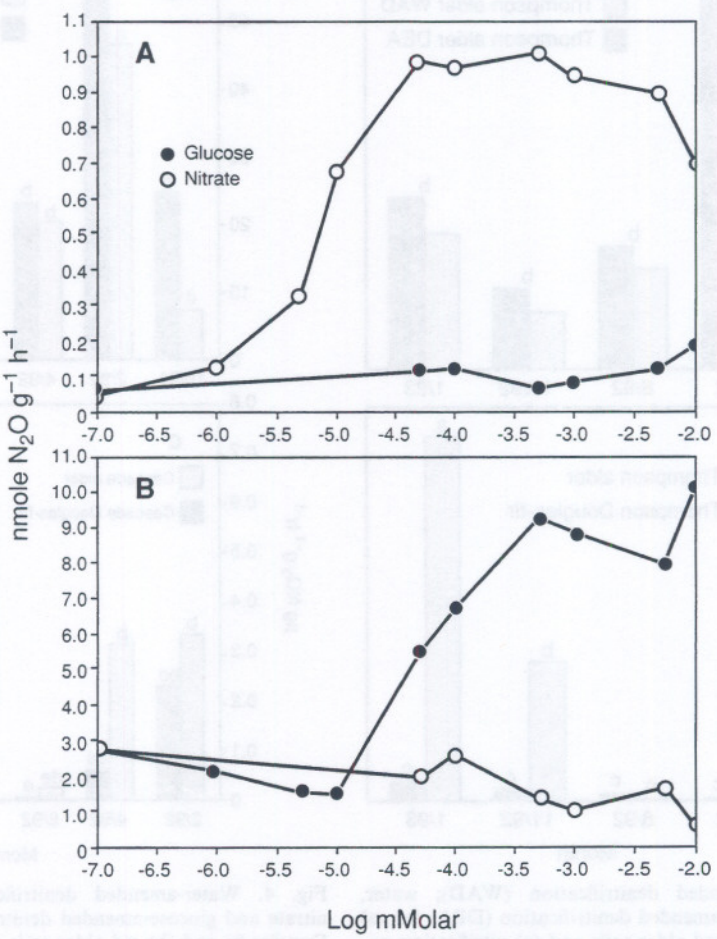


Fig. 5. The effects of nitrate and glucose concentrations on denitrification rates in (a) Douglas-fir and (b) red alder soils from Cascade Head site, October 1991.

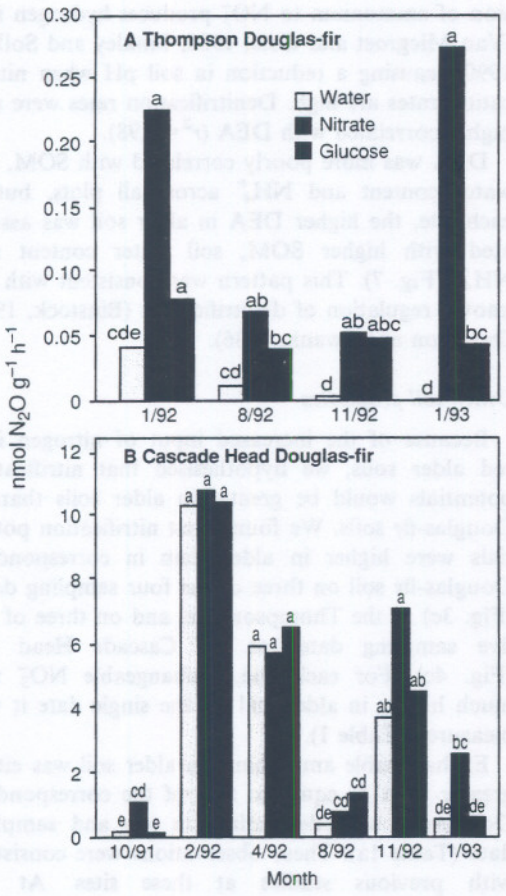


Fig. 6. Denitrification in soils amended with water, nitrate and glucose in Douglas-fir soils from (a) Thompson and (b) Cascade Head. Within each graph, values identified by the same letter are not different at $P < 0.05$.

tion in alder and Douglas-fir soils during these periods.

