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Effects of vegetation regime on denitrification potential in two tropical volcanic soils

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Abstract. Effects of vegetation and nutrient availability on potentail denitrification rates were studied in two volcanic, alluvial-terrace soils in lowland Costa Rica that differ greatly in weathering stage and thus in availability of P and base cations. Potential denitrification rates were significantly higher in plots where vegetation had been left undisturbed than in plots where all vegetation had been removed continuously, and were higher on the less fertile of the two soils. The potential denitrification rates were correlated strongly with respiration rates, levels of mineralizable N, microbial biomass, and moisture content, and moderately well with concentrations of extractable NH_4^+, Kjeldahl N, and total C. In all plots, denitrification rates were stimulated by the removal of O_2 and by the addition of glucose but not by the addition of water or NO_3^-.

Key words: Denitrification – Soil respiration – Nitrous oxide – Tropical volcanic soils – Microbial biomass

Under certain conditions, the conversion of NO_2^- or NO_3^- to NO or N_2O by dissimilatory reduction constitutes a large loss of N from soils in a form that can contribute significantly to atmospheric levels of these greenhouse gasses (Groffman 1991). This flux may be of greatest importance in the tropics (Davidson 1991), where N generally cycles faster than in temperate zones (Vitousek 1984; Vitousek and Sandford 1986; Matson et al. 1987). Denitrification from tropical soils accounts for up to 50% of the global production of N_2O, an important "greenhouse" gas (Weiss 1981; Rosswall and Paustian 1984; Crutzen et al. 1985; Rasmussen and Kahlil 1986; Kellet et al. 1988). Evidence is accumulating that the land-use and vegetation regime can strongly influence rates of N_2O production (Dickinson and Cicerone 1986; Robertson and Tiedje 1988; Groffman 1991) and release into the atmosphere (Jacob and Bakwin 1991); thus, changes in land-use patterns in the tropics have the potential to greatly affect any trend toward global warming.

There are still significant gaps in our knowledge of the mechanisms controlling denitrification. In soils, it requires a population of denitrifying micro-organisms, a supply of energy and NO_2^- or NO_3^-, reducing conditions, and a non-inhibiting temperature and moisture level (Robertson 1989; Tiedje et al. 1989). Most of these factors are affected directly or indirectly by vegetation.

Currently, there is little information concerning the influence of vegetation on denitrification. The main objective of the present study was to determine the degree to which vegetation influences potential denitrification rates in two volcanic-derived tropical soils of contrasting fertility.

Materials and methods

Site description

Soil samples were collected from experimental plots set up as part of a larger study of factors regulating nutrient availability (Sollins and Radulovich 1988; Robertson 1989). The plots lie on two river terraces at the La Selva Field Station in lowland northeastern Costa Rica. Both terraces were first logged in the late 1950s, then farmed extensively until abandonment ca. 1980. At the time of sampling, the control areas supported a mixture of grass, shrubs, and scattered trees. A forested site on the upper terrace near the experimental plots was sampled also. This site, abandoned in the 1970s and regrown in mixed second-growth forest, was that used by Radulovich et al. (1992) for soil water flow experiments.

The lower-terrace soil (Limonal consociation) is a Fluventic Eutropept rich in exchangeable bases and extractable P; the older, more weathered upper-terrace soil (Helechal consociation) is a borderline Oxic Humitropept/Oxic Dystropept, very low both in bases and in P. Both soils have been described more fully elsewhere (Sollins and Radulovich 1988; Sollins et al. 1993); selected data are presented here (Table 1).

The experimental plots were established in spring 1987 on each terrace in four replicate blocks of three treatments: (1) all vegetation and

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Table 1. Characteristics of the two La Selva soils used in the study (after Sollins et al. 1993)

Consociation	Topographic position	Parent material	Texture	pH (H_2O)	Organic matter (%)	Sum of bases ($cmol_c\ kg^{-1}$)	KCl acidity ($cmol_c\ kg^{-1}$)	P ($mg\ kg^{-1}$)
Helechal	Upper alluvial terrace	Andesitic to basaltic sands and silts	Clay	4.3	8.7	1.0	2.7	~4
Limonal	Lower alluvial terrace	Andesitic to basaltic sands and silts	Sandy loam	6.1	5.4	15.7	0.6	~9

Texture and chemical characteristics refer to the A horizon (0 – 10 cm depth).
P extractable with acid ammonium fluoride. Values based on averages for the indicated topographic position across the La Selva Biological Station

litter removed and revegation prevented by periodic hand-weedling (bare), (2) vegetation and litter removed annually with regrowth of native plants allowed in between (annual cut), and (3) an undisturbed control.

