PLANT AND MICROBIAL CONTROLS ON NITROGEN RETENTION AND LOSS IN A HUMID TROPICAL FOREST

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Abstract. Humid tropical forests are generally characterized by the lack of nitrogen (N) limitation to net primary productivity, yet paradoxically have high potential for N loss. We conducted an intensive field experiment with $^{15}NH_4$ and $^{15}NO_3$ additions to highly weathered tropical forest soils in Puerto Rico to determine the relative importance of N retention and loss mechanisms. Over one-half of all the NH_4^+ produced was rapidly converted to NO_3^- via the process of gross nitrification. During the first 24 hours, plant roots took up 28% of the inorganic N produced, dominantly as NH_4^+, and were a greater sink for N than soil microbial biomass. Soil microbes were not a significant sink for added $^{15}NH_4^+$ or $^{15}NO_3^-$ during the first 24 hours, and only for $^{15}NH_4^+$ after 7 days. Patterns of microbial community composition, as determined by terminal restriction fragment length polymorphism analysis (TRFLP), were weakly but significantly correlated with nitrification and denitrification to N_2O. Rates of dissimilatory NO_3^- reduction to NH_4^+ (DNRA) were high in this forest, accounting for up to 25% of gross NH_4^+ production and 35% of gross nitrification. DNRA was a major sink for NO_3^-, which may have contributed to the lower rates of N_2O and leaching losses. Despite considerable N conservation via DNRA and plant NH_4^+ uptake, the fate of ~45% of the NO_3^- produced and 4% of the NH_4^+ produced were not measured in our fluxes, suggesting that other important pathways for N retention and loss (e.g., denitrification to N_2) are important in this system. The high proportion of mineralized N that was rapidly nitrified and the fates of that NO_3^- highlight the key role of gross nitrification as a proximate control on N retention and loss in humid tropical forest soils. Furthermore, our results demonstrate the importance of the coupling between DNRA and plant uptake of NH_4^+ as a potential N-conserving mechanism within tropical forests.

Key words: ^{15}N; *denitrification; DNRA; gross mineralization and nitrification; nitrogen sinks; nitrous oxide; plant–microbial uptake.*

INTRODUCTION

The internal transformations of nitrogen (N) in terrestrial ecosystems exert strong controls on N availability, ultimately affecting net primary productivity (NPP; Melillo et al. 1993, Stark and Hart 1997), NO_3^- leaching into groundwater (Vitousek and Matson 1984, Hedin et al. 1998), and emissions of N-based greenhouse gases (Firestone and Davidson 1989, Hall and Matson 1999, Matson et al. 1999). In north temperate ecosystems N limitation to NPP is common (Vitousek and Howarth 1991). In contrast, the tropics are generally characterized by rapid rates of N cycling and the lack of strong N limitation to NPP (Vitousek and Sanford 1986). Indeed, N fertilization experiments conducted on highly weathered tropical forest soils, typical of large areas of the tropical biome, have demonstrated little evidence of N limitation to NPP (Tanner et al. 1992, Herbert and Fownes 1995, Vitousek and Farrington 1997). This occurs despite large potential N losses via denitrification (Livingston et al. 1988) and leaching (Lewis et al. 1999) from these ecosystems.

The apparent paradox of adequate N availability, despite a high potential for losses, can be explained by either high inputs (i.e., N fixation rates) or effective N retention. Recent studies suggest that ecosystem-level rates of N fixation can be high (Reed et al. 2007), but large amounts of N fixation are unlikely to be pervasive throughout mature tropical forests (Vitousek et al. 2002). There is some evidence in support of highly effective nutrient retention in these ecosystems (e.g., Davidson et al. 2007), but the mechanisms underlying such a response have not been thoroughly examined. Nitrogen can be retained in forest ecosystems via plant and microbial uptake and assimilation into biomass, storage as soil organic N, or as inorganic N adsorbed

onto soil exchange sites and soil organic matter. In temperate soils where N is often a limiting nutrient for plants, microbes outcompete plants for mineral N in the short term (e.g., $\leq$24 h; Jackson et al. 1989, Zogg et al. 2000, Templer et al. 2005). In tropical soils, the relative fate of inorganic N in plants vs. microbial biomass is not well known because most studies have only examined microbial uptake of N in the absence of plant roots. Those studies examining microbial uptake show that microbes take up more NH_4^+ than NO_3^- (Vitousek and Matson 1988, Silver et al. 2001, 2005), possibly due to energetic constraints (Puri and Ashman 1999). Ammonium uptake may be favored because this form of N does not need to be reduced prior to assimilation, unlike NO_3^-.

Nitrate is generally more easily leached than NH_4^+ in soils and is an important substrate for denitrification to N_2O, NO, and N_2. Both leaching and denitrification are important N loss pathways in humid tropical forests (Lewis et al. 1999, Stehfest and Bouwman 2006), which are typically characterized by considerable hydrologic throughput (Schellekens et al. 2004) and fluctuating redox conditions, which can stimulate denitrification (Silver et al. 1999, 2005). Nitrate can be rapidly converted to NH_4^+ during dissimilatory NO_3^- reduction to NH_4^+ (DNRA), a microbial process that occurs under low or fluctuating redox conditions similar to denitrification (Tiedje 1988, Silver et al. 2001). In tropical forests, DNRA could contribute to N retention by decreasing the size of the NO_3^- pool (Silver et al. 2001, 2005), and thus decreasing leaching and denitrification losses. At an ecosystem level, N retention would occur if the rates of DNRA and NH_4^+ uptake exceed N losses from the NO_3^- pool via denitrification or leaching.

The majority of the N transformations in terrestrial ecosystems are controlled by microbes, and thus the rates of N cycling, as well as N retention and loss, may be related to the composition of the soil microbial community. Linking microbial community characteristics with rate processes is challenging, particularly in tropical soils known for their exceptionally high biodiversity and complexity (Borneman and Triplett 1997). However, general relationships between soil microbial community characteristics and soil N fluxes may provide new insights into patterns of soil N retention and loss (Balser and Firestone 2005, Pett-Ridge et al. 2006), and is a critical first step in linking microbial community composition with functional attributes of ecosystems.