Because alder soils contained higher concentrations of SOM than did Douglas-fir soils, we expected to find elevated respiration rates in these soils. With one exception, however, respiration potentials did not differ between alder and corresponding Douglas-fir soils (Table 1).

*DEA and other soil properties*

The potential connections of DEA to microbial indicators, inorganic N forms and other soil properties are shown in Fig. 1. We expected DEA to be temporally correlated with other microbial indicators. DEA and nitrification potential had similar temporal patterns in Thompson Douglas-fir and alder soils (Fig. 3). In contrast, DEA was not well correlated with nitrification potential in Cascade Head soils (Fig. 4), or with mineralizable N or respiration potential in any soil.

Temporal variation in denitrifier communities may be caused by shifts in temperature, water content, and carbon availability that affect the general heterotrophic communities, or these changes may be due specifically to changes in denitrifier communities. We used ratios of DEA to respiration potential as indicators of the proportions of denitrifiers to the general heterotrophic population. If denitrifiers make up a constant proportion of the total heterotrophic population, we would expect that DEA should correlate well with some index of heterotrophic activity. This assumes that DEA reflects concentrations of denitrifying microorganisms (Groffman and Tiedje, 1989). Similarly, respiration

Table 1.

(a) Characteristics of red alder and Douglas-fir soils from the Cascade Head site collected over a 16-month study period

| Date | Forest type | Soil water content (%) | Soil organic matter (%) | pH | Respiration potential (μmol $CO_2$ $g^{-1}$ $h^{-1}$) | Mineralizable nitrogen (μg N $g^{-1}$) | Exchangeable ammonium (μg N $g^{-1}$) | Nitrate (μg N $g^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| Oct 91 | Red alder | 41.8 (4.0)c | NA | NA | 0.29 (0.05)bcd | 111.8 (28.3)bc | 19.3 (3.7)bc | |
| | Douglas-fir | 40.8 (4.2)c | NA | NA | 0.16 (0.05)d | 99.4 (19.1)cd | 15.4 (1.6)bc | |
| Feb 92 | Red alder | 49.0 (3.6)c | 50.1 (9.1)a | 4.0 (0.1)cd | 0.49 (0.15)b | 66.0 (17.8)efg | 13.9 (3.5)c | |
| | Douglas-fir | 45.9 (2.3)c | 40.1 (4.7)abc | 5.0 (0.3)a | 0.33 (0.07)bcd | 93.9 (23.4)cde | 8.3 (1.9)d | |
| Apr 92 | Red alder | 124.6 (39.0)a | 45.1 (16.1)a | 4.0 (0.3)cd | 0.87 (0.28)a | 77.6 (28.4)def | 31.5 (8.6)a | |
| | Douglas-fir | 89.8 (5.47)b | 29.3 (2.1)cd | 5.0 (0.2)a | 0.73 (0.27)a | 149.0 (24.3)a | 20.7 (6.9)b | |
| Aug 92 | Red alder | 35.9 (1.8)c | 42.0 (5.0)ab | 3.9 (0.1)cd | 0.48 (0.21)b | 61.7 (17.1)efg | 3.4 (0.9)cde | |
| | Douglas-fir | 35.1 (3.0)c | 24.2 (4.4)d | 4.6 (0.1)b | 0.40 (0.09)bc | 49.5 (25.2)fg | 2.7 (0.5)de | |
| Nov 92 | Red alder | 128.8 (41.0)a | 50.5 (14.8)a | 3.8 (0.2)d | 0.44 (0.11)bc | 74.0 (32.1)def | 1.5(0.5)e | |
| | Douglas-fir | 105.9 (13.0)ab | 32.7 (2.7)bcd | 4.6 (0.2)b | 0.35 (0.03)bcd | 142.0 (27.9)ab | 1.6 (0.2)e | |
| Jan 93 | Red alder | 105.6 (9.8)ab | 30.4 (3.6)bcd | 4.1 (0.1)c | 0.25 (0.05)cd | 35.0 (11.0)g | 7.3 (3.0)cd | 3.84 (0.39) |
| | Douglas-fir | 54.9 (45.8)c | 26.1 (5.0)d | 4.8 (0.2)ab | 0.26 (0.07)cd | 35.8 (12.3)g | 5.8 (2.8)cde | 0.0012 (0.0011) |

