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$_2$O. 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$_2$O 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 northern 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 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., ≤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 NH₄⁺ than NO₃⁻ (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 NO₃⁻.

Nitrate is generally more easily leached than NH₄⁺ in soils and is an important substrate for denitrification to N₂O, NO, and N₂. 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 NH₄⁺ during dissimilatory NO₃⁻ reduction to NH₄⁺ (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 NO₃⁻ 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 NH₄⁺ uptake exceed N losses from the NO₃⁻ 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 N₂O emissions over 24 h, and to determine the amount of N ultimately assimilated or lost via leaching and denitrification to N₂O 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°40’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 *Cyrra racemiflora* L.

We established three 10 × 30 m plots hereafter referred to as TMF 2, TMF 4, and TMF Iacos. 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 Iacos 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**

**¹⁵N field experiment**

To determine the competitive strengths of internal N-cycling pathways, leaching and denitrification to N₂O, we separately added ¹⁵NH₄⁺ and ¹⁵NO₃⁻ 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 N₂O losses. Plots were randomly subdivided into two sections and assigned to either ¹⁵NH₄⁺ or ¹⁵NO₃⁻ label addition to prevent cross-contamination.

Unlike most ¹⁵N 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 dish lysimeters on the bottom in place of root exclusion cloth. We sampled soils using a soil corer the same size as the
ingrowth cores. All coarse roots ($\geq 2$ 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 $\geq 1$ 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$_2$O fluxes, and microbial community composition, gross N transformations, DNRA, and total soil $^{15}$N content.

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

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

Notes: Values are means ± SE. Different lowercase superscript letters within a row represent statistically significant ($P < 0.05$) differences among plots.
† Data from E. Dubinsky (personal communication).

The $^{15}$N label experiment was carried out in November 2002. We injected $^{15}$NH$_4^+$ or $^{15}$NO$_3^-$ (>$\sim 99$ 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 $\sim 30.6$ atom % $^{15}$N-H$_4$ 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$_2$O 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$_2$O 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 $^{15}\text{N}$ 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-Zellweger 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 $^{15}\text{NO}_3^-$ according to Silver et al. (2005). Briefly, DNRA was determined as the difference in the $^{15}\text{NH}_4^+$ 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 $^{15}\text{NO}_3^-$ atom % during the interval to account for the isotopic composition of source pool. We estimated mean residence times of the $^{15}\text{NH}_4^+$ pool by dividing the initial NH$_4^+$ pool (micrograms per gram) by the rate of gross consumption in days using data from the $^{15}\text{NH}_4^+$ additions (Silver et al. 2001, 2005).

We determined N$_2$O concentration by gas chromatography using a 63Ni detector (GC 8610c; SRI Instruments, Torrence, California, USA), and determined N$_2$O gas isotope ratios using a trace gas module coupled to an IRMS. Rates of N$_2$O flux were estimated after correction for changes in the $^{15}\text{NO}_3^-$ source pool (by dividing by the mean $^{15}\text{NO}_3^-$ excess atom %) over time to make them comparable to other measured N fluxes. Also for comparative purposes, we report N$_2$O fluxes as micrograms N$_2$O-N per gram per day using the mass of soil in the core volume.

Root uptake was estimated as the change in $^{15}\text{N}$ recovered in biomass divided by the average atom % $^{15}\text{N}$ of available NH$_4^+$ and NO$_3^-$ pools over the 24-hour interval. Samples of leachate soil solution were digested with persulfate to determine the concentration of total dissolved N in leachate (Cabrera and Beare 1993) and diffused to determine $^{15}\text{N}$ 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 $^{15}\text{N}$ in soils immediately outside of the cores. In a separate, small study we compared $^{15}\text{N}$ of bulk soils at the center of the cores (230% ± 50% $^{15}\text{N}$) to soils 5–15 cm horizontal distance from the center of the cores (6.1% ± 1.3% $^{15}\text{N}$). 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 $^{15}\text{N}$ 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$_2$SO$_4$; 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 $^{15}\text{N}$ recovered in biomass divided by the average atom % $^{15}\text{N}$ of available NH$_4^+$ and NO$_3^-$ pools over the 24-hour interval. The percentage of $^{15}\text{N}$ recovery from all pools was calculated as the mass of $^{15}\text{N}$ 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°C 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 $^{15}\text{N}$ analyses for roots, microbial biomass, bulk soil,
Table 2. Soil N-cycling processes measured at each site at 24 hours.

