

## DISSIMILATORY NITRATE REDUCTION TO AMMONIUM IN UPLAND TROPICAL FOREST SOILS

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**Abstract.** The internal transformations of nitrogen in terrestrial ecosystems exert strong controls over nitrogen availability to net primary productivity, nitrate leaching into groundwater, and emissions of nitrogen-based greenhouse gas. Here we report a reductive pathway for nitrogen cycling in upland tropical forest soils that decreases the amount of nitrate susceptible to leaching and denitrification, thus conserving nitrogen in the ecosystem. Using <sup>15</sup>N tracers we measured rates of dissimilatory nitrate reduction to ammonium (DNRA) in upland humid tropical forest soils averaging  $\sim 0.6 \mu\text{g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ . Rates of DNRA were three times greater than the combined N<sub>2</sub>O and N<sub>2</sub> fluxes from nitrification and denitrification and accounted for 75% of the turnover of the nitrate pool. To determine the relative importance of ambient C, O<sub>2</sub>, and NO<sub>3</sub> concentrations on rates of DNRA, we estimated rates of DNRA in laboratory assays using soils from three tropical forests (cloud forest, palm forest, and wet tropical forest) that differed in ambient C and O<sub>2</sub> concentrations. Rates of DNRA measured in laboratory assays ranged from 0.5 to 9  $\mu\text{g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$  in soils from the three different forests and appeared to be primarily limited by the availability of NO<sub>3</sub>, as opposed to C or O<sub>2</sub>. Tests of sterile soils indicated that the dominant reductive pathway for both NO<sub>2</sub> and NO<sub>3</sub> was biotic and not abiotic. Because NH<sub>4</sub> is the form of N generally favored for assimilation by plants and microbes, and NO<sub>3</sub> is easily lost from the ecosystem, the rapid and direct transformation of NO<sub>3</sub> to NH<sub>4</sub> via DNRA has the potential to play an important role in ecosystem N conservation.

**Key words:** denitrification; dissimilatory nitrate reduction to ammonium (DNRA); <sup>15</sup>N; nitrogen cycling; nitrous oxide; tropics; upland tropical forest soils.

### INTRODUCTION

Dissimilatory nitrate reduction to ammonium (DNRA) is an anaerobic microbial pathway of the N cycle that transforms NO<sub>3</sub> first to NO<sub>2</sub>, and then on to NH<sub>4</sub>. While the ecology of the process is not well understood (Tiedje et al. 1982), DNRA is known to be catalyzed primarily by facultative and obligately fermentative bacteria, and has been documented in anaerobic sludge, anoxic sediments, and the rumen (Tiedje et al. 1982, Bonin 1996, Nijburg et al. 1997). Dissimilatory nitrate reduction to ammonium is conspicuously absent from most depictions of terrestrial biogeochemical cycling (Schlesinger 1997). This is primarily because DNRA is thought to occur only in highly reducing environments capable of maintaining sustained anaerobic metabolism (Tiedje 1988); these conditions are generally thought to occur only in flooded environments.

Evidence suggests that upland humid tropical forests are good candidates for DNRA activity. Humid tropical forest soils are generally characterized by high N availability and rapid rates of N cycling (Vitousek and Sanford 1986, Vitousek and Matson 1988). These ecosys-

tems also typically have high denitrification potential (Livingston et al. 1988, Veldkamp et al. 1998, Silver et al. 2000) and are the largest global source of N<sub>2</sub>O (Bouwman et al. 1993, Prather et al. 1994), a radiatively important greenhouse gas. The conditions for DNRA (e.g., low redox potential, available NO<sub>3</sub>, and labile C) are similar to those for denitrification, which results in losses of N to the atmosphere as N<sub>2</sub> and N<sub>2</sub>O (Zumft 1997); however, DNRA is thought to be favored by a high ratio of available C to electron acceptors (Tiedje et al. 1982, Tiedje 1988, Fazzolari et al. 1998). Abundant rainfall, high rates of root and microbial activity, and clayey soils create an environment where aerobic and anaerobic microsites occur in a dynamic spatial and temporal mosaic across the landscape. Thus, aerobic processes such as nitrification and highly anaerobic processes such as methanogenesis have recently been shown to co-occur in these environments (Keller et al. 1986, Silver et al. 1999).

