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## Variations in soil N cycling and trace gas emissions in wet tropical forests

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**Abstract** We used a previously described precipitation gradient in a tropical montane ecosystem of Hawai'i to evaluate how changes in mean annual precipitation (MAP) affect the processes resulting in the loss of N via trace gases. We evaluated three Hawaiian forests ranging from 2200 to 4050 mm year<sup>-1</sup> MAP with constant temperature, parent material, ecosystem age, and vegetation. In situ fluxes of N<sub>2</sub>O and NO, soil inorganic nitrogen pools (NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>), net nitrification, and net mineralization were quantified four times over 2 years. In addition, we performed <sup>15</sup>N-labeling experiments to partition sources of N<sub>2</sub>O between nitrification and denitrification, along with assays of nitrification potential and denitrification enzyme activity (DEA). Mean NO and N<sub>2</sub>O emissions were highest at the mesic end of the gradient (8.7±4.6 and 1.1±0.3 ng N cm<sup>-2</sup> h<sup>-1</sup>, respectively) and total oxidized N emitted decreased with increased MAP. At the wettest site, mean trace gas fluxes were at or below detection limit (≤ 0.2 ng N cm<sup>-2</sup> h<sup>-1</sup>). Isotopic labeling showed that with increasing MAP, the source of N<sub>2</sub>O changed from predominately nitrification to predominately denitrification. There was an increase in extractable NH<sub>4</sub><sup>+</sup> and decline in NO<sub>3</sub><sup>-</sup>, while mean net mineralization and nitrification did not change from the mesic to intermediate sites but decreased dramatically at the wettest site. Nitrification potential and DEA were highest at the mesic site and lowest at the wet site. MAP exerts strong control N cycling processes and the magnitude and

source of N trace gas flux from soil through soil redox conditions and the supply of electron donors and acceptors.

**Keywords** Denitrification · Hawai'i · Nitrous oxide · Precipitation · Soil redox

### Introduction

Precipitation and resulting soil water characteristics are among the main factors that regulate the cycling of carbon (C), nitrogen (N), and other elements in terrestrial ecosystems. The accumulation and decomposition of organic matter (Austin 2002; Schuur 2001; Schuur et al. 2001 and others) and the release of mineral nutrients (Chadwick et al. 2003; Silver et al. 1999) are strongly related to soil water across a broad geographic range. Likewise, soil N cycling is influenced by precipitation and soil moisture in terms of hydrologic N losses (e.g. Brooks et al. 1999; Brooks and Williams 1999) and soil N transformations (e.g. Corre et al. 2002; Fierer and Schimel 2002; Franzluebbers et al. 2001; Gilliam et al. 2001). Nitrification and denitrification are thermodynamically favorable oxidation–reduction reactions mediated by soil microbe communities (Hedin et al. 1998; Morel and Hering 1993); oxygen status is, therefore, a primary controlling factor of these processes in soils (Bollmann and Conrad 1998; Robertson 1989). The magnitude and extent of these processes vary widely with changes in environmental characteristic on scales ranging from landscapes to soil microsites.

Nitrification and denitrification within soils are sources of NO and N<sub>2</sub>O trace gases to the atmosphere (Firestone and Davidson 1989 and others). NO regulates the production of tropospheric ozone, while N<sub>2</sub>O is approximately 310 times more efficient a greenhouse gas than CO<sub>2</sub> (IPCC 2001). Soil moisture is considered a key determinant of N trace gas production through stimulation of microbial activity, delivery of electron donors (NH<sub>4</sub><sup>+</sup>, DOC) and acceptors (O<sub>2</sub>, NO<sub>3</sub><sup>-</sup>), and the diffu-