Sampling techniques and storage

All samples were taken with a tubular corer to a depth of 15 cm after any litter layer was scraped away. An initial set of samples for denitrification assays was collected in June 1991. At least four cores were taken within each plot, then composited. The samples were kept refrigerated at La Selva for several days, then transported to Oregon State in a polystyrene cooler (ca. 5 days' travel time). A second set of samples was collected in August 1991 from the upper-terrace plots only and used for amendment experiments. Duplicate subsamples were analyzed from a pooled sample made up of three cores. These cores were taken in each of four plots of each treatment.

Data on NO_3^-, Kjeldahl N, total C, and extractable P were obtained from samples collected routinely at regular intervals since plot establishment in 1987. We used the 22 August 1991 collection data since this was closest to our June soil sampling.

Analytical methods

Microbiological methods were as described by Griffiths et al. (1990) except that denitrification and respiration rates were measured at 25 °C. The dry weight fraction is the ratio of dry to fresh weight.

To measure denitrification, flasks were stoppered, purged with Ar for 4 min at 200 ml min^{-1}, then incubated for 2 h. Checks of selected samples showed that the N_2O production rate was constant over the incubation period. For one-third of the samples, chosen at random, a replicate was run with 10% C_2H_2 atmosphere in order to inhibit reduction of N_2O to N_2. In the amendement experiments, we added 2 ml distilled water or glucose, NO_3^-, or glucose+NO_3^- (all at 1 m*M*) to 5 g field-moist soil placed in a 25-ml Erlenmeyer flask, and then measured denitrification rates as described above.

Respiration rates were determined by monitoring CO_2 concentrations in the headspace of 25-ml Erlenmeyer flasks containing 5 g field-moist soil. The CO_2 concentration was determined by gas chromatography at time 0 and 1 h. Mineralizable N under anerobic conditions, was determined by incubating 10 g field-moist soil in 57-ml screwtop test-tubes that had been filled with de-ionized water and sealed (Griffiths et al. 1991). The tubes were incubated for 7 days at 40 °C. Mineralizable N was calculated as NH_4^+ present after incubation (Corning NH_4^+ electrode) less that present before.

Chloroform – fumigation flush was determined by a modification of the method of Jenkinson and Powlson (1976). Test-tubes (57 ml) containing 10 g field-moist soil were placed in a large desiccator jar with a source of distilled chloroform, and a vacuum was drawn three times. The tubes were then held for 24 h in a water-saturated atmosphere, evacuated three times to remove the chloroform, and sealed with serum bottle caps and two layers of tape. They were then incubated at 25 °C for 10 days. The CO_2 released during this period was assayed by gas chromatography.

Total C was determined with a LECO Carbon analyzer and P by extraction with acid-ammonium fluoride followed by colorimetric analysis (Alpchem Rapid Flow Analyzer). Other results in Table 1 were obtained by standard methods (described more fully by Sollins et al. 1993).

Statistical approaches

Except where indicated, all values are expressed per gram dry weight of soil. Statistics were calculated with Statgraphics (Statistical Graphics Corporation, Rockville, Maryland, USA). The significance of differences between mean values was determined with Fisher's protected least significant difference at the level $P < 0.05$. The data were analyzed for two-way interactions between site and treatment. The significance of linear correlations was calculated by the Spearman rank-correlation method.