The direct and rapid reduction of NO<sub>3</sub> to NH<sub>4</sub> via DNRA has important implications for ecosystem N retention and loss (Fig. 1). In plants and microbes, NH<sub>4</sub> assimilation generally exceeds NO<sub>3</sub> assimilation, due to the energy costs associated with NO<sub>3</sub> reduction in tissues (Gutschick 1981, Smirnov and Stewart 1985, Puri and Ashman 1999). DNRA increases the flux into the NH<sub>4</sub> pool, enhancing NH<sub>4</sub> availability and uptake, and contributing to N retention. In humid environ-

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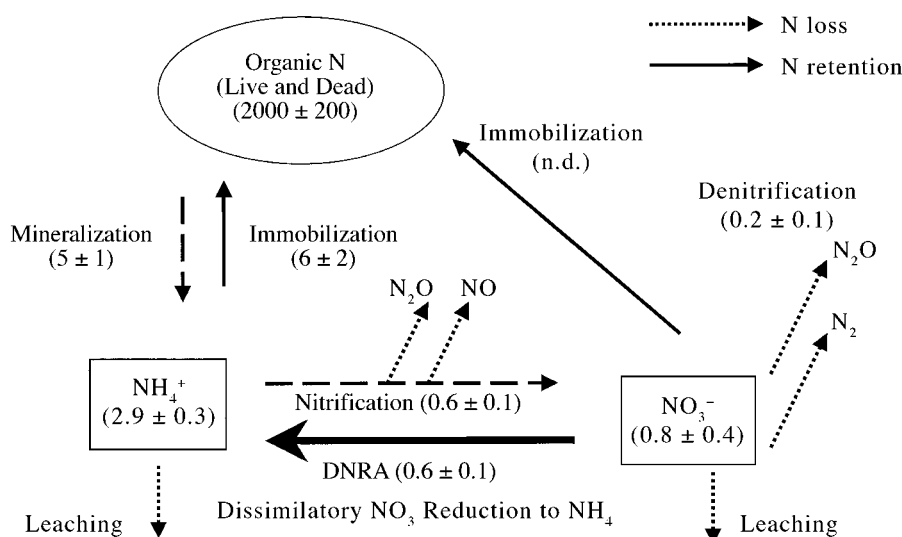


FIG. 1. Loss and retention pathways in the tropical forest N cycle. Rates are in micrograms of N per gram per day for 0–6 h of incubation (n.d. = no data). Pool values are for the standing pools of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , and soil organic N in micrograms of N per gram. Immobilization rates are for assimilation into microbial biomass only and do not include plant uptake. Rates of microbial immobilization of  $\text{NH}_4^+$  are  $\sim 10$  times greater than rates of nitrification, indicating that  $\text{NO}_3^-$  reduction to  $\text{NH}_4^+$  results in N retention in the ecosystem.

ments,  $\text{NO}_3^-$  can be easily leached to groundwater (Vitousek and Matson 1994, Hedin et al. 1998), and N can also be lost as  $\text{N}_2$  and nitrogen oxides (Firestone and Davidson 1989, Hall and Matson 1999, Matson et al. 1999). DNRA decreases the size of the  $\text{NO}_3^-$  pool, and shortens the mean residence time of  $\text{NO}_3^-$  in the soil, both of which are likely to contribute to decreased N losses (Firestone and Davidson 1989).

In this study we used  $^{15}\text{N}$  tracer and pool dilution experiments in the field and laboratory to determine if DNRA occurs in humid tropical forests, and to compare the relative rates of DNRA, denitrification, and microbial  $\text{NO}_3^-$  assimilation. We also addressed how resource availability ( $\text{O}_2$ , C,  $\text{NO}_3^-$ ) affects rates of DNRA, and whether DNRA rates respond to rapid ( $< 24$  h) changes in soil  $\text{O}_2$  availability. In order to determine the importance of abiotic pathways for  $\text{NO}_3^-$  reduction to  $\text{NH}_4^+$  in these ecosystems, we compared rates of biotic  $\text{NO}_3^-$  reduction with rates of abiotic reduction.

#### METHODS

The study was conducted in the Luquillo Long Term Ecological Research Site in Puerto Rico, USA ( $18^\circ 18' \text{N}$ ,  $65^\circ 50' \text{W}$ ). The field experiment was located in an intact, old-growth, lower montane wet tropical forest (Brown et al. 1983). Mean annual temperature is  $19.1^\circ\text{C}$ ; annual precipitation is  $\sim 400$  cm evenly distributed throughout the year. Soils are classified as clay-rich ultisols (Brown et al. 1983). Three replicate  $10 \times 10$  m treatment plots were established on well-drained ridges. For the laboratory experiments, soils were collected from two of the three field campaign sites, as well from two  $10 \times 30$  m plots each in palm forest and

cloud forest. Palm forest is dominated by a single species of palm (*Prestoea montana* (Graham) Nichols), has a mean annual temperature of  $19.5^\circ\text{C}$ , and receives  $\sim 400$  cm of rainfall annually. Cloud forest occurs on the peaks and ridges  $> 750$  m above sea level, has a mean annual temperature of  $18.6^\circ\text{C}$ , and receives  $\sim 400$  cm of precipitation annually, with additional cloud water input (Weaver 1994).

