Denitrification in forest soils of Oregon[1]

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Denitrification represents a potential loss of N from forest soils as well as a source of N oxides to the atmosphere; however, this process has not been closely examined in forest ecosystems of the Pacific Northwest. The objectives of this study were to survey *in situ* denitrification rates in a range of forest ecosystems and to assess the importance of selected soil properties as controlling factors of denitrification in forest soils. Soils from eight mature conifer stands, three recently clear-cut sites, and four *Alnus rubra* Bong. stands were sampled in spring, summer, and autumn. Denitrification potentials (anaerobic soil slurries), *in situ* denitrification rates, soil respiration rates, soil water contents, and soil NO_3^- concentrations were measured. Denitrification potentials ranged from <1 to 1900 ng $N \cdot g^{-1} \cdot h^{-1}$, and *in situ* denitrification rates varied from 0.1 to 40 g $N \cdot ha^{-1} \cdot day^{-1}$. Denitrification potentials were highly correlated with soil NO_3^- concentrations and soil water contents; these two soil variables explained more than 90% of the variation in denitrification potentials. Field denitrification rates were best correlated with soil water contents: using multiple regression, up to 79% of the variation in field denitrification rates was explained by soil water contents. Experiments on the short-term dynamics of denitrification following water addition confirmed the importance of soil water content as a regulator of denitrification and suggested that active denitrification requires formation of anaerobic microsites. Extrapolation of seasonal denitrification measurements suggests that relatively little N (<10 kg $N \cdot ha^{-1} \cdot year^{-1}$) is lost from Oregon forest soils as N gases.

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La dénitrification présente une perte potentielle de l'azote des sols forestiers aussi bien qu'une source d'oxydes azotés pour l'atmosphere. Ce processus n'a pas été examiné de près dans les écosystèmes forestiers du Nord-Ouest pacifique. Les buts de ces recherches ont été de regarder *in situ* les taux de dénitrification dans une série d'écosystèmes forestiers, et d'évaluer l'importance de plusieurs propriétés du sol comme facteurs de contrôle de la dénitrification des sols forestiers. Les sols provenant de huit peuplements mûrs de conifères, de trois parcelles récémment entièrement déboisées et de quatre peuplements d'*Alnus rubra* Bong. ont été prélevés au printemps, en été et en automne. Les potentiels de dénitrification (sol en anaérobie), du taux de dénitrification *in situ*, du taux de respiration du sol, du contenu en eau du sol et des concentrations en ions NO_3^- ont été mesurés. Les potentiels de dénitrification varient de <1 à 1900 ng $N \cdot g^{-1} \cdot h^{-1}$, et les taux de dénitrification varient de 0,1 à 40 g $N \cdot ha^{-1} \cdot jour^{-1}$. Les potentiels de dénitrification sont fortement corrélés avec les concentrations en ions NO_3^- et le contenu en eau du sol; ces deux variables du sol ont expliqué plus de 90% de la variation dans les potentiels de dénitrification. Les taux de dénitrification *in situ* sont plus corrélés avec le contenu en eau du sol, et les analyses de regression multiples ont expliqué jusqu'à 79% de la variation des taux obtenus au champ. L'étude de la dynamique de la dénitrification dans le temps après addition de l'eau, a confirmé l'importance du contenu en eau du sol comme facteur de la dénitrification, et a suggéré que la dénitrification active exige la formation de «micropoches anaérobiques.» L'extrapolation des mesures saisonnières de la dénitrification suggère que relativement peu d'azote (<10 kg $N \cdot ha^{-1} \cdot an^{-1}$) s'échappe des sols forestiers de l'Oregon sous forme de gaz azotés.

Introduction

Biological reduction of NO_3^- to gaseous N_2O and N_2 by denitrifying bacteria is one pathway of N loss from ecosystems. Within forest ecosystems, removal of N by denitrification may be important in reducing site fertility (even small yearly rates may add up to a sizeable loss over the length of a rotation); however, in many cases these losses are probably balanced by small yearly inputs of N from precipitation or nonsymbiotic N_2 fixation. Globally, the production of N oxides by denitrification has been implicated as an important source of greenhouse gases (Bowden 1986; Matson and Vitousek 1990). The importance of N-gas fluxes from forest ecosystems relative to N inputs or to N-oxide fluxes from other ecosystems is not well known because relatively few measurements of *in situ* denitrification have been made in forest soils. The lack of data is partly due to practical limitations in methodology and partly to N cycling in forest ecosystems being generally thought of as conservative and therefore N gas outputs negligible (Vitousek *et al.* 1982).

Denitrification in forest soils has been assessed in only a few different forest ecosystems (Bowden 1986; Davidson *et al.* 1990; Dutch and Ineson 1990). Most studies have measured potential denitrification rates (cf. Davidson *et al.* 1990), which are indicative of denitrifier biomass (Myrold and Tiedje 1985*a*) but may not directly relate to *in situ* losses of N gases. Research on *in situ* denitrification rates has been concentrated in deciduous and coniferous forests of the eastern United States (Robertson and Tiedje 1984; Robertson *et al.* 1987; Groffman and Tiedje 1989*a*, 1989*b*). Except for the studies of Klingensmith (1987) on boreal forests in Alaska, only unpublished data on denitrification rates for Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) ecosystems in British