(b) Characteristics of red alder and Douglas-fir soils from the Thompson site collected over a 16-month study period

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Jan 92 | Red alder | 42.1 (3.8)bc | 26.1 (4.8)a | 4.7 (0.1)cde | 0.57 (0.10)b | 98.6 (26.4)a | 5.1 (0.9)b | |
| | Douglas-fir | 34.9 (3.1)cd | 13.9 (2.2)c | 5.1 (0.4)ab | 0.32 (0.15)c | 56.1 (16.4)dc | 2.8 (0.9)cd | |
| Aug 92 | Red alder | 30.8 (3.2)de | 21.4 (2.6)ab | 4.5 (0.1)ef | 0.69 (0.04)a | 80.0 (24.0)ab | 0.9 (0.2)e | |
| | Douglas-fir | 22.8 (5.7)e | 14.0 (3.9)c | 4.8 (0.3)cd | 0.77 (0.06)a | 63.4 (22.6)bc | 0.9 (0.2)e | |
| Nov 92 | Red alder | 65.8 (8.0)a | 20.6 (2.0)b | 4.6 (0.07)def | 0.22 (0.03)c | 52.5 (10.5)cd | 1.8 (0.3)de | |
| | Douglas-fir | 45.9 (3.6)b | 11.7 (1.5)c | 4.9 (0.1)bc | 0.25 (0.06)c | 28.8 (5.4)de | 1.0 (0.2)e | |
| Jan 93 | Red alder | 65.8 (13.2)a | 23.4 (5.8)ab | 4.3 (0.2)e | 0.32 (0.06)c | 43.2 (21.3)cde | 7.0 (1.3)a | 4.59 (0.54) |
| | Douglas-fir | 47.5 (3.4)b | 10.8 (2.2)c | 5.3 (0.1)a | 0.21 (0.03)c | 22.4 (6.7)e | 3.8 (1.3)c | <0.001 |

Within each site and each column, values identified by the same letter are not different by forest type or date at $P < 0.05$.

potential as we measured it in the presence of glucose should provide a relative index of heterotrophic microorganism concentrations (Smith *et al.*, 1985).

These ratios showed significant differences by both date and forest type (Table 2). DEA-to-respiration ratios were greater on most dates in the red alder soils than in corresponding Douglas-fir soils. This implies that the proportion of denitrifiers in the heterotrophic microbial community was consistently greater in alder soils than in corresponding Douglas-fir soils. A similar approach has been used by others (Myrold and Tiedje, 1985; Groffman and Tiedje, 1989) wherein DEA was normalized to microbial biomass to help explain annual variability in denitrification in north temperate forest soils.

We expected DEA to show a poor temporal correlation with mineralizable N when compared with microbial indicators because mineral N species are small, transient pools that turn over rapidly, and are influenced by a variety of production and consumption processes. There was temporal correlation between DEA and exchangeable $NH_4^+$ in Thompson Douglas-fir soil, but not in other soils (compare Table 1 with Figs 3 and 4).

Across-plot comparison between DEA and other variables showed DEA was positively correlated with nitrification potential ($r^2 = 0.999$), and negatively correlated with pH (Fig. 7). The correlation with nitrification is consistent with previous observations of the relationship of DEA to $NO_3^-$ (Alfani *et al.*, 1983; Melillo *et al.*, 1983; Vermes and Myrold, 1992), and of field denitrification to nitrification potential (Robertson and Tiedje, 1984). The negative correlation ($r^2 = 0.999$) between nitrification potential and pH was expected because the conversion of ammonium to $NO_3^-$ produces hydrogen ions (Van Miegroet and Cole, 1985; Binkley and Sollins, 1990), causing a reduction in soil pH when nitrification rates are high. Denitrification rates were also highly correlated with DEA ($r^2 = 0.98$).