#### Field experiment

In each plot we inserted 50 uncapped,  $6 \times 12$  cm long polyvinyl chloride (PVC) cores into the top 10 cm of soil. Each core was injected with  $^{15}\text{KNO}_3$  solution throughout the core volume ( $0.05 \mu\text{g/g}$  of  $^{15}\text{NO}_3^-$  at 99.7 atom %), yielding a starting soil enrichment of 14 atom %  $\text{NO}_3^-$ . Ten, randomly selected replicate cores were collected from each plot at  $\sim 2.5$ , 6, 24, 48, and 72 h after injection of the  $^{15}\text{N}$  solution. Gas flux was measured immediately before cores were removed from the field at all periods except 48 h. For gas sampling we collected 60 mL of headspace gas from 450-mL PVC chambers four times during 1 h from each of three cores from each plot ( $n = 9$  per sampling period). Following gas sampling, cores were harvested, and immediately extruded into plastic bags, and well mixed. A 30-g oven-dry equivalent (ODE) sample was then measured into 150 mL of 2 mol/L KCl. We kept the slurries on ice until extraction (1–1.5 h). For microbial biomass C and  $^{15}\text{N}$  determinations, we divided additional subsamples into two aliquots. One aliquot was extracted immediately in 0.5 mol/L  $\text{K}_2\text{SO}_4$ ; the other was fumigated with ethanol-free chloroform for 5 d before extraction. Additional subsamples were used to deter-

mine soil moisture. Extracts were shipped frozen to UC Berkeley for analysis. Soil samples for natural abundance  $^{15}\text{N}$  ( $n = 10$  each from plots 1 and 2) were collected adjacent to the  $^{15}\text{N}$  plots using a 2.5 cm diameter corer. Additional subsamples of these soils were used to determine nitrification potentials.

#### Laboratory experiments

The laboratory experiments were designed to examine rates of DNRA from tropical forests with different native C,  $\text{O}_2$ , and soil  $\text{NO}_3$  concentrations, and to determine the effect of altering  $\text{O}_2$  availability on DNRA rates. Ten composite samples of  $\sim 190$  g ODE soil each (five per plot from two plots each and three forest types) were collected at random intervals from the 0–10 cm depth using a 2.5 cm diameter corer. Samples were collected the day prior to the start of the field campaign and were express-mailed at  $5^\circ\text{C}$  within 12 h to UC Berkeley. The samples were split upon arrival at Berkeley. Half of the samples received  $^{15}\text{NH}_4\text{SO}_4$  at a soil concentration of  $0.023 \mu\text{g/g}$  (final soil enrichment of 2.4 atom %  $^{15}\text{NH}_4$ ), and the other half received  $^{15}\text{KNO}_3$  added at a soil concentration  $0.12 \mu\text{g/g}$  (final soil enrichment of 12.4 atom %  $^{15}\text{NO}_3$ ). We incubated 30-g ODE samples from each set of labeled soils in 225-mL jars under ambient conditions for 0, 6, and 24 h, and under an  $\text{N}_2$  headspace for 0, 3, 6, and 24 h. At the end of incubations, the jars were extracted with 150 mL of 2 mol/L KCl. Incubations were done at  $25^\circ\text{C}$ . Additional subsamples were used to determine soil moisture and soil C content. Soil  $\text{O}_2$  was measured in the field according to Silver et al. (1999) from 10 randomly located soil equilibration chambers (0–10 cm depth) in each plot, that have been sampled regularly for 4 yr as part of an ongoing study of soil atmosphere dynamics. Values reported here are from samples that were taken the week prior to the  $^{15}\text{N}$  experiment.