<table>
<thead>
<tr>
<th>Process</th>
<th>TMF 2</th>
<th>TMF 4</th>
<th>TMF Icacos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross NH₄⁺ production (µg·g⁻¹·d⁻¹)</td>
<td>1.07</td>
<td>3.52</td>
<td>1.39</td>
</tr>
<tr>
<td>Gross nitrification (µg·g⁻¹·d⁻¹)</td>
<td>2.26</td>
<td>1.15</td>
<td>0.62</td>
</tr>
<tr>
<td>Gross NH₄⁺ consumption (µg·g⁻¹·d⁻¹)</td>
<td>2.63</td>
<td>11.05</td>
<td>3.08</td>
</tr>
<tr>
<td>Gross NO₃⁻ consumption (µg·g⁻¹·d⁻¹)</td>
<td>3.19</td>
<td>1.06</td>
<td>1.34</td>
</tr>
<tr>
<td>Mean residence time (d), NH₄⁺</td>
<td>0.71</td>
<td>0.43</td>
<td>0.98</td>
</tr>
<tr>
<td>Mean residence time (d), NO₃⁻</td>
<td>0.12</td>
<td>1.27</td>
<td>0.03</td>
</tr>
<tr>
<td>DNRA (µg·g⁻¹·d⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>¹⁵NO₂ flux (ng·g⁻¹·d⁻¹) from ¹⁵NO₃ label</td>
<td>1.93 ± 1.27</td>
<td>0.012 ± 0.012</td>
<td>4.77 ± 5.61</td>
</tr>
<tr>
<td>N₂O flux (ng·g⁻¹·d⁻¹) from ¹⁵NO₃ label</td>
<td>15.28 ± 10.80</td>
<td>0.15 ± 0.13</td>
<td>26.77 ± 25.71</td>
</tr>
<tr>
<td>Plant N uptake (µg·g⁻¹·d⁻¹) from ¹⁵NH₄ label</td>
<td>0.99 ± 0.01</td>
<td>0.09 ± 0.04</td>
<td>0.14 ± 0.12</td>
</tr>
<tr>
<td>Plant N uptake (µg·g⁻¹·d⁻¹) from ¹⁵NO₃ label</td>
<td>0.09 ± 0.01</td>
<td>0.09 ± 0.04</td>
<td>0.14 ± 0.12</td>
</tr>
<tr>
<td>Microbial N uptake (µg·g⁻¹·d⁻¹) from ¹⁵NH₄ label</td>
<td>−0.39 ± 0.21</td>
<td>0.46 ± 0.86</td>
<td>−0.13 ± 0.55</td>
</tr>
<tr>
<td>Microbial N uptake (µg·g⁻¹·d⁻¹) from ¹⁵NO₃ label</td>
<td>0.13 ± 0.11</td>
<td>0.37 ± 0.73</td>
<td>−0.20 ± 0.15</td>
</tr>
</tbody>
</table>

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 ¹⁵N pool size for soil NH₄⁺ and NO₃⁻ 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 ANOVAs in the analyses of environmental variables and fluxes were plotted on top of ordinations; scores with an approximate hour sampling point. Joint biplot scores for secondary environmental variables and fluxes were plotted on top of ordinations; scores with an approximate value across each plot for ¹⁵N, NH₄⁺, and NO₃⁻ was also not statistically significant (P > 0.20 or higher are presented. Significance was determined as P < 0.05 unless otherwise noted.