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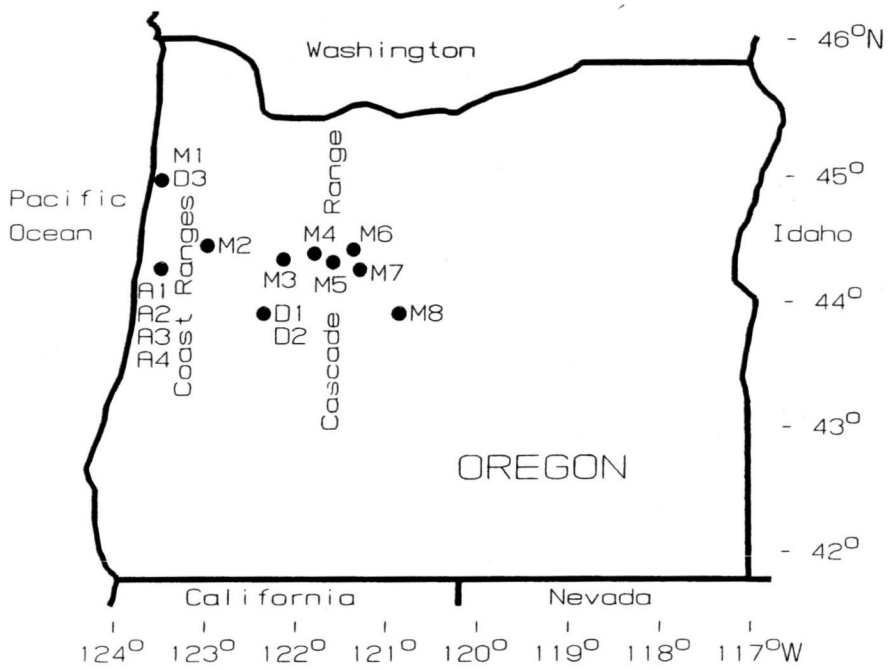

FIG. 1. Location of study sites. A, red alder sites; D, recently clear-cut sites; M, mature conifer sites.

Columbia (Cushon 1985; Martin 1985) are available for western North America. Rates of denitrification in these studies range widely, from <0.01 to 42 kg $N \cdot ha^{-1} \cdot year^{-1}$. More exact information on annual denitrification rates is needed for forest ecosystems in the Pacific Northwest, and high rates should be confirmed to see if such large losses are common. Moreover, some early successional forests in the Pacific Northwest are dominated by N_2-fixing plants, such as red alder (*Alnus rubra* Bong.), which provides large inputs of N (Hibbs and Cromack 1990). These greater N inputs significantly alter N cycling and often enhance nitrification (Van Miegroet and Cole 1985).

The objectives of this study were to (*i*) survey *in situ* denitrification rates in forest soils from a variety of forest ecosystems present in the Pacific Northwest and (*ii*) examine several of the likely regulatory factors and determine their relative importance in controlling denitrification in forest soils.

Study sites

Three groups of forest sites were studied during 1987: eight mature conifer stands representing the major vegetation types found in Oregon (sites M1–M8), four red alder stands of different ages (sites A1–A4), and three recently disturbed conifer sites clear-cut during the summer of 1985 (sites D1–D3). The alder and disturbed sites were chosen to represent soils in which denitrification rates might be enhanced because of greater N availability.

Sites M1–M8 were located along an east–west transect from central Oregon to the Pacific Ocean (Fig. 1). These mature conifer stands, which represent a wide range of vegetation types, soils, climate, and topographic conditions (Table 1), have been studied extensively in previous work (Gholz 1982; Peterson *et al.* 1987; Myrold *et al.* 1989) and serve as a base line for studying effects of forest management on N cycling.

All four alder sites were located within the Siuslaw National Forest, about 15 km from the Pacific Ocean in the Coast Ranges on Bohannon gravelly loam soil (Fig. 1, Table 1). The alder sites differed primarily in stand age, from 8 years (A1) to 78 years (A4); site A1 was located on an area used for storing and loading logs and had compacted soil.

Two of the disturbed sites (D1 and D2) were located on the western flank of the Cascade Range within a few kilometres of each other in the H.J. Andrews Experimental Forest; site D3 was in the Coast Ranges near site M1 in the Cascade Head Experimental Forest (Fig. 1). Site D1 was on MacKenzie River gravelly sandy loam, site D2 was on Carpenter gravelly loam, and site D3 was on an unclassified loam soil (Table 1). Before harvesting, sites D1 and D2 were a mixture of Douglas-fir and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.); site D3 was previously a western hemlock stand. Following clear-cutting and slash burning in 1985, the disturbed sites were planted in 1986 with mixtures of Douglas-fir and red alder (sites D1 and D3) or Douglas-fir and snowbrush ceanothus (*Ceanothus velutinus* Dougl. ex Hook.) on site D2.

Materials and methods

Spring sampling

Alder sites were sampled on 14 May, sites D1 and D2 on 2 June, and site D3 on 4 June for the spring 1987 measurement. The mature conifer stands also were sampled in June. Spring sampling occurred at least 1 week after the last rainfall for all sites.

Denitrification rates were measured using the acetylene inhibition method with intact soil cores (Robertson and Tiedje 1984; Myrold 1988). At each site, 15 soil cores were taken at random locations within a 0.01-ha (5.6-m radius) plot. Soil cores (2.2 cm diameter by 15–20 cm length) encased in acrylic tubes were removed, with minimal disturbance of soil structure, using an impact coring device. The cores were sealed at each end with a rubber septum. Acetylene was added to ensure a partial pressure of at least 10 kPa, and a 4-mL gas sample was taken as an initial sample and stored in a 3-mL evacuated vial. Soil cores were replaced into the hole from which they were collected and incubated *in situ* for about 24 h. The following day, the soil cores were collected, head-space gases were mixed with a 60-mL syringe, and another 4-mL gas sample was taken and stored in a 3-mL evacuated vial. Cores were placed in a cooler, brought to the laboratory, and stored at 4°C until denitrification potentials and NO_3^- concentration were measured within a few weeks. Storage time was not long enough to affect these assays.