DEA was more poorly correlated with SOM, soil water content and $NH_4^+$ across all plots, but at each site, the higher DEA in alder soil was associated with higher SOM, soil water content and $NH_4^+$ (Fig. 7). This pattern was consistent with the known regulation of denitrification (Binstock, 1984; Davidson and Swank, 1986).

*Other soil properties*

Because of the increased input of nitrogen into red alder soils, we hypothesised that nitrification potentials would be greater in alder soils than in Douglas-fir soils. We found that nitrification potentials were higher in alder than in corresponding Douglas-fir soil on three of the four sampling dates (Fig. 3c) at the Thompson site, and on three of the five sampling dates at the Cascade Head site (Fig. 4c). For each site, exchangeable $NO_3^-$ was much higher in alder soil on the single date it was measured (Table 1).

Exchangeable ammonium in alder soil was either greater than or equal to that of the corresponding Douglas-fir soil, depending on site and sampling date (Table 1a). These observations were consistent with previous studies at these sites. At the Thompson site, Van Miegroet *et al.* (1992) found exchangeable ammonium to be higher in alder than Douglas-fir soils only on one of five sampling dates, whereas exchangeable $NO_3^-$ was always higher in alder soil. At Cascade Head, Binkley *et al.* (1992a,b) found resin-collected and soil-solution

Table 2. Ratios of DEA-to-respiration potentials

| Site | Date | Forest type | DEA-to-Respiration ratio |
|---|---|---|---|
| Cascade Head | Oct 91 | Red alder | 84.8ab |
| | | Douglas-fir | 21.5cde |
| | Feb 92 | Red alder | 111.8a |
| | | Douglas-fir | 45.8c |
| | Apr 92 | Red alder | 26.7cde |
| | | Douglas-fir | 12.2d |
| | Aug 92 | Red alder | 30.4cd |
| | | Douglas-fir | 4.4d |
| | Nov 92 | Red alder | 50.2bc |
| | | Douglas-fir | 23.7cde |
| | Jan 93 | Red alder | 44.7c |
| | | Douglas-fir | 12.9d |
| Thompson | Jan 92 | Red alder | 40.2a |
| | | Douglas-fir | 0.9d |
| | Aug 92 | Red alder | 10.8b |
| | | Douglas-fir | 1.2c |
| | Nov 92 | Red alder | 22.1ab |
| | | Douglas-fir | 0.4e |
| | Jan 93 | Red alder | 33.4a |
| | | Douglas-fir | 1.7c |

Within each site and each column, values identified by the same letter are not different at $P < 0.05$. The units used are (nmol $N_2O$ N $g^{-1}$ $h^{-1}$)/(μmol $CO_2$ $g^{-1}$ $h^{-1}$) for DEA/respiration.