Results

Fates of ¹⁵N over 24 hours

The mean residence time for both NH₄⁺ and NO₃⁻ was less than one day (Table 2). Gross NH₄⁺ production rates averaged 1.99 ± 0.77 µg·g⁻¹·d⁻¹ over the first 24 hours (values reported are mean ± SE of three forest plots; Fig. 1, Table 2). The dominant fate of NH₄⁺ was NO₃⁻ (Fig. 1; Table 2). Gross nitrification averaged 1.34 ± 0.48 µg·g⁻¹·d⁻¹, ~65% of gross NH₄⁺ production. Microbial uptake of NH₄-N and NO₃-N was not significantly different from zero (0.099 ± 0.34 and 0.10 ± 0.25 µg·g⁻¹·d⁻¹, respectively; Fig. 2). The rate of root ¹⁵NH₄⁺ uptake was 0.45 ± 0.17 µg·g⁻¹·d⁻¹. Root uptake of ¹⁵NO₃⁻ was ~24% of ¹⁵NH₄⁺ uptake (0.11 ± 0.017 µg·g⁻¹·d⁻¹; Figs. 1 and 2). Although not statistically significant (P = 0.09), root biomass was a larger sink than microbial biomass following ¹⁵NH₄⁺ addition. Similarly, root biomass was a larger sink for added ¹⁵NO₃⁻ (2.37 ± 0.75%¹⁵N recovery) than microbial biomass (0.69 ± 3.02%¹⁵N recovery), but this pattern was also not statistically significant (P > 0.05). N₂O production accounted for a very small proportion of the added ¹⁵NO₃⁻ label during the first 24 h (Table 2). Rates of DNRA averaged 0.47± 0.40 µg·g⁻¹·d⁻¹ and were ~35% of the rate of NO₃⁻ production (Table 2, Fig. 1). Although spatially variable, DNRA rates exceeded rates of N₂O production from the NO₃⁻ label by approximately two orders of magnitude (P < 0.05).
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 l g$^{-1}$ 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 l g$^{-1}$ 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 ± 1.03% $^{15}$N recovery for NH$_4^+$ and NO$_3^-$, respectively) than microbial biomass (1.91 ± 0.52 and 0.26 ± 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 l g$^{-1}$d$^{-1}$), and an even smaller amount of $^{15}$NH$_4^+$ leached out of the soils (0.015 ± 0.007 l g$^{-1}$d$^{-1}$). Nitrous oxide fluxes from label additions were extremely low in both $^{15}$NH$_4^+$ and $^{15}$NO$_3^-$ labeled cores (<0.001 l g$^{-1}$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$_2$O 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$_2$O 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
NH$_4^+$ production averaged 1.99 ± 0.77 μg g$^{-1}$ d$^{-1}$. If we sum the direct rates of NH$_4^+$ (nitrification, microbial uptake, root uptake, and leaching), the resulting value of 1.90 ± 0.37 μg g$^{-1}$ 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 $^{15}$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 μg g$^{-1}$ d$^{-1}$. Summing the measured rates of NO$_3^-$ (root uptake, microbial uptake, denitrification to N$_2$O, leaching, and DNRA) yields a value of 0.74 ± 0.55 μg g$^{-1}$ 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 $^{15}$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 g$^{-1}$ d$^{-1}$ to NH$_4^+$, and roots took up approximately the same amount of NH$_4^+$ over a 24-hour period. In contrast, N$_2$O 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 g$^{-1}$ 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 μg g$^{-1}$ 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 $-0.47 \pm 0.17 \mu g$ 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$_4^+$ and NO$_3^+$ (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$_2$O

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$_2$O 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$_2$O globally (Stehfest and Bouwman 2006), and N$_2$O 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$_2$O production from denitrification. DNRA coupled with root and microbial uptake, N$_2$O 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$_2$O to N$_2$ ratio would contribute to the low N$_2$O 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 N₂ 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 N₂O and CH₄ 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 N₂O. As mentioned previously, NO₃⁻ production and the fates of NO₃⁻ 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 O₂ 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 onphases 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 NO₃⁻ in N retention and loss in humid tropical forest soils. We account for ~96% of the NH₄⁺ produced and found that two-thirds of the NH₄⁺ produced is converted to NO₃⁻ during nitrification over 24 hours. Over one-third of the NO₃⁻ produced is re-reduced to NH₄⁺ via DNRA where it is available for plant uptake. Rates of DNRA, while spatially variable, were two orders of magnitude greater than N₂O production from the ¹⁵NO₃⁻ label. Plant uptake into biomass is the likely fate of the remaining NH₄⁺, while the other possible major fate of NO₃⁻ in this system is N₂ 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 NO₃⁻ 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 N₂ and N₂O during denitrification.

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