In the laboratory, head-space volume was determined using a pressure transducer (Parkin *et al.* 1984), and the cores were extruded,

TABLE 1. Site characteristics of forest stands and soils used to study denitrification in Oregon

Site	Tree species	Soil type	Elevation (m)	Mean annual air temperature (°C)	Mean annual precipitation (cm)	Soil respiration ($mg\ C \cdot kg^{-1} \cdot day^{-1}$)	Soil NO_3^- ($mg\ N \cdot kg^{-1}$)
Mature†							
M1	Western hemlock	Haplohumult	290	10	253	49	0.09
M2	Douglas-fir	Xerochrept	230	11	108	15	0.02
M3	Douglas-fir	Xerumbrept	490	10	158	19	0.02
M4	Mixed conifer	Haplohumult	1220	8	185	23	<0.01
M5	Mountain hemlock	Cryandept	1460	5	212	11	0.03
M6	Ponderosa pine	Vitrandept	915	9	26	4.6	<0.01
M7	Mixed conifer	Vitrandept	1240	7	40	12	0.03
M8	Juniper	Torriorthent	1315	6	26	1.3	0.7
Disturbed							
D1	Douglas-fir, 1 year	Xerumbrept	630	9	165	12	0.95
D2	Douglas-fir, 1 year	Haplohumult	865	9	175	19	0.22
D3	Douglas-fir, 1 year	Dystrochrept	330	10	250	24	2.6
Alder							
A1	Red alder, 8 years	Haplohumult	75	10	200	15	12
A2	Red alder, 31 years	Haplohumult	80	10	200	7.7	17
A3	Red alder, 51 years	Haplohumult	85	10	200	9.5	18
A4	Red alder, 78 years	Halpohumult	110	10	200	39	39

†Data are from Myrold *et al.* (1989).

weighed, mixed, and divided into three subsamples for measurements of NO_3^- concentration (5 g soil in 50 mL of 2 M KCl), denitrification potential (10 g), and gravimetric water content determination.

Nitrate concentrations were determined colorimetrically (diazotization following Cd reduction) using an autoanalyzer. A gas chromatograph equipped with a ^{63}Ni electron capture detector was used to measure the concentrations of N_2O and CO_2 in the gas samples stored in evacuated vials (Parkin 1985; Robertson and Tiedje 1985). The difference between 1-day and initial N_2O and CO_2 concentrations was used to calculate soil denitrification and respiration rates, respectively.

Denitrification potentials were measured using a slight modification of the phase I assay of Smith and Tiedje (1979). A subsample of soil (10 g) was put into a 160-mL serum bottle, and 25 mL of a chloramphenicol solution (500 mg/kg soil), 1 mL of a KNO_3 solution (100 mg N/kg soil), and 1 mL of a glucose solution (1 g C/kg soil) were added. The serum bottle was capped with a butyl rubber stopper, crimp sealed, evacuated, and flushed several times with argon; 10 mL of acetylene was added, the excess gas pressure was relieved, and the serum bottle was placed on an orbital shaker for 2.5 h at room temperature. During this incubation period, five 0.5-mL samples were taken at 30-min intervals and immediately analyzed for N_2O with a gas chromatograph equipped with a ^{63}Ni electron capture detector. Five samples were sufficient to determine the rate (denitrification potential) by linear regression, which was constant over the first 3 h.

Summer sampling

Alder and disturbed sites were sampled during the first 2 weeks of August. No rainfall had been received at any site for at least 2 weeks prior to sampling. Determinations of *in situ* denitrification and soil respiration rates were made at the alder and disturbed sites as described for the spring sampling.

After head-space volume and total weight of soil in the soil cores were determined, soil cores for each site were bulked, mixed, and subdivided for measurement of water content, pH (1:1 soil to water), NO_3^- concentration, and denitrification potential. Denitrification potentials were measured by four different types of anaerobic incubations to determine whether NO_3^- or available C limited expression of denitrification potentials. The four treatments were as follows: only chloramphenicol solution, chloramphenicol solution plus glucose, chloramphenicol solution plus KNO_3, and chloramphenicol solution plus both glucose and KNO_3. The amounts of solutions added and all other aspects of the denitrification potential determinations were as described for the spring sampling. Only three replicates of each treatment per site were performed, because the variability was low using a composite sample.

Autumn sampling

At the time of autumn sampling (26 October for alder sites, 27 October for disturbed sites) no rainfall had occurred since before the summer sampling period. For this reason, no *in situ* incubations were done. Instead, 15 cores from each site were taken to the laboratory to study the temporal response (0–52 h) of denitrification to simulated rainfall. Water was added to each core at the rate of 3 mm per centimetre of soil, which is less than half the average September and October rainfall. Acetylene was added (10 kPa), head-space gases were mixed, and an initial 0.5-mL sample was taken for measurement of N_2O. Soil cores were incubated at 12°C (the ambient soil temperature) and sampled for N_2O concentration after 6, 24, 30, 48, and 54 h of incubation, and denitrification rates were calculated for each of these intervals. Head-space volumes were measured, a 15-g subsample was removed from each core to form a composite sample by site, which was used to measure denitrification potential, and the remainder was dried to determine water content. Denitrification potentials of composite samples were measured as described for the spring samples, with three replicates per site.