In this study we explored mechanisms of N retention and loss in a humid tropical forest on highly weathered soils. We used stable isotope tracers to follow the fate of N through the dominant internal N-cycling processes as well as N_2O emissions over 24 h, and to determine the amount of N ultimately assimilated or lost via leaching and denitrification to N_2O over 7 days. Our measurements of process rates allow us to provide an estimate for the potential role of internal biological processes in

retaining N, as well as a comparison of plant vs. microbial sinks for N in tropical forest soils. Finally, we generated soil microbial "fingerprints" to determine whether spatial or temporal N dynamics could be linked to patterns in belowground microbial community composition.

Study site

The study was conducted in the Luquillo Experimental Forest (LEF), a National Science Foundation sponsored Long Term Ecological Research Site in northeastern Puerto Rico (latitude 18°30′ N; longitude 65°80′ W). Sites occur within the subtropical lower montane wet forest life zone, which receives ~4500 mm of rainfall annually and has an average annual temperature of 18.5°C, both with little temporal variation throughout the year (Weaver and Murphy 1990). The study sites were located on soils derived from volcanoclastic sediments (ultisols) with high clay content (up to 70%). This forest (referred to here as TMF, tropical montane forest) has ~40 tree species/ha >10 cm diameter at breast height (Brown et al. 1983), and is dominated by *Cyrilla racemiflora* L.

We established three 10 × 30 m plots hereafter referred to as TMF 2, TMF 4, and TMF Icacos. Plots were located within a 1-km² area at 650–750 m elevation. The TMF 2 and TMF 4 plots are part of on-going studies of N and C cycling in humid tropical forest soils (Silver et al. 1999, 2001, 2005, Pett-Ridge and Firestone 2005, Teh et al. 2005, Pett-Ridge et al. 2006). The TMF Icacos site is part of a long-term study of catchment-scale N cycling (McDowell et al. 1992). The plots have similar rainfall, elevation, slope, and plant cover. They differed slightly in soil moisture, total N, total C, and C:N ratio representing some of the inherent spatial heterogeneity of the ecosystem (Table 1).

METHODS

^{15}N field experiment

To determine the competitive strengths of internal N-cycling pathways, leaching and denitrification to N_2O, we separately added $^{15}NH_4^+$ and $^{15}NO_3^-$ tracers to root ingrowth cores in each of our three plots and measured N-cycling processes and fates after 24 h. We measured assimilation into roots and microbial biomass on additional cores after 7 d, as well as leaching and N_2O losses. Plots were randomly subdivided into two sections and assigned to either $^{15}NH_4^+$ or $^{15}NO_3^-$ label addition to prevent cross-contamination.

Unlike most ^{15}N tracer experiments that use harvested soil or intact cores with excised roots (Booth et al. 2005), root ingrowth cores allowed us to estimate N-cycling processes in the presence of active live roots. Cores (9 cm diameter; 10 cm depth) were constructed from 2-mm nylon mesh on the sides and root exclusion cloth on the bottom; a subset of cores was fitted with disk lysimeters on the bottom in place of root exclusion cloth. We sampled soils using a soil corer the same size as the

TABLE 1. Background soil characteristics of each of our three sites in the Luquillo Experimental Forest in northeastern Puerto Rico.

	Tropical montane forest site		
Characteristic	TMF 2	TMF 4	TMF Icacos
Topographic position	windward slope	windward slope	leeward slope
Bulk density (g/cm^3)	0.50 ± 0.033	0.48 ± 0.034	0.52 ± 0.046
% soil moisture	$46.7^a \pm 2.2$	$52.7^b \pm 1.0$	$49.2^{ab} \pm 1.4$
Soil %N	$0.28^a \pm 0.02$	$0.38^b \pm 0.02$	$0.27^a \pm 0.01$
Soil %C	$5.66^a \pm 0.51$	$9.21^b \pm 0.57$	$5.29^a \pm 0.32$
Soil C:N	$20.01^a \pm 0.18$	$24.50^b \pm 0.58$	$19.64^a \pm 0.63$
Total Fe oxides† (µg/g)		21.7 ± 6.2	

Notes: Values are means $\pm$ SE. Different lowercase superscript letters within a row represent statistically significant ($P < 0.05$) differences among plots.

† Data from E. Dubinsky (*personal communication*).

ingrowth cores. All coarse roots (≥ 2 mm), and as many fine roots as possible, were removed by hand picking prior to repacking the cores to field bulk density. Packed cores were then placed back into the same holes from which they were taken. Cores were placed in the field ≥ 1 m apart and assigned to experimental categories for two labels ($^{15}NH_4^+$, $^{15}NO_3^-$) and two incubation periods (24 h and 1 week). All experimental cores were inserted in December 2001 and allowed to equilibrate over an 11-month period before the ^{15}N tracer experiment began in November 2002. At the time of the experiment, 10 cores per plot were used to determine background levels of soil moisture, C, N, microbial biomass N, N_2O fluxes, and natural abundance $\delta^{15}N$. We destructively sampled a subset of ingrowth cores in June ($n = 3$ cores per plot), October ($n = 3$ per plot), and November 2002 ($n = 10$ per plot) to estimate root ingrowth over time. To determine root biomass, soils were extruded from ingrowth cores and washed through three sieves of successively smaller pore sizes (all <1 mm). Roots were then handpicked, sorted as live or dead based on visual characteristics and tensile strength, and then dried at 65°C and weighed. We report on fine roots <2 mm diameter here. During the 11-month period of root ingrowth, total fine root biomass initially increased and then stabilized in the last two months. The mass of total fine roots (live + dead) at the time of label addition (November 2002) was 59% $\pm$ 4% of the background root biomass (live + dead roots) in December 2001, and did not differ among plots or treatments (ANOVA, $P = 0.38$ for $^{15}NH_4^+$ vs. $^{15}NO_3^-$). At the time of the ^{15}N addition, there was ~160 g/m^2 live fine roots, which is similar to the live fine root biomass of surface soils at a lower elevation in this forest (~170 g/m^2; Silver and Vogt 1993).