#### Sterile soils

Using five replicate soil samples from the wet tropical forest site, we measured 60 g ODE soil into each of 15 225-mL jars, autoclaved the soils for 1 h, and re-autoclaved them 24 h later. Plate counts on tryptic soy agar indicated  $>99.9\%$  kill. We also prepared a set of 10 jars that were not autoclaved. We added  $\text{NO}_2$  or  $\text{NO}_3$  (both 99.7 atom %  $^{15}\text{N}$ ) at a soil concentration of  $0.93 \mu\text{g }^{15}\text{N/g}$  separately to each of five autoclaved soils and live soils. These concentrations yielded a minimum detectable change of 0.14% in the  $^{15}\text{NH}_4$  pool. Five autoclaved jars were used as a control with no label added. We incubated all soils for 2.5 h at  $25^\circ\text{C}$ , extracted the soils with 150 mL of 2 mol/L KCl, and immediately measured  $\text{NH}_4$ ,  $\text{NO}_2$ , and  $\text{NO}_3$  concentrations prior to preparation for isotopic analysis.

#### Analytical and statistical procedures

We determined  $\text{NH}_4$ ,  $\text{NO}_3$ , and  $\text{NO}_2$  concentrations colorimetrically. Extracts were prepared for isotope

analysis by diffusion (Herman et al. 1995), and N-isotope ratios measured using an automated nitrogen-carbon analyzer coupled to an isotope ratio mass spectrometer (ANCA-IRMS; PDZ Europa, Limited, Crewe, UK). We determined  $\text{N}_2\text{O}$  by gas chromatography using a  $^{63}\text{Ni}$  detector (Europa Scientific, Cheshire, UK), and determined N-gas isotope ratios using a trace gas module coupled to an IRMS. Total organic C in microbial biomass was measured using a TOC analyzer (OI Analytic, College Station, Texas), and total N by Kjeldahl digestion and colorimetry (Colorimeter: Lachat Quik Chem flow injection analyzer, Lachat Instruments, Milwaukee, Wisconsin). Microbial C and N were calculated as the difference between the fumigated and unfumigated soils. We calculated rates of DNRA as the difference in the  $^{15}\text{NH}_4$  atom % between sampling periods, multiplied by the mean  $\text{NH}_4$  pool size during the interval, and corrected for the mean residence time (MRT) of the  $\text{NH}_4$  pool. This was then divided by the mean  $^{15}\text{NO}_3$  atom % during the interval to account for the isotopic composition of the source pool. For the field experiment we estimated MRT of the  $^{15}\text{NH}_4$  pool by dividing the initial  $\text{NH}_4$  pool (in micrograms per gram) by the rate of gross consumption using the 0–24 interval data from the simultaneous laboratory experiment. The MRT value from the 0–24 h interval was chosen because it reduced the impact of short-term soil disturbance, and as it was the largest MRT value measured, its use in the DNRA calculation provided the most conservative estimates of DNRA. For the laboratory experiment, we used individual MRT values generated from each treatment. Gross mineralization, nitrification, and  $\text{NH}_4$  and  $\text{NO}_3$  consumption were calculated according to Kirkham and Bartholomew (1954). Soil C was estimated on a CE Elantec CN analyzer (Lakewood, New Jersey) at UC Berkeley. Soil  $\text{O}_2$  was analyzed in the field using a YSI  $\text{O}_2$  meter and electrode (Yellow Springs Instrument, Yellow Springs, Ohio; Silver et al. 1999). Soil moisture was determined gravimetrically after drying subsamples at  $105^\circ\text{C}$  to a constant mass. Natural abundance  $^{15}\text{N}$  was measured as described above after extracting 30 g ODE soils with 150 mL of 2 mol/L KCl. Nitrification potential was measured according to Hart et al. (1994).

Statistical analyses were performed using Systat (Wilkinson 1990). We used analysis of variance (ANOVA) to determine if changes in pool sizes or rates occurred among treatments. Data were log-transformed when necessary to meet assumptions for ANOVA. Significant differences were determined as  $P < 0.05$  unless otherwise noted.

## RESULTS AND DISCUSSION

### Rates of DNRA and denitrification in tropical forest soils

Nitrate pools in the wet tropical forest were small ( $0.78 \pm 0.36 \mu\text{g N/g soil}$ ), but nitrification potential was high ( $13 \pm 3 \mu\text{g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ ; mean  $\pm 1$  SE), indicating that oxic periods allowed substantial  $\text{NO}_3$  production. There was a rapid and significant increase in the  $^{15}\text{NH}_4$