Estimation of annual rates

Annual rates of *in situ* denitrification losses from the alder and disturbed sites were estimated from mean values of the spring, summer, and autumn denitrification rates. Annual rate for the mature conifer sites came from monthly (less frequently when snow covered) denitrification measurements (Myrold *et al.* 1989). Because denitrification rates were measured at relatively few points in time, these estimates are speculative but still useful for comparative purposes. Annual rates were extrapolated by numerically integrating the area under the rate versus time curve. This was done by connecting data points with a straight line, calculating the area of the resulting trapezoids using the standard trigonometric formula, and summing these areas. This procedure is often called trapezoidal quadrature or integration using the trapezoidal rule.

Statistical analysis

As found in many previous studies (Parkin *et al.* 1987; White *et al.* 1987; Myrold 1988), denitrification rates, denitrification potentials, and soil NO_3^- concentrations were lognormally distributed when

measurements were made on individual cores. Means and 95% confidence intervals were therefore calculated on log-transformed data (Sichel 1966; Parkin *et al.* 1988, 1990). Because some *in situ* denitrification rates were zero (or in some cases slightly negative because of measurement error), a small constant value was added to all data before log transformation and was subsequently subtracted after back transformation to obtain the final mean value. This constant was set equal to the following: 2 × [the 95% confidence interval around the mean atmospheric N_2O concentration (~25 ng N per core)] ÷ (the mass or surface area of soil) ÷ (the length of the incubation period). The choice of this constant was arbitrary, and tests with several values did not greatly affect the final mean estimate. Data from composited soil samples were not lognormally distributed, so standard statistical methods were used to compute the means and 95% confidence intervals for these data.

One-way ANOVA was done on denitrification rates and denitrification potentials for each of the three sampling dates, using log-transformed data when necessary. If the ANOVA showed significant site differences, the Student–Newman–Keuls test was used to compare mean values within sites.

Correlation coefficients and forward multiple regressions were calculated to determine the relationships between denitrification rates or denitrification potentials and other soil properties. Variables were introduced into the regression model according to the contribution of those variables to the preceding R^2. New variables were selected based on their ability to increase the R^2. A Fisher test gave the probability of having no linear relationship between the independent variables introduced in each regression model and the dependent variable.

Results and discussion

Soil properties

Soil properties that affect denitrification vary greatly among the sites (Table 1). Mean daily soil respiration rates ranged from about 1 to 50 mg $C \cdot kg^{-1} \cdot day^{-1}$, but this variation is about as wide within the mature, disturbed, and alder site groups as among these groups. Differences in mean soil NO_3^- concentrations were even greater, ranging over three orders of magnitude. Soil NO_3^- concentrations were 10 times greater in the alder soils compared with soils from the other sites; NO_3^- concentrations were generally higher in disturbed sites than in mature stands. Differences in soil NO_3^- undoubtedly resulted from (*i*) the greater input of N from N_2 fixation, which subsequently enhanced net N mineralization and nitrification in the alder stands (Van Miegroet and Cole 1985), and (*ii*) the greater rates of net N mineralization and nitrification often observed in recently disturbed soils (Vitousek *et al.* 1982).

Denitrification potentials

Denitrification potentials varied among sites and sampling dates (Table 2). Most sites showed an increase in denitrification potentials from spring to summer followed by a decrease in autumn to levels than were generally lower than those measured in spring. These seasonal changes were usually significant for the alder sites. Such temporal shifts have often (Alfani *et al.* 1983; Groffman 1987; Rashid and Schaefer 1987), but not always (McClellan 1987), been observed and are presumably linked to fluctuations in environmental conditions, which control the development of active denitrifying populations.

Differences in denitrification potentials among sites were more pronounced than seasonal changes at a site (Table 2). The lowest denitrification potentials were measured at sites D1 and D2 in the Cascades. No detectable denitrification potential was measured at site D2, which indicates that if denitrifying bacteria are present at site D2, environmental conditions were not suitable to induce denitrifying activity. Site D3 in the Coast Ranges had significantly higher denitrification potentials than other disturbed sites, and these were similar to the denitrification potentials of the younger alder stands. The higher denitrification potential found at site D3 reflects higher soil NO_3^- concentrations, somewhat higher soil respiration rates, and wetter soil conditions (Table 1). Denitrification potentials were generally highest on the alder sites, which showed increasing denitrification potentials with stand age. The upward trend of higher denitrification potentials in older alder stands followed similar trends in soil NO_3^- concentrations and, except for site A1, soil respiration rates.

Regression analysis gave significant correlations between denitrification potentials and soil NO_3^- concentrations and, to a lesser extent, soil water content and soil respiration rate (Table 3). Using multiple regression, 94 to 99% of the variation in spring and summer denitrification potentials was accounted for by either soil NO_3^- concentrations alone or a combination of NO_3^- and soil water contents. Presumably, high correlations also would have been obtained in the autumn if soil NO_3^- concentrations had been measured. For example, denitrification potentials measured in autumn were significantly correlated with mean soil NO_3^- concentrations from the spring and summer samples ($r = 0.89$, $p < 0.01$), and multiple regression of denitrification potentials with mean soil NO_3^- concentrations and with soil water contents was

TABLE 2. Denitrification potentials (ng $N \cdot g^{-1} \cdot h^{-1}$) of forest soils from red alder and recently clear-cut sites

Season	Site	Mean†	95% confidence limits Min.	Max.
Spring‡	A1	104*c*	77	171
	A2	201*b*	172	254
	A3	186*b*	149	263
	A4	1010*a*	790	1500
	D1	7*d*	4	16
	D2	<1*e*		
	D3	81*c*	60	129
Summer§	A1	102*d*	83	121
	A2	264*c*	220	309
	A3	587*b*	553	622
	A4	1860*a*	1630	2080
	D1	8*e*	4	11
	D2	<1*e*		
	D3	211*c*	100	324
Autumn§	A1	34*d*	−3	72
	A2	81*c*	54	108
	A3	147*b*	87	207
	A4	704*a*	641	767
	D1	<1*e*		
	D2	<1*e*		
	D3	118*b*	52	184

NOTE: Mean values presented as <1 were below the detection limit.