To estimate patterns of near surface leaching, we fitted the bottom of 10 ingrowth cores per plot (five per label) with 9 cm diameter disk tension lysimeters (Prenart Soil Disc Samplers, Prenart Equipment, Frederiksberg, Denmark). Cores with lysimeters were among those incubated for one week following ^{15}N label addition. Lysimeters were maintained under tension (20 PSI) throughout the duration of the seven-day experimental period.

The ^{15}N label experiment was carried out in November 2002. We injected $^{15}NH_4^+$ or $^{15}NO_3^-$ (>99 atom % ^{15}N) at rates of 4.60 and 0.92 µg N/g soil, respectively, to individual cores. The ^{15}N addition rates were determined from previous studies in this forest (Silver et al. 2001) and brought the labeled pools to ~30.6 atom % ^{15}N-NH_4^+ and 5.3 atom % ^{15}N-NO_3^-, which optimized the ^{15}N signal while minimizing perturbation of the standing N pools. Adding different amounts of label was thus necessary, but could have had additional impacts on N-cycling estimates. Kirkham and Bartholomew (1954) and laws of first order kinetics argue that gross rates should not be affected by the size of the product pool. The other processes (uptake, N_2O fluxes, leaching, DNRA) may have been, and thus label additions could result in overestimates in uptake and leaching, particularly following the NH_4^+ label additions.

The ^{15}N-labeling solution was added with a syringe to each core in six 1-mL injections. The injections were distributed evenly throughout the core volume. We destructively sampled a set of soil cores ($n = 5$ per plot) immediately following the ^{15}N addition to collect our T_0 samples. We sampled gases at 24 h and one week. These cores were fitted with removable static chamber tops to measure $^{15}N_2O$ fluxes across the soil–atmosphere interface. Nitric oxide (NO) fluxes from these soils have been shown to be negligible (probably due to high soil moisture; Erickson et al. 2001), and therefore were not measured in this study. For gas fluxes, we collected 60 mL of headspace gas four times over 1 h from each core chamber (992 mL volume). Gas samples were injected into two pre-evacuated 20-mL Wheaton vials fitted with geomicrobial septa.

At each harvest time point, a subset of 10 cores per label was removed from each plot. Soils were extruded into plastic bags and immediately handpicked for the subsample of root biomass used for tissue ^{15}N analysis as an estimate of root uptake. The remaining soil was processed in the field and laboratory for determination of gravimetric moisture content, microbial biomass ^{15}N, microbial community composition, gross N transformations, DNRA, and total soil ^{15}N content.

Analytical methods

Initial processing of samples was done in the field and at the International Institute of Tropical Forestry laboratory, part of the USDA Forest Service in Puerto Rico. Final processing and analyses were conducted at the University of California–Berkeley. For mineral ^{15}N pool determination, a 60-g oven-dry equivalent (ODE) sample was measured into 162 mL of 2 mol/L KCl in the field. Samples were shaken for 60 minutes, filtered, and stored frozen until analyses were conducted. We determined NH_4^+ and NO_3^- concentrations colorimetrically (Lachat QuikChem 8000 Flow Injector Analyzer; Latchat-Zellwger Instruments, Milwaukee, Wisconsin, USA). Extracts were prepared for isotope analysis by diffusion (Herman et al. 1995), and N-isotope ratios were measured using an automated nitrogen–carbon analyzer coupled to an isotope ratio mass spectrometer (ANCA-IRMS; PDZ Europa Limited, Crew, UK). Gravimetric soil moisture was determined for all soil samples by drying 10 g soil at 105°C to a constant mass.

Gross NH_4^+ production and gross nitrification rates were determined for the soil cores taken at 24 h according to Kirkham and Bartholomew (1954) and Hart et al. (1994). We also calculated rates of DNRA over the first 24 h using the soil cores that received $^{15}NO_3^-$ according to Silver et al. (2005). Briefly, DNRA was determined as the difference in the $^{15}NH_4^+$ atom % between sampling periods, multiplied by the mean NH_4^+ pool size during the interval, and corrected for the mean residence time of the NH_4^+ pool during the interval. This was then divided by the mean $^{15}NO_3^-$ atom % during the interval to account for the isotopic composition of source pool. We estimated mean residence times of the $^{15}NH_4^+$ pool by dividing the initial NH_4^+ pool (micrograms per gram) by the rate of gross consumption in days using data from the $^{15}NH_4^+$ additions (Silver et al. 2001, 2005).

We determined N_2O concentration by gas chromatography using a 63Ni detector (GC 8610c; SRI Instruments, Torrence, California, USA), and determined $^{15}N_2O$ gas isotope ratios using a trace gas module coupled to an IRMS. Rates of N_2O flux were estimated after correction for changes in the $^{15}NO_3^-$ source pool (by dividing by the mean $^{15}NO_3^-$ excess atom %) over time to make them comparable to other measured N fluxes. Also for comparative purposes, we report N_2O fluxes as micrograms N_2O-N per gram per day using the mass of soil in the core volume.

Root uptake was estimated as the change in ^{15}N recovered in biomass divided by the average atom % ^{15}N of available NH_4^+ and NO_3^- pools over the 24-hour interval. Samples of lysimeter soil solution were digested with persulfate to determine the concentration of total dissolved N in leachate (Cabrera and Beare 1993) and diffused to determine ^{15}N content (Herman et al. 1995). We calculated rates of N leaching by assuming only vertical flow of water through the soil core over the seven-day experimental period. Although this is a largely untested assumption, we did not detect significant

amounts of enriched ^{15}N in soils immediately outside of the cores. In a separate, small study we compared ^{15}N of bulk soils at the center of the cores (230‰ ± 50‰ ^{15}N) to soils 5–15 cm horizontal distance from the center of the cores (6.1‰ ± 1.3‰ ^{15}N). For comparison with other fluxes, we divided the mass of N in the lysimeter solution by the total mass of soil in each ingrowth core to determine the rates of leaching per unit soil. Our estimate of water volume leached is an upper estimate because we used tension lysimeters, which can pull water from a larger volume of soil water than just the soil above it.