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sion of N trace gases from soils (Firestone and Davidson 1989; Stark and Firestone 1995). A number of studies have reported dramatic shifts in NO and N<sub>2</sub>O emissions from soils with changes in soil moisture (Davidson 1992; Davidson et al. 1991, 1993; Garcia-Mendez et al. 1991; Garcia-Montiel et al. 2003; Keller and Reiners 1994 and others), with NO emissions typically highest under relatively dry conditions while N<sub>2</sub>O emissions increase with increasing soil moisture content. Theoretical models of trace gas flux predict that with soil water content sufficient to stimulate microbe activity but less than field capacity (approximately 10–60% water filled pore space (WFPS)), nitrification dominates as a source of N trace gases. For soils above field capacity, however, denitrification dominates as the source of N gases with N<sub>2</sub>O being the dominant flux from 60% to 80% WFPS and N<sub>2</sub> most important under more saturated conditions (Davidson 1991; Davidson and Verchot 2000).

The relative importance of the nitrification versus denitrification as the source of N trace gas emissions is often inferred from gas flux responses to water treatments, but has rarely been measured directly in the field. Panek et al. (2000) used stable isotope methods to measure sources of N<sub>2</sub>O from irrigated agricultural systems in Sonora, Mexico and showed that after irrigation N<sub>2</sub>O was generated by denitrification, but as soils dried nitrification became an increasingly important source. Other studies using isotopic labeling have indicated that denitrification can be a critical source of N<sub>2</sub>O even though soil moisture conditions predicted nitrification as the dominant mechanism (Muller et al. 2002, 2004; Wolf and Brumme 2002). Finally, the analysis of natural abundance of <sup>15</sup>N in ecosystems has begun to offer insights into the relative importance of different microbial sources (Houlton 2005; Perez et al. 2001).

Many parts of the tropics are highly seasonal and show pronounced changes in magnitude and form of N oxide fluxes between wet and dry seasons (Davidson et al. 1991; Keller and Reiners 1994; Verchot et al. 1999); however, this is not universally the case as human induced alterations such as pasture conversion tends to reduce seasonal variation (Garcia-Montiel et al. 2003). Similarly, N cycling and trace gas emissions in dry tropical forests differ markedly from humid and wet tropical forests (Matson and Vitousek 1987; Vitousek and Sanford 1986). To better understand how factors control N oxide emission at very large spatial scales, it is necessary to parse out the individual affects of controlling factors (precipitation, soils, and vegetation) and incorporate them into models of spatial variability (e.g. Martin and Asner 2005).

We sought to enhance the mechanistic understanding of N trace gas emissions by evaluating processes and gas fluxes along a rainfall gradient from 2200 to 4050 mm year<sup>-1</sup> in tropical montane forest on the island of Maui, Hawai'i. This gradient is characterized by strong soil redox changes from relatively oxic to anoxic soil conditions (Table 1). We measured the magnitude and sources of N trace gas emissions (NO and N<sub>2</sub>O), soil

inorganic N pools (NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>), and N transformations including net mineralization, nitrification, and denitrification at three sites along this gradient. Using isotopic labeling, we traced the source of N<sub>2</sub>O from soil to air. We expected that as rainfall and soil moisture increased across the sites, the importance of nitrification to trace gas production and overall N cycling would decrease in favor of denitrification with an accompanying shift from NO to N<sub>2</sub>O as the important gas flux at the soil–air surface.

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### Site description and study design

Our study sites are arrayed along a rainfall gradient located on the north flank of Haleakala volcano in the Makawao and Koolau Forest Reserves, Maui, Hawai'i (Fig. 1). The gradient was described by Schuur and Matson (2001), Schuur et al. (2001), and Miller et al. (2001). Mean annual rainfall at these sites increases systematically from 2200 mm year<sup>-1</sup> (mesic) to 4050 mm year<sup>-1</sup> (wet) over a distance less than 5 km (Giambelluca et al. 1986). In this region, aspect changes rapidly from relatively windward to relatively leeward establishing a strong rain shadow effect, and as a result of the prevailing northeast trade winds, rainfall is largely aseasonal with precipitation spread evenly across the year and more than 100 mm in any month (Schuur and Matson 2001). Soil moisture content measured at Sites 1 and 4 by time-domain reflectometry confirm long-term aseasonality but also show shorter-term changes in soil moisture on the order of days to weeks (data courtesy of B. Houlton, not shown). Volumetric soil moisture (5 cm depth) averaged 47% at our “mesic” Site 1 and 73% at Site 4 indicating dry conditions likely did not limit microbial activity at these sites. For logistical reasons, we selected three of the original six sites along the gradient (Sites 1, 4, and 5), which spanned broad changes in soil moisture and thermodynamic conditions (Table 1).