†Significance was determined by the Newman and Keuls test. Means within a season followed by the same letter are not significantly different ($p = 0.05$).

‡Means of 15 cores per site. Confidence limits were calculated with Sichel's estimator for lognormally distributed data.

§Means of three replicates from a composite sample of 15 cores per site. Confidence limits were calculated using the *t*-statistic.

TABLE 3. Factors explaining the variation in denitrification potentials of forest soils from red alder and recently clear-cut sites ($n = 7$)

Season	Factor	r†	Additive R^2‡	p-value§
Spring	NO_3^-	0.90***	0.82	0.005
	H_2O	0.84**	0.99	0.002
	CO_2	0.72*	0.99	0.324
	pH	nd		
Summer	NO_3^-	0.96***	0.91	0.001
	H_2O	0.76**	0.94	0.320
	CO_2	0.72*	0.94	0.876
	pH‖	−0.93***		
Autumn	CO_2	0.85**	0.71	0.018
	H_2O	0.67*	0.72	0.694
	NO_3^-	nd		
	pH	nd		

†Significance level (p) of r-values: *, **, ***, 0.10, 0.05, and 0.01, respectively; nd, not determined.

‡Proportion of the variation explained by the combination of the factor plus all preceding factors.

§Significance level.

‖pH was not included in the multiple regression analysis.

significant ($R^2 = 0.94$, $p = 0.0310$). These correlations suggest that the establishment of actively denitrifying populations in forest soils depends upon (*i*) the presence of NO_3^-, an alternative electron acceptor used by denitrifiers under anaerobic conditions, and (*ii*) wet conditions conducive to the establishment of anaerobic microsites in the soil. The availability of C, as indexed by soil respiration rates, appears less critical to the development of actively denitrifying populations in these soils. This is in contrast with studies in agricultural soils (Myrold and Tiedje 1985*a*) and likely reflects the higher C content often found in forest soils. The greater importance of NO_3^- and water over C in controlling the denitrification potential in forest soils agrees with studies from eastern temperate forests (Davidson and Swank 1987; Groffman and Tiedje 1989*a*, 1989*b*).

A significant negative correlation of denitrification potentials with soil pH was observed in summer. This high correlation probably reflects the lower pH of soils with higher NO_3^- concentrations ($r = -0.97$) rather than any cause and effect relationship, because denitrifying bacteria generally are inhibited at low soil pH (Firestone 1982). The inverse relationship between soil NO_3^- concentrations and pH is likely caused by acidification associated with the nitrification process (Van Miegroet and Cole 1985).

Denitrification potentials vary widely among forest soils in Oregon (Table 6). The range of denitrification potentials from <1 to almost 2000 ng $N \cdot g^{-1} \cdot h^{-1}$ agrees with the compilation given by Davidson *et al.* (1990). In particular, the denitrification potentials for red alder stands found in this study are very similar to those reported by Klingensmith (1987) for alder stands in Alaska; those for Douglas-fir stands are similar to denitrification potentials measured by McClellan (1987) for Douglas-fir soils in Oregon. Denitrification potentials measured in this study for red alder stands in the Coast Ranges are about 10 times those reported by McClellan (1987) for red alder stands in the Cascades and probably reflect lower NO_3^- concentrations in Cascade soils and possibly differences in other soil properties. Such differences were apparent in soil properties of the disturbed sites in this study (Table 1).

TABLE 4. Field denitrification rates (g $N \cdot ha^{-1} \cdot day^{-1}$) of forest soils from red alder and recently clear-cut sites (data are based on 15 cores per site per season)

			95% confidence limits‡	
Season	Site	Mean†	Min.	Max.
Spring	A1	1.01*a*	0.65	1.79
	A2	0.435*ab*	0.360	0.544
	A3	0.518*ab*	0.318	0.914
	A4	0.064*bc*	−0.053	0.292
	D1	0.263*abc*	0.163	0.436
	D2	0.139*bc*	0.035	0.490
	D3	0.022*c*	−0.141	0.400
Summer	A1	0.622*ab*	0.335	1.26
	A2	0.113*bc*	0.012	0.294
	A3	0.513*abc*	0.120	1.70
	A4	0.542*ab*	0.309	1.03
	D1	0.200*bc*	0.113	0.349
	D2	0.003*c*	−0.070	0.128
	D3	1.98*a*	0.82	7.19
Autumn§	A1	36.5*a*	12.0	344
	A2	0.138*b*	0.061	0.267
	A3	1.34*b*	0.44	4.44
	A4	16.9*a*	7.3	99.6
	D1	0.192*b*	−0.020	0.694
	D2	−0.032*b*	−0.076	0.040
	D3	40.1*a*	16.8	285

†Significance was determined by the Newman and Kuels test. Means within a season followed by the same letter are not significantly different ($p = 0.05$).