We determined soil microbial biomass ^{15}N at the 24-hour and seven-day sampling points using the chloroform fumigation method (Cabrera and Beare 1993). Specifically, we divided subsamples into two aliquots. One aliquot was extracted immediately in 0.5 mol/L K_2SO_4; the other fumigated with ethanol-free chloroform for five days before extraction and digestion. Twenty-five-mL extracts were digested with 25 mL potassium persulfate, tightly capped, and autoclaved for 40 minutes. Microbial biomass N was calculated as the difference in total dissolved N between the fumigated and unfumigated soils (Brookes et al. 1985, Vance et al. 1987). Microbial N uptake was estimated as the change in ^{15}N recovered in biomass divided by the average atom% ^{15}N of available NH_4^+ and NO_3^- pools over the 24-hour interval. The percentage of ^{15}N recovery from all pools was calculated as the mass of ^{15}N label divided by the amount added at the beginning of the experimental period.

For microbial community analysis, we used terminal restriction fragment length polymorphism analysis (TRFLP; Blackwood et al. 2003). One gram of homogenized soil from each replicate core collected at time zero from each of the three forest types was sampled and immediately frozen in dry ice until returned to the laboratory and stored at −80°C until analysis. Community analysis was performed on the cores to which labeled NH_4^+ had just been added. DNA extraction and TRFLP profiling procedures follow those in Pett-Ridge and Firestone (2005), with minor modifications. Briefly, extracted DNA was amplified with eight replicate PCR amplifications, performed at a range of annealing temperatures from 49° to 55°C. Bacterial 16S rRNA PCR products were cleaned up using a Qiagen PCR Miniprep Kit (Qiagen Sciences, Valencia, California, USA). MspI restriction digests were performed overnight with 400 ng PCR product in a reaction mixture containing 2u restriction enzyme and appropriate buffers. Electrophoresis of amplicons and GeneScan 500-ROX size standards (Applied Biosystems, Foster City, California, USA) was performed on an ABI 3100 automated capillary sequencer (Applied Biosystems, Foster City, California, USA).

Statistical analysis

Individual cores were treated as independent samples for ^{15}N analyses for roots, microbial biomass, bulk soil,

TABLE 2. Soil N-cycling processes measured at each site at 24 hours.

Process	TMF 2	TMF 4	TMF Icacos
Gross NH_4^+ production ($\mu g \cdot g^{-1} \cdot d^{-1}$)	1.07	3.52	1.39
Gross nitrification ($\mu g \cdot g^{-1} \cdot d^{-1}$)	2.26	1.15	0.62
Gross NH_4^+ consumption ($\mu g \cdot g^{-1} \cdot d^{-1}$)	2.63	11.05	3.08
Gross NO_3^- consumption ($\mu g \cdot g^{-1} \cdot d^{-1}$)	3.19	1.06	1.34
Mean residence time (d), NH_4^+	1.07	0.99	0.95
Mean residence time (d), NO_3^-	0.71	0.43	0.98
DNRA ($\mu g \cdot g^{-1} \cdot d^{-1}$)	0.12	1.27	0.03
$^{15}N_2O$ flux ($ng \cdot g^{-1} \cdot d^{-1}$) from $^{15}NO_3$ label	1.93 ± 1.27	0.012 ± 0.012	4.77 ± 5.61
N_2O flux ($ng \cdot g^{-1} \cdot d^{-1}$) from $^{15}NO_3$ label	15.28 ± 10.80	0.13 ± 0.13	26.77 ± 25.71
Plant N uptake ($\mu g \cdot g^{-1} \cdot d^{-1}$) from $^{15}NH_4$ label	0.35[a] ± 0.11	0.21[a] ± 0.07	0.79[b] ± 0.15
Plant N uptake ($\mu g \cdot g^{-1} \cdot d^{-1}$) from $^{15}NO_3$ label	0.09 ± 0.01	0.09 ± 0.04	0.14 ± 0.12
Microbial N uptake ($\mu g \cdot g^{-1} \cdot d^{-1}$) from $^{15}NH_4$ label	−0.039 ± 0.21	0.46 ± 0.86	−0.13 ± 0.55
Microbial N uptake ($\mu g \cdot g^{-1} \cdot d^{-1}$) from $^{15}NO_3$ label	0.13 ± 0.11	0.37 ± 0.73	−0.20 ± 0.15

Notes: Values are means ± SE. No standard error was estimated for gross N-cycling rates and mean residence times as data were generated at the plot level. Different lowercase superscript letters indicate statistically significant differences among plots.

gas, and leachate ($n = 10$ per plot). Mineral N flux rates could not be determined from individual cores because the analysis required destructive harvests at each time point, and flux rates were determined from multiple (unpaired) randomly selected cores over time. Thus, we averaged the N and ^{15}N pool size for soil NH_4^+ and NO_3^- within each sampling point for each plot prior to calculation of DNRA and gross N-cycling rates ($n = 3$) (Silver et al. 2001, 2005). We conducted one-way analyses of variance (ANOVA) using SAS JMP software version 3.2.5 (1999) with ingrowth core as the experimental unit for analyses of ^{15}N in roots, microbial biomass, bulk soil, gas, and leachate. We used experimental plot as the experimental unit for ANOVAs in the analyses of DNRA and gross N-cycling rates. Data that were not normally distributed were log-transformed prior to statistical analysis to meet the assumptions for ANOVA.