Sites along this gradient were selected to control for ecosystem state factors while varying only in precipitation amount. Elevation among the sites ranges from 1300 m to 1370 m and mean annual temperature is constant at 16°C. All of the sites are situated on the Kula volcanic series lava flow (approximately 400,000-year old) in generally flat areas (slope < 5%). Soils are classified as Inceptisols (2200 mm year<sup>-1</sup> site) and Andisols (3350 and 4050 mm year<sup>-1</sup> sites) (Miller et al. 2001; Schuur et al. 2001). Above-ground, tree biomass was dominated by *Metrosideros polymorpha* and averaged 23,772 ± 222 g m<sup>-2</sup> (± SE) across the sites. *Cheirodendron trigynum* was also found within the sites but comprised a relatively small fraction of the total biomass while N-fixing *Acacia koa* was found within the watershed but not within the study sites (Schuur and Matson 2001). Understory biomass increased from 692 ± 58 g m<sup>-2</sup> at Site 1 to 1431 ± 861 g m<sup>-2</sup> and 1579 ± 679 g m<sup>-2</sup> at Sites 4 and 5, respectively. Ferns

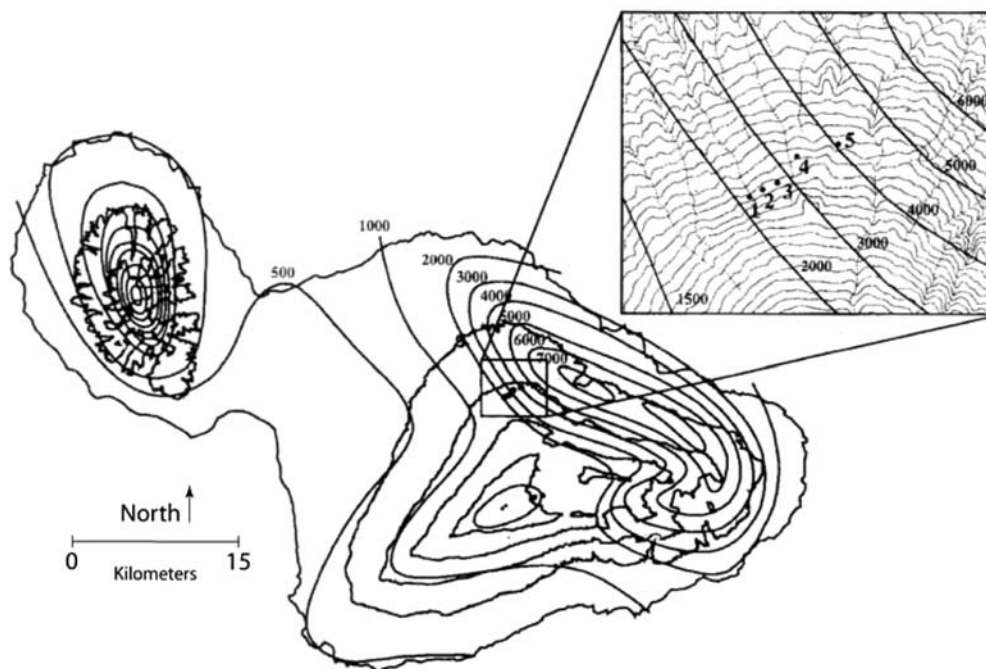
**Table 1** Site characteristics

Site	Coordinates <sup>a</sup> Latitude (deg min sec), Longitude (deg min sec)	Elevation <sup>a</sup> (m)	Mean annual precipitation <sup>a</sup> (mm yr <sup>-1</sup> )	Redox potential <sup>a</sup> (mV) n = 120	Soil water content <sup>b</sup> (% wet weight) n = 4	Bulk density <sup>b</sup> (g cm <sup>-3</sup> ) n = 24–40	Water filled pore space <sup>b</sup> ( % ) n = 4
1	20° 48' 21.0", 156° 15' 19"	1370	2200	417, 499, 304	62 ± 4	0.28 ± 0.02	57 ± 9
4	20° 48' 47.5", 156° 14' 49.5"	1320	3350	10, 224, -229	76 ± 2	0.17 ± 0.01	63 ± 8
5	20° 48' 47.5", 156° 14' 25"	1300	4050	-139, 86, -270	82 ± 2	0.10 ± 0.02	54 ± 5

<sup>a</sup>Site characteristics for mesic to wet precipitation gradient from Schuur et al. (2001). Soil redox shown as median, upper quartile, lower quartile from monthly measurements over 1 year

<sup>b</sup>Measured soil water content (percent of wet weight) and water filled pore space (WFPS) is from 4 sampling days with eight replicates per day. Bulk density (BD) is from 2 to 3 sampling events with 12 replicates. WFPS was derived from average BD for each site, gravimetric water content (percent of dry weight) for each replicate, and calculated particle density (see text for explanation of particle density calculation). Values are mean ± 1 SE