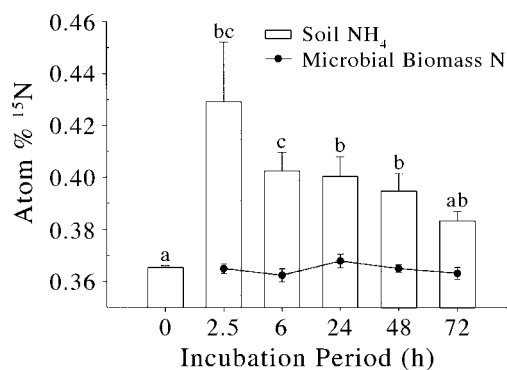


FIG. 2. Changes in the  $^{15}\text{NH}_4$  atom % of the soil and the  $^{15}\text{N}$  of the microbial biomass following addition of a  $^{15}\text{NO}_3$  label in a wet tropical forest soil in the Luquillo Experimental Forest. Values are means + 1 SE; lowercase letters signify statistically significant differences ( $P < 0.05$ ). The  $^{15}\text{N}$  atom % of the microbial biomass did not change significantly during the experiment. Time 0 represents natural abundance values prior to the label addition.

atom % within 2.5 h of adding the  $^{15}\text{NO}_3$  tracer to field soils (Fig. 2). The  $^{15}\text{NH}_4$  atom % then decreased over time, but remained significantly elevated above natural abundance levels for 48 h of the 72-h field experiment ( $P < 0.01$ ). The microbial biomass  $^{15}\text{N}$  did not change during the experiment (Fig. 2), indicating that assimilatory reduction of  $\text{NO}_3$  was not responsible for the patterns observed. The lack of  $\text{NO}_3$  uptake by microbial biomass is not surprising for this ecosystem. Ammonium availability in this ecosystem is relatively high; others have reported that as mineral N availability increases, microbial N immobilization decreases (Vitousek and Matson 1988, Hall and Matson 1999). The  $\text{NH}_4$  pool size also did not change significantly during the field assay and averaged  $2.92 \pm 0.32 \mu\text{g/g}$ . The stability of this pool means that  $\text{NH}_4$  consumption approximately equaled inputs from mineralization and DNRA. The  $\text{NO}_3$  pool increased as a result of our  $^{15}\text{NO}_3$  addition, and then decreased significantly for the first 24 h; thereafter, it did not differ significantly from background levels. Rates of dissimilatory  $\text{NO}_3$  reduction to  $\text{NH}_4$  were greatest during the initial 2.5 h ( $0.9 \pm 0.2 \mu\text{g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ ) and decreased to  $0.5 \pm 0.04 \mu\text{g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$  at 24 h; DNRA averaged  $0.6 \mu\text{g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$  over the 72-h period (Table 1).

To determine rates of denitrification, we measured the efflux of both  $^{15}\text{N}_2$  and  $^{15}\text{N}_2\text{O}$  from field cores. Production of  $^{15}\text{N}_2$  was not detectable (detection limit = 0.0006 atom %  $^{15}\text{N}$ ), so we estimated denitrification as the efflux of  $^{15}\text{N}_2\text{O}$ . We converted the surface flux measurements to a mass per unit volume estimate to compare with DNRA, and to determine how much of the added  $^{15}\text{NO}_3$  label was used in denitrification vs. DNRA in the top 10 cm of mineral soil. Nitrous oxide fluxes were very high ( $34.0 \pm 9.6 \text{ ng}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ ), and denitrification averaged  $0.19 \mu\text{g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$  over 72 h; however, this was a factor of

3 lower than DNRA rates (Table 1). Denitrification rates were relatively stable over the first 24-h period, then decreased significantly at the 72-h measurement ( $P < 0.01$ ). Nitrous oxide production can also occur via nitrification, although in wet soils, denitrification is thought to predominate (Davidson et al. 1986). Any  $\text{N}_2\text{O}$  produced by nitrification during the assay period would have led to an overestimate of denitrification. In soils with low  $\text{NO}_3$  concentrations, denitrification is strongly dependent upon the size and turnover rate of the  $\text{NO}_3$  pool (Firestone and Davidson 1989, Paul and Clark 1996). DNRA accounted for 75% of the turnover of the nitrate pool during the first 6 h. Small standing  $\text{NO}_3$  pools combined with high rates of  $\text{NO}_3$  reduction by DNRA limit the  $\text{NO}_3$  available for denitrification, and lead to decreased N loss through this pathway.