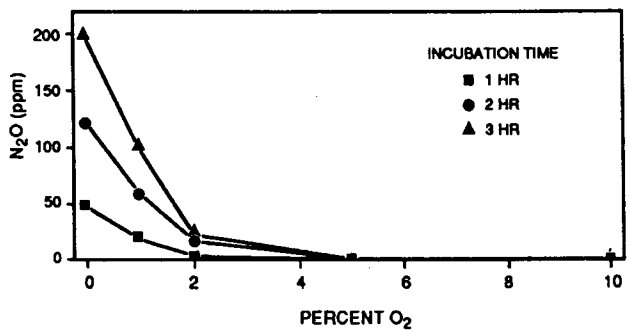

Fig. 1. Effects of headspace O_2 concentration on denitrification rates

Results and discussion

Experiment 1: Unamended soils

The denitrification rate was highest (Fig. 1) when the headspace was maintained O_2-free (100% Ar). After a 3-h incubation under an atmosphere of 1% O_2, the concentration of N_2O in the headspace dropped to approximately half that when O_2 was absent. At 2% O_2, N_2O was barely detectable above the background level.

The presence or absence of C_2H_2 had no effect on the rate of N_2O production ($r = 0.98$ by paired *t*-test). This finding indicates that N_2O was the major product of denitrification in these assays. Since these soils were not analyzed within 24 h of sampling, it is possible that the ability of the microbial population to reduce N_2O to N_2 was impaired (Erich et al. 1984). Thus, it is not clear whether the dominant product in situ is N_2O . We are not aware of any other studies of denitrification in tropical soils in which N_2O release was monitored with and without the acetylene block in the same samples.

Vegetation regime had a clear effect on potential denitrification (Fig. 2A) with higher rates in the control plots than in the bare plots on both terraces. This was ex-