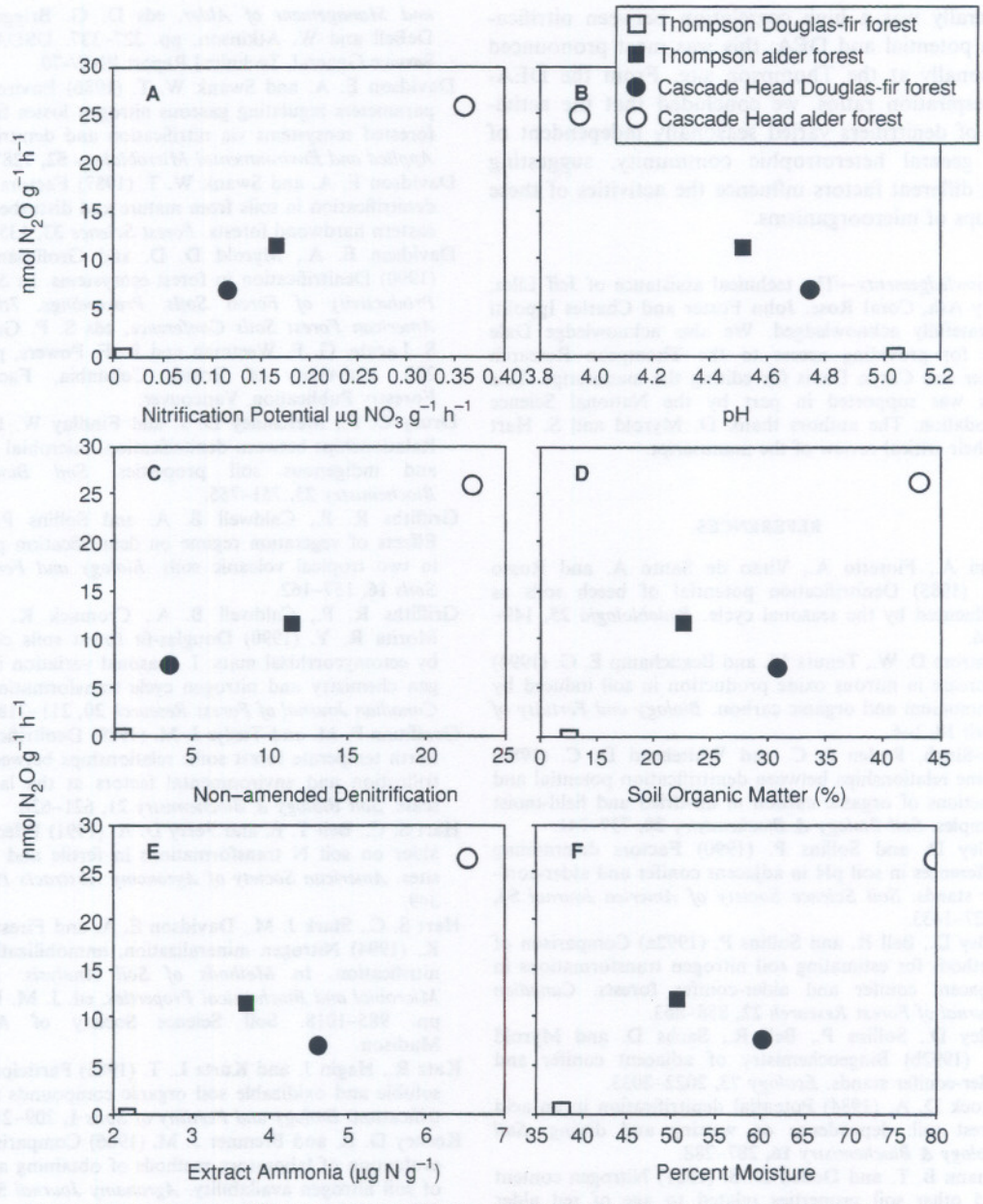


Fig. 7. Mean values for DEA *versus* (a) nitrification potentials, (b) pH, (c) non-amended denitrification, (d) soil organic matter, (e) exchangeable ammonium, and (f) percent soil water content in alder and Douglas-fir soils from Thompson and Cascade Head sites. Only the four common seasonal studies for the two sites were used to determine the means. The $r^2$ percentages for the seasonal means of the variable pairings listed above are: a = 99.9, b = 99.9, c = 97.5, d = 81.6, e = 85.2, and f = 85.3 using simple linear correlation analysis.

ammonium to be slightly lower in alder-influenced soil, but $NO_3^-$ to be much higher.

Mineralizable N did not differ consistently between alder and Douglas-fir soils (Table 1). At Cascade Head, the Douglas-fir soil had higher mineralizable N on two dates; at the Thompson site, the red alder soil had higher value on one date. Binkley *et al.* (1992a) found similar values of mineralizable N in Cascade Head conifer and alder-conifer soils.

## CONCLUSIONS

We found that DEA in red alder soils was greater than or equal to that in corresponding Douglas-fir soils. Denitrification in red alder soils was generally not limited by either $NO_3^-$ or energy, although it could be limited by both $NO_3^-$ and energy in Douglas-fir soils. The responses of the denitrifier populations to the addition of nitrate were greater during some months, primarily in the winter. There

generally was a high correlation between nitrification potential and DEA; this was most pronounced seasonally at the Thompson site. From the DEA-to-respiration ratios, we concluded that the activities of denitrifiers varied seasonally independent of the general heterotrophic community, suggesting that different factors influence the activities of these groups of microorganisms.

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