#### Resource availability and DNRA rates

The occurrence of DNRA has been documented in soils incubated at low  $\text{O}_2$  partial pressures under laboratory conditions (Stanford et al. 1975, Fazzolari et al. 1998, Bengtsson and Bergwall 2000). These studies have suggested that DNRA is less sensitive to  $\text{O}_2$  than denitrification, and more sensitive to the amount of labile C. Our simultaneous laboratory experiments were designed to determine if DNRA occurred in other humid tropical forest types, as well as whether DNRA was sensitive to ambient levels of C,  $\text{O}_2$ , and  $\text{NO}_3$ . Soil C increased significantly from the wet tropical forest site to the palm and cloud forests, respectively ( $P < 0.01$ ), while soil  $\text{O}_2$  was significantly higher in the wet tropical forest than the cloud and palm forests (Table 2). Soil moisture was significantly lower in the wet tropical forest than in the other forest types ( $P < 0.01$ , Table 2). In the laboratory assays we also modified soil  $\text{O}_2$  availability by incubating soils under an  $\text{N}_2$  atmosphere.

The addition of  $^{15}\text{NO}_3$  resulted in a significant increase in  $^{15}\text{NH}_4$  atom % in soils from all three sites incubated under both ambient and anaerobic atmospheres. The atom %  $^{15}\text{N}$  of the  $\text{NO}_3$  pool declined linearly over time (Fig. 3). The  $^{15}\text{NH}_4$  atom % was significantly greater under  $\text{N}_2$  atmospheres than under ambient atmospheres in wet tropical forest soils, but not in the other sites. Cloud and palm forest soils are frequently saturated and experience a high proportion of anaerobic microsites relative to the wet tropical forest (Silver et al. 1999), and may not have experienced

TABLE 1. Rates of dissimilatory nitrate reduction to ammonium (DNRA) and denitrification ( $^{15}\text{N}_2\text{O} + ^{15}\text{N}_2$  flux) in a humid tropical forest soil.

Time interval (h)	DNRA rate ( $\mu\text{g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ )	Denitrification rate ( $\mu\text{g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ )
0–2.5	$0.9 \pm 0.2$	$0.3 \pm 0.1$
0–6	$0.6 \pm 0.05$	$0.2 \pm 0.1$
0–24	$0.5 \pm 0.04$	$0.2 \pm 0.1$
0–72	$0.5 \pm 0.1$	$0.1 \pm 0.04$

Note: Rates are means  $\pm$  1 SE.

TABLE 2. Site characteristics, nitrogen transformations, and rates of DNRA in three wet tropical forest soils in Puerto Rico.

Measure	Wet tropical forest	Palm forest	Cloud forest
Site characteristics			
Soil carbon (%)	6.8 ± 1.1 <sup>a</sup>	10.9 ± 1.5 <sup>b</sup>	13.9 ± 0.3 <sup>c</sup>
Soil oxygen (%)	14.5 ± 1.6 <sup>a</sup>	4.4 ± 1.8 <sup>b</sup>	6.0 ± 2.3 <sup>b</sup>
Soil moisture (%)	51 ± 2 <sup>a</sup>	62 ± 2 <sup>b</sup>	55 ± 1 <sup>b</sup>
Nitrogen transformations			
Gross mineralization	4.8 ± 0.9	8.2 ± 2.4	9.6 ± 2.4
Gross NH <sub>4</sub> consumption	6.4 ± 2.2	7.1 ± 2.1	6.4 ± 1.9
Mean residence time of NH <sub>4</sub> (d)	0.48 ± 0.09	1.16 ± 0.38	0.72 ± 0.15
Gross nitrification	0.57 ± 0.12	0.59 ± 0.13	0.63 ± 0.16
DNRA			
Ambient atmospheres	1.2 ± 0.8	0.9 ± 0.3	0.5 ± 0.2
N <sub>2</sub> atmospheres	2.8 ± 2.1	8.7 ± 7.7	3.0 ± 1.5

Notes: Nitrogen transformations are in  $\mu\text{g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$  with the exception of mean residence time in days. Superscript lowercase letters signify statistically significant differences among forest types. Values are for the 0–6 h incubation. Nitrogen transformations reported are under ambient atmospheric conditions. Values are means ± 1 SE.

significant O<sub>2</sub> reduction in soil microsites under N<sub>2</sub> atmospheres. Rates of DNRA were highly variable and did not differ significantly among forest types, or between ambient and anaerobic treatments (Table 2).