**Fig. 1** Location of mesic-to-wet precipitation gradient sites, Maui, Hawai'i, USA. Map includes isohyets of similar mean annual precipitation (in mm) and elevation contours of 610 m (full scale) or 12.2 m (inset). Figure adapted from Schuur (2001)



dominated the understory at Sites 1 and 4 while shrubs dominated at Site 5 (Schuur and Matson 2001).

## Methods

### N trace gas collection and analyses

Fluxes of nitrous oxide (N<sub>2</sub>O) and nitric oxide (NO) were measured across the gradient once in June 2000, twice in November 2000, and once in July 2001 with eight replicates per sampling using methods described in Matson et al. (1996). Soils within each chamber were destructively sampled following flux measurements, which allowed us to more accurately relate N trace gas emissions to N cycling parameters. As a result, the location of soil measurements was changed with each sampling date. Soil moisture was typical of long-term averages at these sampling times (Table 1). Because of the highly consistent climate and lack of seasonal

variation reported in this region (Schuur and Matson 2001) and in other Hawaiian sites (Hedin et al. 2003), we expected temporal variation within sites to be relatively small compared to differences between sites.

For N trace gas measurements, we inserted polyvinylchloride (PVC) bases (25 cm diameter × 10 cm height) approximately 5 cm into the soil surface and allowed the soil to equilibrate for a minimum of 15 min before initiation of flux measurements. Previous research in similar soils lacking a developed root mat has indicated that this delay is sufficient to obtain accurate results (Hall and Matson 2003; Hedin et al. 2003; Riley and Vitousek 1995). Bases were then fitted with molded acrylonitrile-butadiene-styrene (ABS) covers (10 cm height) fitted with gas-tight septa. Eight to 10 ml air samples were removed at four approximately equally spaced time points over the 30–45 min incubation and N<sub>2</sub>O concentrations in these samples were analyzed by gas chromatography (Shimadzu GC8A configured with a <sup>63</sup>Ni electron capture detector; Kyoto, Japan). Fluxes

were calculated by least-squares regression of  $\text{N}_2\text{O}$  concentration versus time, correcting for differences in air temperature and chamber volume. The minimum detectable flux was  $0.1 \text{ ng N cm}^{-2} \text{ h}^{-1}$ .