Microbial fingerprint TRFLP data from the MspI digest were analyzed with PCORD v4 (MJM Software Design, Gleneden Beach, Oregon, USA) according to guidelines described in Pett-Ridge and Firestone (2005) and Pett-Ridge et al. (2006). Principal components analysis was chosen for the TRFLP data because it is well suited for environmental data (McCune and Grace 2002). The cross-products matrix was constructed using the variance/covariance method, which centers the data but does not standardize the column variables (TRFs). The scores for samples were calculated by weighted averaging. ANOVA and Tukey pairwise comparison tests were used to test for significant differences between treatments. One sample each from the TMF 2 and TMF 4 sites failed quality control guidelines following sequencing and was therefore dropped from the analysis. To examine correlations among environmental variables or N-cycling rates and microbial community structure (represented by principal components) we used multiple regressions using JMP software (SAS Institute, Cary, North Carolina, USA; see Balser and Firestone 2005 for further discussions of this technique). Regression analysis of environmental variables against principal components scores was done using data collected in ^{15}N-

NH_4^+ label cores, except nitrification and DNRA, which were from cores that received ^{15}N-NO_3^- label. In order to examine potential correlations between the microbial communities and N-cycling rates, we used the average value across each plot for ^{15}N, NH_4^+, and NO_3^- at the T_0 sampling point and determined an N-cycling rate on a "per core" basis using the N and ^{15}N content at the T_{24}-hour sampling point. Joint biplot scores for secondary environmental variables and fluxes were plotted on top of ordinations; scores with an approximate r^2 value of 0.20 or higher are presented. Significance was determined as $P < 0.05$ unless otherwise noted.

RESULTS

Fates of ^{15}N over 24 hours

The mean residence time for both NH_4^+ and NO_3^- was less than one day (Table 2). Gross NH_4^+ production rates averaged $1.99 ± 0.77 \mu g \cdot g^{-1} \cdot d^{-1}$ over the first 24 hours (values reported are mean ± SE of three forest plots; Fig. 1, Table 2). The dominant fate of NH_4^+ was NO_3^- (Fig. 1; Table 2). Gross nitrification averaged $1.34 ± 0.48 \mu g \cdot g^{-1} \cdot d^{-1}$, ~65% of gross NH_4^+ production. Microbial uptake of NH_4-N and NO_3-N was not significantly different from zero ($0.099 ± 0.34$ and $0.10 ± 0.25 \mu g \cdot g^{-1} \cdot d^{-1}$, respectively; Fig. 2). The rate of root $^{15}NH_4^+$ uptake was $0.45 ± 0.17 \mu g \cdot g^{-1} \cdot d^{-1}$. Root uptake of $^{15}NO_3^-$ was ~24% of $^{15}NH_4^+$ uptake ($0.11 ± 0.017 \mu g \cdot g^{-1} \cdot d^{-1}$; Figs. 1 and 2). Although not statistically significant ($P = 0.09$), root biomass was a larger sink than microbial biomass following $^{15}NH_4^+$ addition. Similarly, root biomass was a larger sink for added $^{15}NO_3^-$ ($2.37 ± 0.75\%^{15}N$ recovery) than microbial biomass ($0.69 ± 3.02\%^{15}N$ recovery), but this pattern was also not statistically significant ($P > 0.05$). N_2O production accounted for a very small proportion of the added $^{15}NO_3^-$ label during the first 24 h (Table 2). Rates of DNRA averaged $0.47 ± 0.40 \mu g \cdot g^{-1} \cdot d^{-1}$ and were ~35% the rate of NO_3^- production (Table 2, Fig. 1). Although spatially variable, DNRA rates exceeded rates of N_2O production from the NO_3^- label by approximately two orders of magnitude ($P < 0.05$)
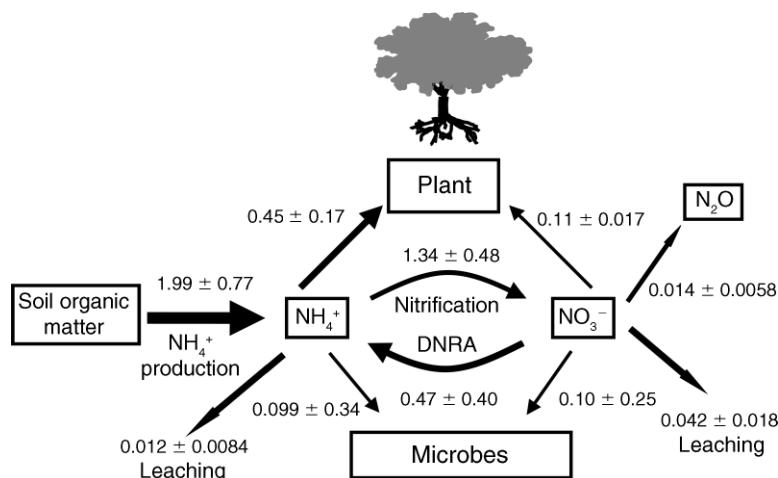
FIG. 1. Plant and microbial processes in a wet tropical forest soil during the first 24 hours following ^{15}N addition. The value for NH_4^+ production includes all sources of ^{14}N (gross mineralization and DNRA). DNRA is the rate of dissimilatory NO_3^- reduction to NH_4^+. The value included for NO_3^- leaching was measured at the seven-day sampling point. Values are means $\pm$ SE, given in $\mu g \cdot g^{-1} \cdot d^{-1}$.

Nitrogen uptake and losses over seven days

After seven days, there was detectable ^{15}N in microbial biomass from the $^{15}NH_4^+$ label (0.083 ± 0.022 μg excess $^{15}N/g$ soil; Fig. 2), but not from the $^{15}NO_3^-$ label. There was more root uptake from the $^{15}NH_4^+$ label compared to the $^{15}NO_3^-$ label at day 7 ($P < 0.001$). The roots were a significant sink for ^{15}N from the $^{15}NH_4^+$ label (0.19 ± 0.051 μg excess $^{15}N/g$ soil), but not the $^{15}NO_3^-$ label. Root biomass was a significantly larger sink for added $^{15}NH_4^+$ and $^{15}NO_3^-$ (6.28 ± 1.05 and $3.90 \pm 1.03\%$ ^{15}N recovery for NH_4^+ and NO_3^-, respectively) than microbial biomass (1.91 ± 0.52 and $0.26 \pm 0.83\%$ ^{15}N recovery for NH_4^+ and NO_3^-, respectively; $P < 0.05$).