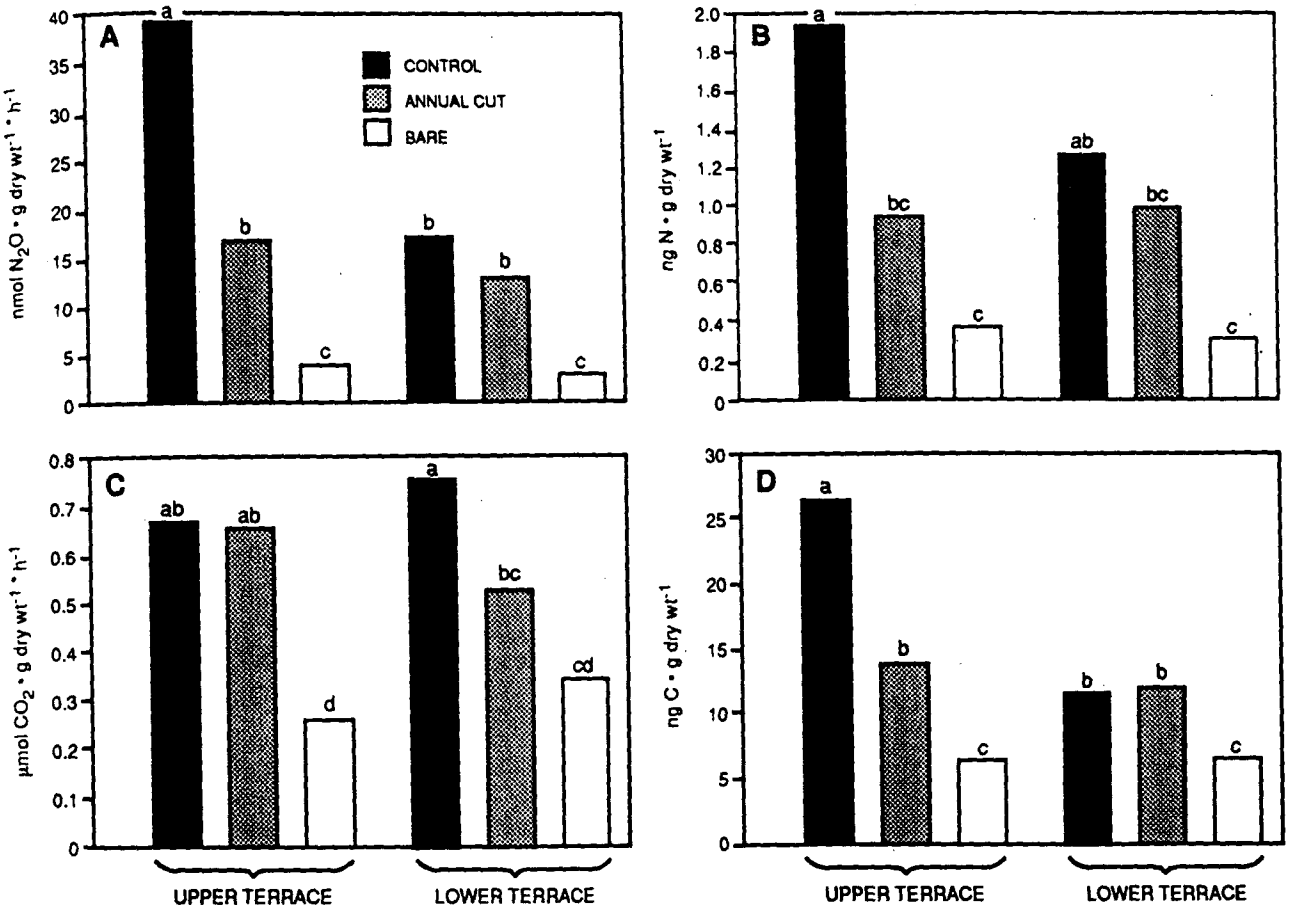

Fig. 2 A–D. Effects of vegetation on four microbial processes: **A** potential denitrification; **B** mineralizable N; **C** respiration; and **D** chloroform fumigation-flush CO_2. The sites were two differing alluvial terraces at La Selva, Costa Rica; vegetation regimes were annual removal of vegetation and litter with revegetation allowed (*ANNUAL CUT*); complete removal of vegetation and litter with no revegetation allowed (*BARE*); and no disturbance (*CONTROL*); *dry wt*, dry weight

pected because denitrifiers require a C source in order to reduce NO_2^- or NO_3^- to N_2O. The same general pattern held for mineralizable N (Fig. 2B), respiration (Fig. 2C), and microbial biomass as indexed by chloroform fumigation-flush CO_2 (Fig. 2D). Working also at La Selva, and comparing eight sites that differed with respect to both vegetation and soil type, Robertson and Tiedje (1988) found large differences in denitrification rates measured in situ, but it was unclear to what extent the differences might be due to soil type as opposed to vegetation regime.

In general, total C and Kjeldahl N concentrations were highest in the control plots, intermediate in the annual cut plots, and lowest in the bare plots (Fig. 3A, B). NO_3^- levels were roughly similar on both terraces and showed no consistent pattern with vegetation regime (Fig. 4). The denitrification rates were well correlated with concentrations of total C and Kjeldahl N but not with NO_3^- levels (Table 2). C,N, and nitrate, however, had been measured on a separate set of samples, so changes through time might have affected the correlations, especially for NO_3^-.

The denitrification rates were also correlated strongly with respiration, anerobically mineralizable N, chloroform fumigation flush, and dry weight fraction (Table 2). The high correlations with mineralizable N and chloroform fumigation flush CO_2 may reflect a high correlation with the microbial biomass, which these factors are thought to measure (Myrold 1987).

Microbial activity was generally highest on the upper terrace, which is the less fertile and productive of the two. P availability may explain this pattern; it is much lower on the upper terrace (Fig. 5). Perhaps soil organic matter is decomposed more rapidly where P is a limiting factor as the microflora attempt to obtain P. Plant growth was shown to be P-limited on some La Selva soils similar to our upper terrace soil (Vitousek and Denslow 1986; 1987), and Caldwell et al. (1993) found higher levels of activity of several soil enzymes on the upper-terrace than on the lower-terrace plots.

Experiment 2: Amended soils

After our first experiment, which showed large effects of vegetation regime on denitrification rates, we conducted a second experiment in which soils from the upper-terrace study plots, and from an adjacent forested area, were amended with water or NO_3^- and/or glucose. As shown in Fig. 6, unamended controls gave rates similar to those obtained in the first experiment, and the forest and control plots gave roughly similar results.