Gross nitrification rates averaged  $0.60 \pm 0.08 \mu\text{g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$  at 24 h under ambient atmospheric conditions (Table 2). In general, rates of gross nitrification were lower than DNRA rates measured using added NO<sub>3</sub>. This indicates that DNRA activity is limited by NO<sub>3</sub> availability in these soils. Gross nitrification rates measured here are in the low to intermediate range compared to other forests (Stark and Hart 1997). Rates of DNRA under ambient atmospheric conditions ranged from 11% to 25% of gross mineralization rates. This high percentage is due in part to the extra, albeit small amount, of NO<sub>3</sub> available to DNRA from the labeled NO<sub>3</sub> addition. A summary of the rate of DNRA relative to other N cycling pathways in upland tropical forest soils illustrates the role of DNRA in N retention (Fig. 1). Rates of DNRA were approximately equal to rates of gross nitrification. Gross nitrification rates, however, were only 1/10 that of NH<sub>4</sub> assimilation. If denitrification is dependent on NO<sub>3</sub> availability (and our data support this), then removal of NO<sub>3</sub> via DNRA decreases the loss of NO<sub>3</sub> by this pathway.

#### *Biotic vs. abiotic nitrate reduction*

Smith and Chalk (1982) suggested that NO<sub>2</sub> might be reduced to NH<sub>4</sub> by nonbiological mechanisms, although these mechanisms are not well understood. To determine the potential role of abiotic processes, and the relative importance of the first reductive step to NO<sub>2</sub>, we incubated sterilized and live soils for 2.5 h after adding either a <sup>15</sup>NO<sub>2</sub> or <sup>15</sup>NO<sub>3</sub> tracer. There was a very small increase in the atom % <sup>15</sup>NH<sub>4</sub> in sterile soils, detectable only because of the high enrichment of the added tracer (Table 3). The ratio of <sup>15</sup>N accumulated in the NH<sub>4</sub> pool in live soils to that in sterile

soils was  $4.1 \pm 2.4$  for NO<sub>2</sub>, and  $2.3 \pm 1.6$  for NO<sub>3</sub>. However, since these values are not corrected for turnover of the NH<sub>4</sub> pool, the ratios of live:dead are clearly underestimates. In live soils, reduction of NO<sub>2</sub> to NH<sub>4</sub> was greater than for NO<sub>3</sub> ( $P < 0.01$ ), suggesting that reduction of NO<sub>3</sub> to NO<sub>2</sub> was rate limiting. These results demonstrate that the dominant pathway for NO<sub>3</sub> reduction to NH<sub>4</sub> in these soils is microbial.

#### *Conclusions*

Why has this process been overlooked by terrestrial biogeochemists? The answer may lie in the difficulty in measuring DNRA, the common assumption that the process is limited to highly reducing environments, and the relatively few studies of microbial soil N dynamics that have been conducted in tropical ecosystems. Recent work has shown that microbial diversity is vast in tropical forest soils, with many organisms new to science (Borneman and Triplett 1997). The highly diverse and potentially novel character of tropical soil microbial communities may set the stage for new variations in microbially mediated biogeochemical cycling.

Much of the N reduced from NO<sub>3</sub> to NH<sub>4</sub> will be assimilated by plants and microbes, and this is likely to result in N conservation. Perhaps the best test of the role of DNRA in N retention would be to selectively inhibit DNRA, and determine the N loss in the absence of this process. However, we know of no inhibitor that acts exclusively on DNRA while not also inhibiting other N cycling pathways. Numerical modeling may provide an alternative approach to explore the role of DNRA under a variety of scenarios. Previous models of the N cycle show two possible fates of NO<sub>3</sub> in terrestrial ecosystems: immobilization by plants and microbes, and loss via denitrification and leaching. In temperate, low-NO<sub>3</sub> systems, loss pathways are strongly dependent upon the size and turnover time of the NO<sub>3</sub> pool (Firestone and Davidson 1989, Paul and