Lysimeter samples taken at seven days indicated that a small amount of $^{15}NO_3^-$ leaching occurred (0.048 ± 0.024 $\mu g \cdot g^{-1} \cdot d^{-1}$), and an even smaller amount of $^{15}NH_4^+$ leached out of the soils (0.015 ± 0.007 $\mu g \cdot g^{-1} \cdot d^{-1}$). Nitrous oxide fluxes from label additions were extremely low in both $^{15}NH_4^+$ and $^{15}NO_3^-$ labeled cores (<0.001 $\mu g \cdot g^{-1} \cdot d^{-1}$).

Microbial community patterns

Microbial community profiling by TRFLP revealed a diverse microbial assemblage with 143 distinct terminal restriction fragments (TRFs). Following ordination, 50% of the data variance could be explained on the first two principal component axes. Secondary analysis by regression of environmental variables and rate processes against the first two principal components (PC) axes revealed weak (though statistically significant) correlations among environmental variables, N-cycling rates, and shifts in microbial community structure. The variables that correlated best with the microbial community first principal component axis were gross nitrification ($R^2 = 0.2$, $P < 0.03$) and N_2O flux ($R^2 = 0.22$, $P < 0.01$). Gross nitrification was also significantly

correlated with the second principal component axis ($R^2 = 0.18$, $P < 0.02$). We measured as much community variability within sites as across sites (Fig. 3). The most significant correlates differed when analyzed on a site-by-site basis. In TMF 2, with its relatively drier soils and significantly higher gross nitrification rates, soil moisture was most closely aligned with the microbial community patterns ($R^2 = 0.2$, $P < 0.051$). In TMF 4, where soil moisture was higher, the strongest correlates with microbial community patterns were N_2O flux, root recovery, and gross nitrification ($R^2 = 0.6$, 0.4, 0.2, $P < 0.001$, 0.054, 0.003, respectively). In the TMF Icacos site, the strongest correlate was soil C:N ($R^2 = 0.6$, $P < 0.011$).

Discussion

Nitrogen retention and loss: a soil nitrogen budget

We present a relatively complete budget for soil N-cycling in this tropical forest (Fig. 1). The gross rates of
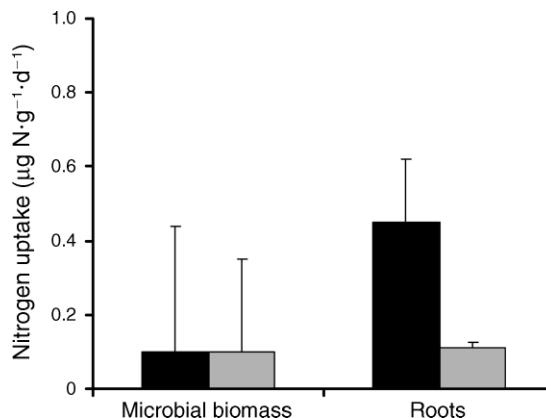

FIG. 2. Ammonium (solid bars) and NO_3^- (gray bars) uptake (mean + SE) by microbial biomass and roots over 24 h following ^{15}N addition.
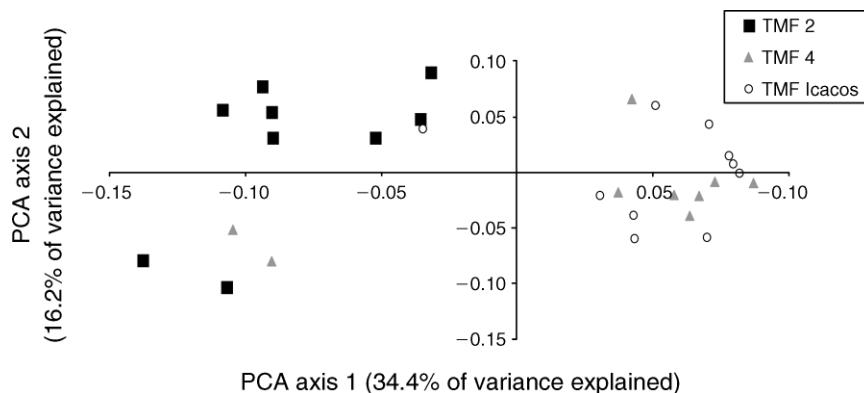
FIG. 3. Principal component analysis of soil bacterial terminal restriction length polymorphism (T-RFLP) fragment patterns from three 10×30 m plots (tropical montane forest 2, 4, and Icacos) in upland humid tropical soils, Puerto Rico. Replicate samples are indicated by symbols, and axes are scaled to percentage variance explained.

NH_4^+ production averaged 1.99 ± 0.77 $\mu g \cdot g^{-1} \cdot d^{-1}$. If we sum the direct fates of NH_4^+ (nitrification, microbial uptake, root uptake, and leaching), the resulting value of 1.90 ± 0.37 $\mu g \cdot g^{-1} \cdot d^{-1}$ agrees quite well with the value for gross NH_4^+ production determined by pool dilution. The measured rates of gross NH_4^+ production include all ^{14}N entering the NH_4^+ pool (gross mineralization and DNRA). The dominant fate for NH_4^+ in this system was nitrification with a rate of 1.34 ± 0.48 $\mu g \cdot g^{-1} \cdot d^{-1}$. Summing the measured fates of NO_3^- (root uptake, microbial uptake, denitrification to N_2O, leaching, and DNRA) yields a value of 0.74 ± 0.55 $\mu g \cdot g^{-1} \cdot d^{-1}$, accounting for ~55% of the rate of NO_3^- production. The fate of the remaining 45% of N entering the NO_3^- pool is likely to have been lost as N_2 in these humid, C-rich soils. There is good agreement (95.7%) between the measured rates of ^{14}N-NH_4^+ production and NH_4^+ consumption. Not accounting for 100% of $^{15}NO_3^-$ applied is a common challenge in tracer experiments. The lack of complete recovery does not alter our basic conclusions because we document the relative importance of several important soil processes.