‡Confidence limits were calculated with Sichel's estimator for lognormally distributed data.

§Rate was determined over a 24-h period that began 30 h after the addition of water.

Field denitrification rates

Rates of denitrification measured *in situ* (coefficients of variation (CV) of 100–300%) were inherently more variable than denitrification potentials (CV of 10–100%). Seasonal changes were less consistent for field denitrification rates than for denitrification potentials (Tables 2 and 4). For example, denitrification rates increased from spring to summer at sites A4 and D3 but decreased or stayed the same at all other sites; whereas denitrification potentials during this interval increased at all sites except site A1, which showed no change. The simulated rainfall for the autumn sample stimulated denitrification over summer rates at most sites, although sites A2, D1, and D2 maintained similar rates.

Site differences in field denitrification rates generally did not follow trends observed in denitrification potentials among sites. The Cascade sites D1 and D2, however, which had very low denitrification potentials, also had consistently low denitrification rates of less than 0.2 g $N \cdot ha^{-1} \cdot day^{-1}$, and these values often were not significantly different from zero (Table 4). Site D3 had the highest denitrification rate in summer and autumn but had the lowest rate in spring. The low spring rate was likely related to the low soil NO_3^- concentration measured at that time (0.48 mg $N \cdot kg^{-1}$). Among the alder stands, the youngest (A1) and oldest (A4) usually had the highest rates of denitrification, which seemed to be related to higher soil respiration rates (Table 1).

TABLE 5. Factors explaining the variation in field denitrification rates of forest soils from red alder and recently clear-cut sites ($n = 7$)

Season	Factor†	r‡	Additive R^2§	p-value‖
Spring	H_2O	–0.68*	0.46	0.095
	NO_3^-	0.11	0.79	0.066
	CO_2	–0.66	0.81	0.587
	DP	–0.27	0.85	0.559
	pH	nd		
Summer	H_2O	0.58	0.33	0.174
	DP	0.04	0.71	0.083
	CO_2	0.40	0.86	0.164
	pH	–0.11		
	NO_3^-	0.01		
Autumn	CO_2	0.49	0.24	0.260
	DP	0.12	0.55	0.172
	H_2O	0.47	0.64	0.469
	NO_3^-	nd		
	pH	nd		

†DP, denitrification potential.
‡Significance level (p) of r-values: *, 0.10; nd, not determined.
§Proportion of the variation explained by the combination of the factor plus all preceding factors.
‖Significance level.

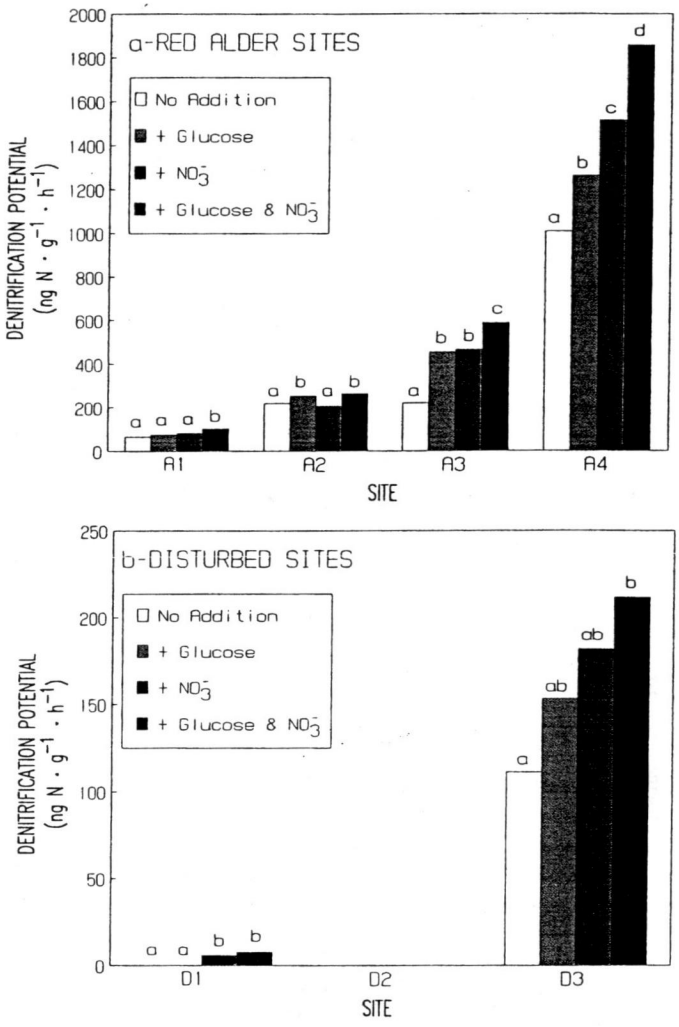

FIG. 2. Effects of glucose and NO_3^- on denitrification potentials for soils sampled in summer. Within a site, different letters denote significant treatment differences ($p < 0.05$) using the Student–Newman–Keuls multiple range test. A, red alder sites; D, recently clear-cut sites.

Correlations of field denitrification rates with individual soil properties were highest for soil water content followed by CO_2 production rates, but these correlations were rarely significant (Table 5). Soil respiration rates were, to some degree, reflective of soil water contents (these two soil properties are correlated at $r = 0.92$, 0.80, and 0.70 for the spring, summer, and autumn sample times, respectively). The lack of significant correlations and the negative correlation of water content and soil respiration with denitrification activity in spring preclude drawing any conclusions regarding cause and effect. It is generally accepted, however, that high soil water contents function to promote denitrification by reducing O_2 diffusion, stimulating microbial activity, and promoting diffusion of NO_3^- and soluble C. Because soil respiration indicates C availability and O_2 utilization, soil water content and microbial respiration are thought to act together to produce anaerobic sites in soils.