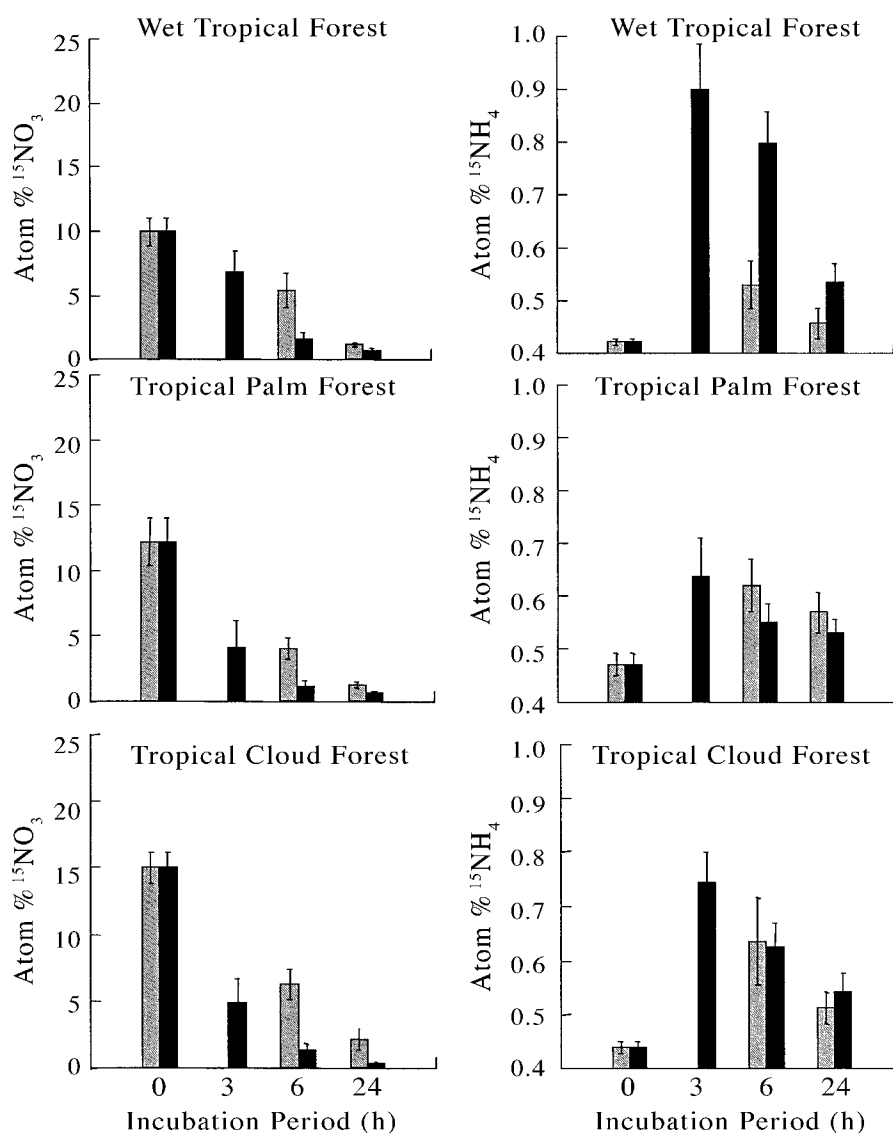


FIG. 3. Changes in the  $^{15}\text{NO}_3$  and  $^{15}\text{NH}_4$  atom % after adding the  $^{15}\text{NO}_3$  label to soils from three upland humid tropical forests. Shaded bars represent assays conducted under ambient atmospheres, and solid bars represent assays conducted under an  $\text{N}_2$  atmosphere. The  $^{15}\text{N}$  atom % in the  $\text{NO}_3$  pool decreased significantly over time, and there was significantly lower  $^{15}\text{NO}_3$  atom % in all forest types under  $\text{N}_2$  vs. ambient atmospheres at 6 and 24 h ( $P < 0.01$ , ANOVA). The  $^{15}\text{NH}_4$  atom % was significantly elevated above background levels in all forest types after the label was added and was significantly greater under  $\text{N}_2$  atmospheres ( $P < 0.01$ , ANOVA) than ambient in the wet tropical forest soils. The label was added just prior to the time-0 measurement.

TABLE 3.  $^{15}\text{NH}_4$  derived from separate additions of  $^{15}\text{NO}_2$  and  $^{15}\text{NO}_3$  in humid tropical forest in Puerto Rico.

Label added	Atom % excess $^{15}\text{NH}_4$		$^{15}\text{N}$ reduced to $\text{NH}_4$ (live:dead)
	Sterile	Live	
$^{15}\text{NO}_2$	$0.02 \pm 0.006^{\text{Aa}}$	$0.66 \pm 0.11^{\text{Ab}}$	$4.1 \pm 2.4^{\text{A}}$
$^{15}\text{NO}_3$	$0.01 \pm 0.003^{\text{Aa}}$	$0.25 \pm 0.06^{\text{Bb}}$	$2.3 \pm 1.6^{\text{A}}$

Notes: Values are means  $\pm$  1 SE. Samples were incubated for 2.5 h under ambient conditions. Superscript lowercase letters signify statistically significant differences between sterile and live treatments. Superscript uppercase letters signify statistically significant differences between labels added.

Clark 1996). In humid tropical forest soils, we found that high rates of DNRA resulted in rapid turnover of a small  $\text{NO}_3$  pool, thus limiting  $\text{NO}_3$  availability to denitrification and leaching.

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