Within 30 min of  $\text{N}_2\text{O}$  collection, in situ measurements of NO were performed on the same replicates using methods described in Matson et al. (1996). PVC bases were covered individually with a vented ABS plastic cover connected to the detector in a continuous flow design. In this method, sample air from an enclosed chamber is blended with ambient air in a ratio of 4–6 to 1 (approximately  $1.0 \text{ L min}^{-1}$  ambient air to  $0.2 \text{ L min}^{-1}$  sample air). NO in the mixture is converted to  $\text{NO}_2$  and detected in real time with a Scintrex LMA–3 chemiluminescence detector (Concord, ON, Canada) through a photochemical reaction with Luminol solution. Readings were made every 15 s for a minimum of 4 min and NO fluxes were calculated by least-squares regression using the most linear segment of NO concentration versus time, correcting for differences in air temperature and chamber volume. The minimum detectable flux was  $0.1 \text{ ng N cm}^{-2} \text{ h}^{-1}$ .

#### Soil sampling and analyses

Once normal gas sampling was completed, two soil cores 10 cm deep and  $260 \text{ cm}^3$  in volume were collected from inside each chamber base. Within 4–6 h these soils were homogenized and sieved by hand to 2 mm, removing all roots and rocks. Subsamples (10 g wet weight) of sieved soils were extracted for inorganic N in 100 ml 2 N KCl as per Hart et al. (1994). Ammonium-N ( $\text{NH}_4^+\text{-N}$ ) and nitrate-N ( $\text{NO}_2^-\text{-N} + \text{NO}_3^-\text{-N}$ ; referred to herein by the primary component  $\text{NO}_3^-\text{-N}$ ) concentrations in 2 N KCl extracts were measured colorimetrically with an Alpkem Flow Solution IV running Win Flow 4.01 software (OI Analytical, College Station, TX, USA). To estimate net N transformations, a second 10 g subsample was incubated aerobically in the dark for 7–10 days at approximately  $25^\circ\text{C}$ , after which it was extracted in 2 N KCl as above. Net mineralization was calculated, as the increase of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  divided by duration of incubation. Net nitrification was calculated, as the increase of  $\text{NO}_3^-\text{-N}$  divided by duration of incubation. A minimum of 50 g wet weight of the remaining soil was dried at  $105^\circ\text{C}$  for 48 h to determine percent moisture content.

#### $\text{N}_2\text{O}$ source experiments

The relative proportions of  $\text{N}_2\text{O}$  from nitrification and denitrification were determined through application of  $^{15}\text{N}$ -labeled  $\text{NH}_4^+$  or  $\text{NO}_3^-$  as a tracer. This experiment was originally conducted in December of 2001 and repeated in July of 2002. Methods for the labeling study were modified from Panek et al. (2000) and briefly described here.

In four replicate blocks within each site, three chamber bases (plots) identical to those used for N trace gas measurements were inserted into the soil roughly 30 cm–50 cm apart; close enough to minimize differences resulting from spatial heterogeneity within the site yet far enough apart so that cross contamination of the tracer was unlikely. Plots within each block were injected with 100 ml of either  $^{15}\text{N}$ -labeled ammonium sulfate solution ( $(^{15}\text{NH}_4)_2\text{SO}_4$ ),  $^{15}\text{N}$ -labeled potassium nitrate solution ( $\text{K}^{15}\text{NO}_3$ ), or unlabeled water (control). To maximize the amount of label in each plot while minimizing stimulation of microbe activity through fertilization, solution concentrations were 10% of the estimated native soil extractable  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{N}$  pools at 99 atom percent  $^{15}\text{N}$ . To ensure adequate label at Site 5, where  $\text{NO}_3^-$  concentrations were very small, solutions were prepared at 50% of the estimated background pool. Nitrogen additions ranged from 58 to  $344 \mu\text{g KNO}_3\text{-N}$  and  $130\text{--}352 \mu\text{g } (^{15}\text{NH}_4)_2\text{SO}_4\text{-N}$  per plot. Label solution was injected from 0 to 10 cm depth in a standard grid pattern across the plot. To evenly distribute the tracer throughout the soil profile, injections were made using a modified spinal needle, which was slowly retracted as the label solution was injected.