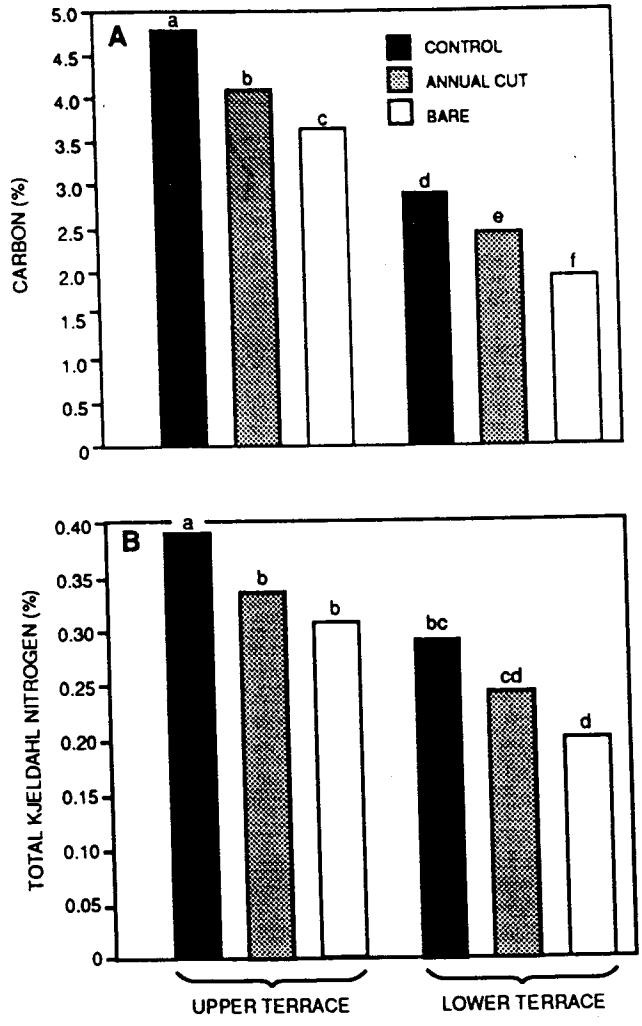

Fig. 3. Total soil C and Kjeldahl N concentrations for the study site and vegetation combinations; for further explanations, see Fig. 2

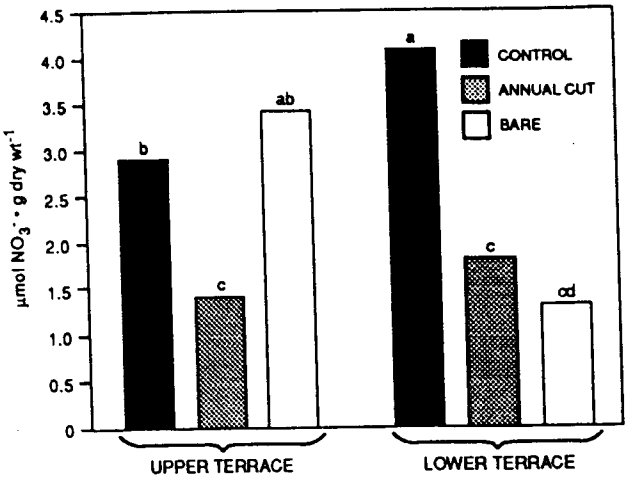

Fig. 4. NO_3^- concentrations for the study site and vegetation combinations; for further explanations, see Fig. 2

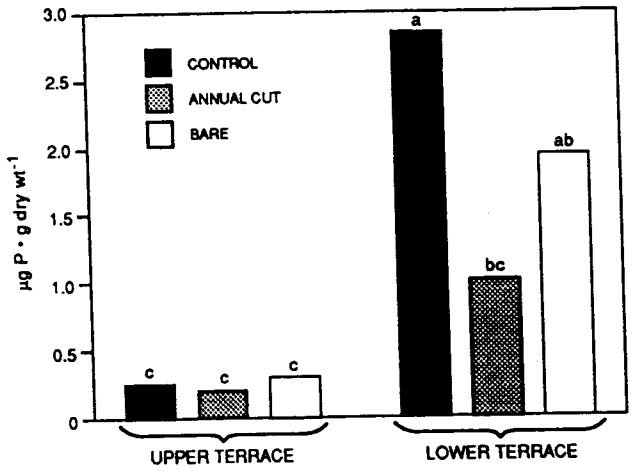

Fig. 5. Extractable P (acid-ammonium fluoride) concentrations for the study site and vegetation combinations; for further explanations, see Fig. 2