Several previous studies have found soil respiration rate and soil water content to be good predictors of field denitrification in both agricultural (Rice *et al.* 1988; Robertson *et al.* 1988; Myrold 1988) and forest soils (Robertson and Tiedje 1984; Davidson and Swank 1986). Other soil properties, such as nitrification rates (Robertson and Tiedje 1984) or denitrification potentials (Groffman and Tiedje 1989*b*), are sometimes better predictors, however. The low correlation of field denitrification rates with soil NO_3^- concentration (Table 5) agrees with studies in eastern temperate forests, which found NO_3^- production potentials to be a better predictor than soil NO_3^- pool sizes (Robertson and Tiedje 1984; Davidson and Swank 1986).

Multiple regression analyses were significant ($p < 0.1$) in the spring and summer, with two-variable models accounting for 71 to 79% of the variation in field denitrification rates (Table 5). Soil water content was the most important factor in these regressions, with either soil NO_3^- concentration or denitrification potential being of secondary importance. The amount of variation explained by the multiple regressions compares favorably with other studies in which 36 to 53% of the variation could be explained by soil properties (Robertson and Tiedje 1984; Myrold 1988; Robertson *et al.* 1988; Groffman and Tiedje 1989*b*).

Upon the establishment of anaerobic microsites, the supply of either NO_3^- or available C will likely become limiting for denitrification. Figure 2 shows the response of denitrification activity in anaerobic soil slurries to additions of NO_3^- and glucose. All soils that had measurable activity responded to the combined addition of NO_3^- and glucose with increased rates of N_2O production, three of the soils responded positively to the addition of glucose alone, and two soils had significantly higher denitrification rates when NO_3^- alone was added. Positive responses to glucose or NO_3^- were not related to soil NO_3^- concentrations or soil respiration rates (Table 1), making these results difficult to interpret. Nevertheless, it is clear that either NO_3^- or C will ultimately limit denitrification rates when anaerobic conditions prevail.

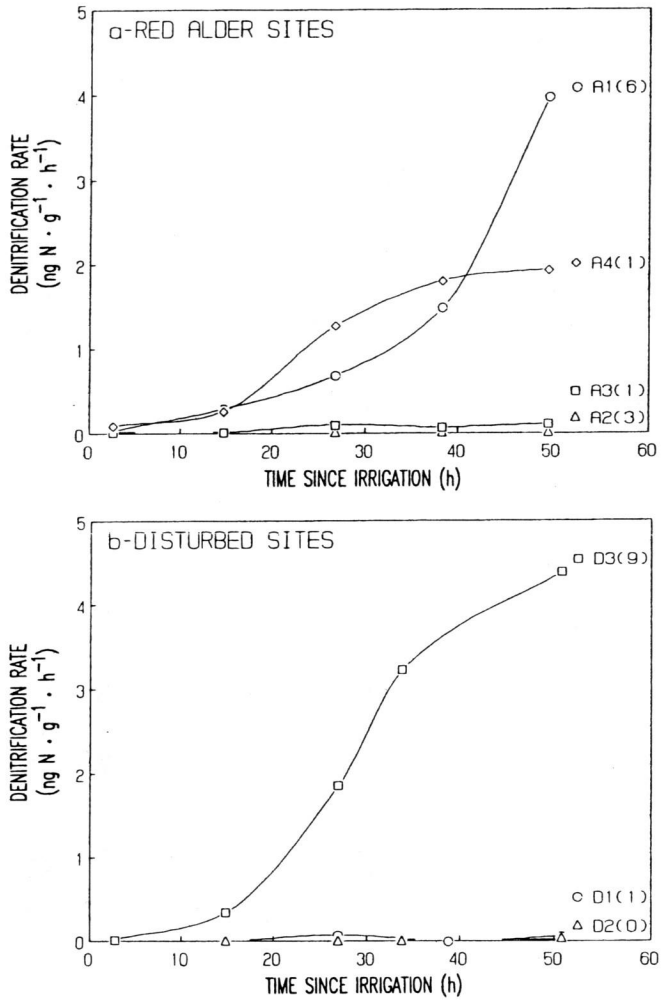

FIG. 3. Temporal responses of denitrification rates to simulated rainfall during the autumn incubation. Each data point is the mean of 15 observations. Numbers in parentheses are the number of cores with denitrification rates significantly greater than zero. A, red alder sites; D, recently clear-cut sites.

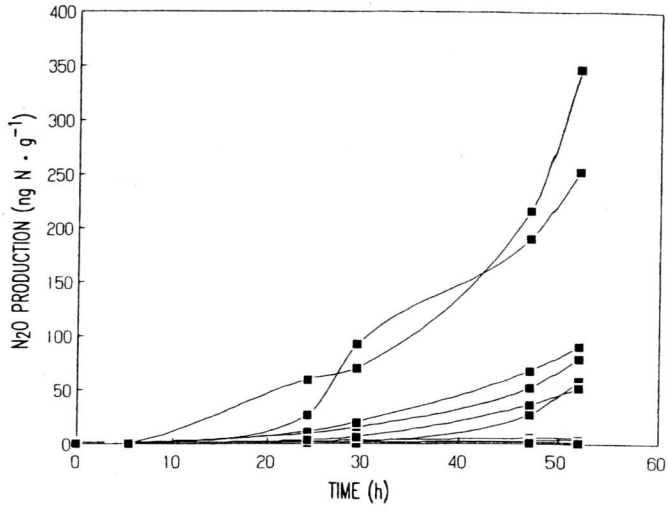

FIG. 4. Variation in temporal response of denitrification to simulated rainfall during the autumn incubation. Data are for 15 soil cores from site A1 (a red alder site); similar variability was shown at other sites where denitrification responded to water addition.