Two hours after labeling,  $\text{N}_2\text{O}$  flux measurements were made on all plots as described above. Immediately after the final flux sample (30 or 45 min), 60 ml of chamber headspace was removed and stored in 50 ml vials (Wheaton No. 223745 with Geo-Microbial Technologies septa No. 1313, Ochelata, OK, USA) previously flushed with  $\text{N}_2$  gas and evacuated to roughly 26 mm Hg. Septa were sealed with a small amount of silicone sealant. Samples were kept at room temperature while transported to University of California at Berkeley for analysis on a Europa Scientific GC mass spectrometer (Europa Scientific, Crewe, Cheshire, UK).

After chamber headspace was sampled, three soil cores were taken from inside each plot to 10 cm depth. In December 2001, soils were transported to the lab (approximately 2–6 h) to be homogenized and extracted as described above. In July 2002, soils were weighed (25–57 g wet weight) and extracted with 150 ml 2 N KCl in the field, then taken to the lab for filtering and storage to minimize N transformations during the time between soil sampling and processing. Upon returning to Stanford University, soil KCl extracts from the  $^{15}\text{N}$  tracer experiment were diffused for  $^{15}\text{NH}_4^+$  and  $^{15}\text{NO}_3^-$  using the methods of Brooks et al. (1989) and analyzed at the University of California at Berkeley with a Europa Scientific mass spectrometer. In addition, a 10 cm deep,  $181 \text{ cm}^3$  volume core sample was taken just outside each plot and dried at  $105^\circ\text{C}$  to calculate bulk density (BD). BD values were repeatable but extremely low, probably due to their high organic content (see Table 1). Water-filled pore space was calculated as per Elliott et al. (1999) using average BD for each site and particle density (PD) based on percent C of the top 10 cm (from Schuur and Matson 2001), assumed PD of  $1.3 \text{ g cm}^{-3}$  for the or-

ganic fraction and  $2.65 \text{ g cm}^{-3}$  for the mineral fraction (Linn and Doran 1984).

Isotope mass ratios 44/45/46 ( $\text{N}_2\text{O}$ ) and 28/29/30 ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ) were used to calculate  $\mu\text{mol } ^{15}\text{N}_2\text{O-N}$  evolved to the headspace and then divided by  $\mu\text{mol } ^{15}\text{NH}_4^+\text{-N}$  or  $^{15}\text{NO}_3\text{-N}$  in the soil pool for each chamber. Data are presented as the ratio of this value from ( $^{15}\text{NH}_4$ ) $_2\text{SO}_4$  labeled plots to that from the  $\text{K}^{15}\text{NO}_3$  labeled plots for each block (see appendix for equations and details on the calculations). This method assumes that loss of  $^{15}\text{N}$  from soil to the headspace does not significantly affect soil enrichment and that labeled  $^{15}\text{NH}_4^+$  is the sole source of  $^{15}\text{N}$  for nitrification while labeled  $^{15}\text{NO}_3^-$  is the source for denitrification. In addition, fractionation of  $^{15}\text{N}$  by soil microbial processes is considered negligible, relative to the high isotopic enrichment of soil pools.

#### Nitrification potential and denitrification enzyme activity

To assess potential rates of nitrification and denitrification, we performed nitrification potential and denitrification enzyme activity (DEA) assays as per Hart et al. (1994) from control plots for both  $^{15}\text{N}$  tracer experiments. For nitrification potential assays, a soil slurry consisting of 10 g soil wet weight and 100 ml solution of ( $\text{NH}_4$ ) $_2\text{SO}_4$ ,  $\text{K}_2\text{HPO}_4$ ,  $\text{KH}_2\text{PO}_4$  at pH 7.2 was shaken to maintain aerobic conditions for a period of 24 h. At five time points over the 24 h, 15 ml of soil slurry was filtered and stored frozen for colorimetric analysis of  $\text{NO}_3^-$ -N. Nitrification potential rates were calculated as the least-squares regression of  $\text{NO}_3^-$ -N accumulation over 24 h and expressed on an areal basis using measured bulk densities. The minimum detectable flux was approximately  $0.2 \text{ mg N kg}^{-1} \text{ dry soil h}^{-1}$ .