Table 2. Spearman rank correlations among denitrification rates, respiration rates, mineralizable (N_{min}), extractable NH_4^+, microbial biomass (chloroform fumigation-flush CO_2), fraction dry wt. (dry wt./wet wt.), nitrate, phosphorus, Kjeldahl N_2, and total C

	Denitrification	Respiration	N_{min}	Extractable NH_4^+	Biomass	Dry weight	NO_3^-	P	Kjeldahl N	Total C
Denitrification	1.000	0.697***	0.755***	0.583**	0.771***	−0.968***	0.223	−0.335	0.635**	0.639**
Respiration		1.000	0.603**	0.386	0.549**	−0.750***	0.144	0.071	0.250	0.237
N_{min}			1.000	0.625**	0.647**	−0.763***	0.126	−0.137	0.431*	0.429*
Extractable N				1.000	0.553**	−0.600**	−0.104	−0.277	0.281	−0.683**
Biomass					1.000	−0.828***	0.094	−0.262	0.580**	0.611**
Dry weight						1.000	−0.170	0.344	−0.671**	0.237
NO_3^-							1.000	0.194	0.322	0.252
P								1.000	−0.524*	−0.666**
Kjeldahl N									1.000	0.910***
Total C										1.000

* $P<0.05$, ** $P<0.01$, *** $P<0.001$

Since the first experiment showed that denitrification rates increased with soil moisture content, we wanted to determine whether the addition of water alone would stimulate denitrification, as reported previously (Binstock 1984; Davidson and Swank 1986). In fact, water alone decreased the denitrification rate in all four sets of samples (Fig. 6), perhaps because a portion of the N_2O produced became dissolved in the added water instead of being released into the headspace. In any case, the result showed clearly that water had not been a limiting factor

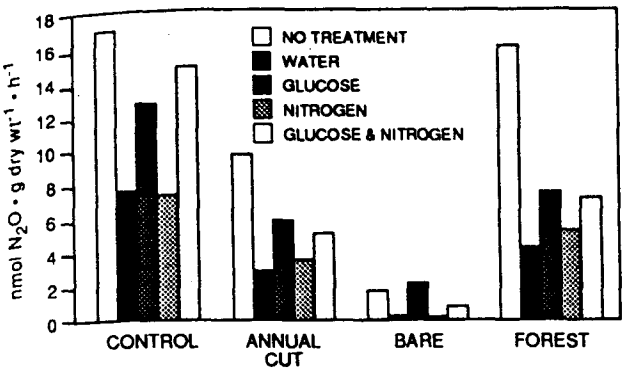

Fig. 6. Effects of glucose and NO_3^- amendment on potential denitrification rates; for further explanations, see Fig. 2

in the first experiment. Of the various amendments, only glucose stimulated denitrification compared to the water-only treatment. (NO_3^- gave the same rate as water alone, and NO_3^- +glucose was the same as glucose alone.)

Other studies have reported varying results. Robertson and Tiedje (1988) found that the addition of NO_3^- stimulated denitrification (relative to a water-only control) at some of their La Selva sites (see above) but not at others. Matson et al. (1987) found that glucose but not NO_3^- increased denitrification rates in tropical soils. Livingston et al. (1988), however, found that NO_3^-, but not water or NH_4^+, increased N_2O production in Amazon soils by a factor of 2–20 over controls. Keller et al. (1988) also reported that adding NO_3^- increased N_2O production in tropical forest soils.

Our study shows that denitrification rates in these soils were strongly influenced by vegetation. The high correlation between denitrification potential and other indicators of microbial activity and microbial biomass suggests that differences in vegetation directly influence microbial function in soils, most likely by altering the quality and quantity of organic matter input. Regardless of the vegetation, however, NO_3^- did not limit denitrification, although C may become limiting if the O_2 tension is sufficiently reduced. This supposition is supported by the low correlation between denitrification rates and NO_3^- concentrations and the high correlation with total C. The data also suggest that if these soils become anerobic, they have the potential to denitrify NO_3^- very rapidly and that vegetation management has the potential to influence the N_2O efflux from these tropical soils.

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