Nitrate reduction via DNRA does not, in itself, serve as an N retention mechanism unless the NH_4^+ produced is assimilated into plants and/or microbial biomass. Assimilation would take the N "out of play" over the short term, decreasing the chances for re-nitrification and subsequent loss via denitrification and leaching. At a system level, N retention occurs when N uptake exceeds N losses. In this study DNRA reduced ~0.47 μg NO_3-$N \cdot g^{-1} \cdot d^{-1}$ to NH_4^+, and roots took up approximately the same amount of NH_4^+ over a 24-hour period. In contrast, N_2O production combined with leaching from the $^{15}NO_3^-$-labeled cores (assuming similarity between 24-hour and seven-day rates) resulted in the loss of only 0.056 μg NO_3-$N \cdot g^{-1} \cdot d^{-1}$. If we assume that all of the remaining NO_3^- produced was denitrified to N_2 (a liberal assumption as some may have been reduced to organic N; Dail et al. 2001, although see Colman et al. 2007), then NO_3^- losses amounted to 0.66 $\mu g \cdot g^{-1} \cdot d^{-1}$.

Rates of DNRA and subsequent NH_4^+ uptake decreased the potential NO_3^- losses. The absence of DNRA would likely increase N losses by 40% (assuming similar rates of plant and microbial uptake as currently observed) to 72% (if all of the additional NO_3^- was lost via leaching and denitrification). It is important to note that these estimates do not account for the response of plant and microbial processes to changes in NO_3^- concentrations.

Gross N cycling and N uptake

Few data are available for gross N transformations in tropical forests. Our rates of gross NH_4^+ production fall at the low end of the range of estimates of gross mineralization in tropical forest soils reviewed by Booth et al. (2005). This may result from the fact that most other assays were likely conducted with severed roots in the field or under laboratory settings where soil disturbance is likely to increase mineralization rates. The seemingly low rates of NH_4^+ production might also reflect an important role for small molecular mass organic N compounds (Schimel and Bennett 2004). Our rates of gross nitrification fall within the range found in other tropical forest soils (Booth et al. 2005), and highlight the large and rapid throughput of organic N to NO_3^-.

We are not aware of any studies that have measured in situ plant N uptake in tropical forest soils, or any that have compared plant vs. microbial N assimilation. Our results show that root uptake of $^{15}NH_4^+$ and $^{15}NO_3^-$ was much greater than microbial uptake over the seven days in these soils. We speculate that the fact that root N uptake exceeded microbial uptake may be a consequence of the high N availability in these soils, where microbes may be N saturated while plants may require greater N uptake for biomass maintenance and growth.

Roots preferred $^{15}NH_4^+$ over $^{15}NO_3^-$ uptake in this forest. Plant species differ in their capacity to take up NH_4^+ or NO_3^- (Gharbi and Hipkin 1984, Horsley 1988, Crabtree and Bazazz 1993, Garnett and Smethurst 1999). Many temperate plants show a strong preference

for NH_4^+ over NO_3^- (Wallander et al. 1997, Gessler et al. 1998, Garnett and Smethurst 1999). Nitrate is often more available to roots than NH_4^+ due to the high mobility of NO_3^- in soil water, but may be in low supply relative to NH_4^+ in these tropical forest soils due to the large number of strongly competing sinks. The preference of tropical plant species for NH_4^+ vs. NO_3^- can parallel the availability of either form of inorganic N (Houlton et al. 2007). Furthermore, plants must use energy to incorporate NO_3^- into their amino acids (Gutschick 1981, Smirnoff and Stewart 1985).

Our estimate of plant N uptake is conservative given that we did not measure movement of N into aboveground plant biomass or transport by roots outside of the cores. However, we can account for ~96% of the NH_4^+ produced over 24 h. Based on 24-hour root measurements, plant roots in our study took up ~0.47 ± 0.17 μg NH_4-N·g^{-1}·d^{-1}. Plant roots accessed and took up 23% of NH_4^+ produced during gross NH_4^+ production. Plant roots therefore represent a significant sink for NH_4^+, reducing the likelihood of N being lost from soils via nitrification and subsequent leaching or denitrification. In addition it is possible that roots in these soils take up N as amino acids, although the relatively high N availability in these soils is likely to decrease the importance of this pathway (Schimel and Bennett 2004). Direct root uptake of organic N from these soils would also serve to reduce N available for nitrification and subsequent loss from the NO_3^- pool.

We found that microbial uptake and assimilation of $^{15}NH_4^+$ and $^{15}NO_3^-$ was extremely low within the first 24 h. In 15 tropical forest soils, Vitousek and Matson (1988) found a strong preference for NH_4^+ over NO_3^- by the microbial biomass, and decreased net N immobilization, regardless of the form added, with increasing mineral N concentrations. As with plants, the process of assimilatory NO^- reduction by microbes requires energy for the conversion of NO_3^- to NH_4^+, and therefore is metabolically repressed when NH_4^+ is available (Puri and Ashman 1999). In soils with relatively high NH_4^+ availability, such as the tropical forest soils we studied, microbial assimilation of NO_3^- is often negligible (Vitousek and Matson 1988, Silver et al. 2001, 2005).

We calculated relatively high rates of consumption for NH_4-N and NO_3-N (Table 2). Calculated rates of consumption include several processes including abiotic sinks, gross nitrification (for NH_4^+), denitrification and DNRA (for NO_3^-), and microbial and plant N uptake. Because the calculated rate of consumption includes processes in addition to microbial uptake, it makes sense that our direct measure for microbial uptake was significantly less than that calculated for consumption. These results show the importance of actually measuring microbial N uptake directly (e.g., fumigation extraction techniques). Similarly low rates of microbial uptake of N have been found in another study conducted in wet tropical forests of Puerto Rico (Silver et al. 2001).