DEA was measured using the methods of Tiedje (1982). In this method, 10 g of wet soil was combined with a 25 ml solution of  $\text{C}_4\text{H}_4\text{Na}_2\text{O}_4 \cdot 6\text{H}_2\text{O}$  (sodium succinate) and  $\text{KNO}_3$  so that both C and  $\text{NO}_3^-$  were in excess. Flasks were capped and made anaerobic by bubbling  $\text{N}_2$  gas through the soil slurry and acetylene ( $\text{C}_2\text{H}_2$ , 20 ml) was injected into the headspace to block production of  $\text{N}_2$  and allow only the production of  $\text{N}_2\text{O}$  by denitrification. Soil slurries were shaken continuously for 1 h during which 5 ml samples of headspace were removed at 15 min intervals and analyzed for  $\text{N}_2\text{O}$  as described above. Denitrification potential rates were calculated as the least-squares regression of  $\text{N}_2\text{O}$  accumulation over 1 h and expressed on an areal basis using measured bulk densities. All fluxes were well above detection limits.

#### Statistical analyses

All data were checked for normality (D'Agostino-Pearson  $K^2$ -test) and homoscedasticity (Bartlett's test) and if

necessary, were log transformed [ $X_{\text{log}} = \log(X + 1)$ ] prior to statistical analysis (Zar 1999). Trace gas fluxes, soil inorganic N, and rates of N transformation were analyzed using means of eight replicate chambers for each sampling day ( $n=4$  for all sites and variables with the exception of net mineralization and nitrification where  $n=3$  and NO for Site 4 where  $n=3$ ) with JMP IN statistical software Version 3.2.1 (SAS Institute Inc., 1997). Unless otherwise noted, the effect of mean annual precipitation (MAP) on soil and trace gas data were evaluated by one-way analysis of variance while comparisons between individual sites were made using Tukey-Kramer HSD and considered to be significant at the 0.05 level. Correlation coefficients were calculated using Spearman's rho ( $\rho$ ) rank correlation.