Short-term response of denitrification to water addition

There was a temporal response in denitrification to water additions at all alder sites and at site D3 (Fig. 3), which were the only sites with detectable denitrification potentials. During the first 24 h after water addition, denitrification rates were lower for all sites than rates measured in summer, thereafter denitrification rates increased for all sites except D1 and D2, which had no detectable denitrification activity. Typically, denitrification rates were tenfold higher after 48 h than they were at 24 h, but these rates are still <10%, and in most cases <1%, of their respective denitrification potentials.

Temporal responses to water addition were slower and more sustained than those reported for coarse-textured forest (Robertson and Tiedje 1984) or agricultural (Sexstone *et al.* 1985) soils. The soils from the alder and D3 sites were well-aggregated loams and displayed lag times more similar to those observed by Sexstone *et al.* (1985) for an aggregated clay loam agricultural soil. The length of the lag time before induction of higher denitrification activity and the duration of the higher rate is probably related to the rate of water infiltration through the soil core and concurrent water movement into soil aggregates. Unlike the clay loam soil, the loam soils from the alder and D3 sites maintained high denitrification rates 48 h after water addition. This sustained level of active denitrification may be a result of greater C availability in forest compared with agricultural soils (Myrold and Tiedje 1985*b*).

Water content of the wetted cores was much less variable (CV of 9–35%) than denitrification rates (CV of 150–350%). In part, this was because, even within a site, not all cores responded to addition of water (Fig. 4). Most soil cores had little denitrification activity, a few had high rates, and there were some cores with intermediate rates. This core to core variation in denitrification following water addition is probably because of uneven development of anaerobic microsites, or hot spots, in the cores (Parkin 1987). It is interesting that the number of cores per site that responded to water addition (Fig. 3) was linearly related to the mean denitrification rate ($r = 0.97$). This relationship also supports the idea of hot spot formation and suggests that the occurrence of denitrification is perhaps best thought of as being discrete rather than continuous in time and space, i.e., if a hot spot forms, a high rate results; if one does not form, only a background level of denitrification occurs.

Annual denitrification losses from Oregon forest soils

Estimates of denitrification rates for Oregon forest soils range from <0.01 to about 6 kg $N \cdot ha^{-1} \cdot year^{-1}$ (Table 6). These annual denitrification rates are within the range of literature values for forest soils (Bowden 1986; Davidson *et al.* 1990; Dutch and Ineson 1990). Losses of N from red alder stands via denitrification agree with measurements of losses from alder stands in Alaska (0–3.7 kg $N \cdot ha^{-1} \cdot year^{-1}$; Klingensmith 1987) and Denmark (4.9 kg $N \cdot ha^{-1} \cdot year^{-1}$; Struwe and Kjøller 1989, 1990). All alder sites used in this study were in upland positions, and it is possible that greater losses could occur near stream channels (Davidson and Swank 1986). McClellan (1987) did find greater denitrification potentials in floodplain soils than in upslope soils.

TABLE 6. Summary of denitrification data for the Pacific Northwest

Site	Denitrification potential† (ng $N \cdot g^{-1} \cdot h^{-1}$)	Denitrification rate‡ (kg $N \cdot ha^{-1} \cdot year^{-1}$)
M1	216 (9.4)	<0.01
M2	21 (1.6)	<0.01
M3	4 (0.4)	<0.01
M4	1 (0.5)	<0.01
M5	<1	<0.01
M6	<1	<0.01
M7	<1	<0.01
M8	2 (0.3)	<0.01
D1	1–8	0.08
D2	<1	0.02
D3	81–211	6.1
A1	34–104	5.4
A2	81–264	0.09
A3	147–587	0.3
A4	704–1860	2.5

NOTE: Values presented as <1 and <0.01 were below detection limits.

†Mean and standard error of the mean (n=3) are shown for mature sites; range of spring, summer, and autumn data are shown for disturbed and alder sites.

‡Annual rates were extrapolated from monthly (mature sites) or seasonal (disturbed and alder sites) rate measurements using a trapezoidal integration method. Rates for mature sites are from Myrold *et al.* (1989).

Denitrification was below detection limits for all mature conifer stands studied, and rates ranged from 0.02 to 6.1 kg $N \cdot ha^{-1} \cdot year^{-1}$ in recently clear-cut sites. These rates are in agreement with all reported values except those of Martin (1985) for Douglas-fir on Vancouver Island, which are about 10 times higher. Differences in soil properties may account for the much higher denitrification rate observed by Martin (1985), because soil NO_3^- concentrations were about five times higher in the Vancouver Island soils than those measured at site D3, the most actively denitrifying non-alder soil.

Conclusions

The results from this study suggest that although significant potential for denitrification exists in many forest soils of Oregon, particularly in the Coast Ranges and under red alder vegetation, this potential is seldom realized. There are only minimal losses from mature forests. In most cases, losses of N from denitrification are probably <10 kg $N \cdot ha^{-1} \cdot year^{-1}$, even on recently harvested sites or under red alder.

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