The fates of nitrate: DNRA vs. denitrification to N_2O

We found that 67% of the N entering the inorganic N pool was subsequently nitrified during the first 24 h. This, coupled with the significant potential for NO_3^- losses from humid tropical forest soils (Lewis et al. 1999, Stehfest and Bouwman 2006), highlights the importance of determining the fates of NO_3^- in order to understand patterns of N retention and loss in this ecosystem. We measured surprisingly low denitrification to N_2O from the $^{15}NO_3^-$ label. Nitrous oxide is an important greenhouse gas and a precursor for stratospheric ozone destruction (Cicerone 1987, Prather et al. 1995). Tropical forests are the largest natural source of N_2O globally (Stehfest and Bouwman 2006), and N_2O fluxes from humid soils are thought to be dominated by denitrification (Davidson et al. 1986). In this study, rates of DNRA were ~35% of gross nitrification, and were much greater than rates of N_2O production from denitrification. DNRA coupled with root and microbial uptake, N_2O fluxes, and leaching losses explained 55% of the NO_3^- produced, leaving 45% unexplained. We expect that denitrification to N_2 is the likely fate of the remaining NO_3^-. The lower montane subtropical wet forests in the LEF are characterized by variable redox conditions (Silver et al. 1999), high C availability (McGroddy and Silver 2000), and considerable NO_3^- production (Silver et al. 2001 and see Results), all of which contribute to high denitrification potential. High denitrification, coupled with a low N_2O to N_2 ratio would contribute to the low N_2O fluxes we observed. It is difficult to measure denitrification to N_2 in natural terrestrial systems due to the high background N_2 concentrations in the atmosphere. Current isotope ratio mass spectrometry analysis of N_2 is generally not sufficiently sensitive to detect added ^{15}N label in relatively low-level tracer experiments (Groffman et al. 2006). New methods for measuring N_2 fluxes are needed to better quantify the relative importance of denitrification and DNRA in tropical forests.

Leaching losses

We found small amounts of NH_4^+ leaching from these soils (0.015 ± 0.007 μg·g^{-1}·d^{-1}). While NO_3^- losses via leaching were greater, they were still small compared to the other processes we measured (Fig. 1). There has been research on the loss of stream water N from tropical forested watersheds (McDowell et al. 1992, 1996, McDowell and Asbury 1994). Approximately 50% of the total N exports (NH_4^+ + NO_3^- + dissolved organic nitrogen + particulate N) from tropical watersheds globally are in the form of NO_3^- (Lewis et al. 1999). Previous research in Puerto Rico shows that export from streams for three forested humid tropical watersheds averaged only 1.6 ± 0.2 kg NO_3-N·ha^{-1}·yr^{-1} (McDowell and Asbury 1994). Approximately 3.1% of NO_3^- produced via gross nitrification was leached during our week long assay. These relatively low leaching rates are likely a result of the other strong sinks for NO_3^-, which

decrease its susceptibility to leaching including DNRA and N_2 fluxes.

Microbial communities

Nitrogen-cycling processes are controlled by the interactions of microbes and their environment. Our data and previous studies have demonstrated considerable spatial and temporal heterogeneity of N-cycling processes in tropical forests (Vitousek and Matson 1988, Silver et al. 2001, 2005, Pett-Ridge 2005). Our microbial community data (Fig. 3) are consistent with this, showing high variability across sites. In addition, we found considerable variation within sites, particularly in TMF 4 where soil edaphic characteristics such as moisture, iron (Table 1), percentage clay, and belowground N_2O and CH_4 production (Pett-Ridge 2005) exhibit a wide range of values. The TRFLP bacterial fingerprints were correlated with N-cycling processes, particularly gross nitrification and denitrification to N_2O. As mentioned previously, NO_3^- production and the fates of NO_3^- appear to play a particularly important role in N retention and loss in this ecosystem. It is important to note that while the microbial community data show interesting trends, the correlations were relatively weak. TRFLP is a low-resolution approach of characterizing the complex microbial community structure, and thus should not be expected to yield strong correlations with specific N transformations. New techniques promise spatially and temporally explicit, deep phylogenetic and functional analyses of complex microbial communities. Unpublished clone library analysis (E. Brodie, personal communication) and high density microarray deep phylogenetic analysis of the communities in the TMF-4 site (E. Dubinsky, personal communication) show an abundance of phylogenetically diverse organisms not previously described. Under the fluctuating redox characteristics of these soils, a range of energy-generating metabolisms (from O_2 respiration to methanogenesis) are available to these complex communities over time and space. Specific microbial populations possess the appropriate metabolic capacities to exploit specific redox niches. Thus the processes that we measure (e.g., DNRA, denitrification, nitrification) turn on and off in these soils. Their on-phases are controlled in time and space by the localized redox conditions and the localized microbial community composition (Pett-Ridge 2005, Pett-Ridge and Firestone 2005).

CONCLUSIONS

Our research highlights the importance of rapid gross nitrification and the fates of NO_3^- in N retention and loss in humid tropical forest soils. We account for ~96% of the NH_4^+ produced and found that two-thirds of the NH_4^+ produced is converted to NO_3^- during nitrification over 24 hours. Over one-third of the NO_3^- produced is re-reduced to NH_4^+ via DNRA where it is available for plant uptake. Rates of DNRA, while

spatially variable, were two orders of magnitude greater than N_2O production from the $^{15}NO_3^-$ label. Plant uptake into biomass is the likely fate of the remaining NH_4^+, while the other possible major fate of NO_3^- in this system is N_2 production.

Nitrogen deposition rates in the tropics are expected to increase significantly over the next 10 to 20 years (Galloway et al. 1994, Matthews 1994, Holland et al. 1999). Matson et al. (1999) caution that, unlike some temperate forests, tropical forests are unlikely to act as a greater C sink with increased N deposition due to the fact that most tropical forests are limited by nutrients other than N. In fact, Matson et al. (1999) predict that tropical forests will ultimately respond to increased N deposition with reduced productivity because of the negative consequences of excess N (e.g., soil acidity, cation loss). Future research should focus on the fates of NO_3^- under increased N deposition scenarios in humid, highly weathered tropical forest soils. Key research needs include a more thorough understanding of the N retention and loss under increasing N deposition, and the factors controlling the ratio of N_2 and N_2O during denitrification.

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