## Results and discussion

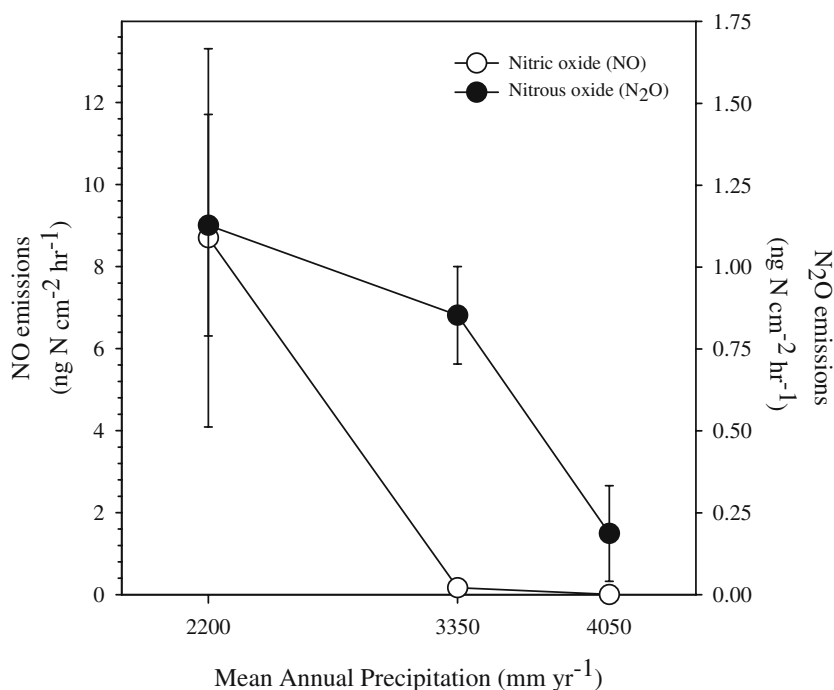
### Nitrogen trace gases

Patterns of N trace gas flux and N cycling changed dramatically along this mesic to wet precipitation gradient in Maui, Hawai'i. Analysis of variance showed MAP to have a significant effect on  $\text{N}_2\text{O}$  production across these sites ( $F_{2,9}=4.5$ ,  $P<0.05$ ) with Site 1 significantly different only from Site 5. Total losses of N via trace gases were highest at Site 1 and lowest at Site 5 (Fig. 2). Mean  $\text{N}_2\text{O-N}$  fluxes of eight replicate chambers ranged from  $-0.2$  to  $1.8 \text{ ng N cm}^{-2} \text{ h}^{-1}$  across all sites. Sites 1 and 4 typically exhibited  $\text{N}_2\text{O}$  production while Site 5 consistently had fluxes at or below detection limits. Mean NO-N fluxes ranged from 0.0 to  $21.5 \text{ ng N cm}^{-2} \text{ h}^{-1}$ . Only Site 1 had substantial fluxes of NO, and non-parametric analysis of variance (Kruskal-Wallis) showed MAP to have a significant effect on NO production ( $H=8.9$ , d.f.=2,  $P=0.01$ ). The  $\text{N}_2\text{O-N}$  to NO-N ratio from chambers with measurable fluxes changed significantly from  $0.4 \pm 0.2$  ( $\pm \text{SE}$ ) at Site 1 to  $5.2 \pm 1.2$  at Site 4 ( $t_5=4.5$ ,  $P<0.01$ ). Site 5 did not have measurable NO fluxes so we could not calculate the  $\text{N}_2\text{O-N/NO-N}$ . Total losses of trace gases were lower at Sites 4 and 5 but reflected a greater proportion  $\text{N}_2\text{O-N}$ .

$\text{N}_2\text{O}$  emissions did not correlate with WFPS but were positively correlated with  $\text{NO}_3^-$  (per unit area;  $\rho=0.44$ ,  $P<0.001$ ,  $n=78$ ), net mineralization ( $\rho=0.49$ ,  $P<0.001$ ,  $n=63$ ), and net nitrification ( $\rho=0.41$ ,  $P<0.001$ ,  $n=63$ ). NO emissions also did not correlate with WFPS and were positively correlated with  $\text{NO}_3^-$  ( $0.87$ ,  $P<0.001$ ,  $n=88$ ), net mineralization ( $\rho=0.66$ ,  $P<0.001$ ,  $n=64$ ), and net nitrification ( $\rho=0.58$ ,  $P<0.001$ ,  $n=64$ ). NO emissions were, however, correlated with percent moisture ( $\rho=-0.63$ ,  $P<0.001$ ,  $n=88$ ).

The trend of decreasing net N transformations corresponding with lower total trace gases flux fits well with models suggesting that the rate of N cycling in soils is a primary factor controlling soil N emissions (e.g. Davidson et al. 2000; Firestone and Davidson 1989). We expected that trace gases and soil N transformations

**Fig. 2** Nitrous oxide ( $\text{N}_2\text{O}$ ) and nitric oxide (NO) emissions across mesic to wet precipitation gradient. Values are means  $\pm$  1 SE from 6/2000, 11/2000 (twice), and 7/2001 with five to eight replicates per sampling date, with the exception of NO where Site 4 was sampled once in 11/2000. MAP had a significant effect on NO and  $\text{N}_2\text{O}$  emissions from soils (ANOVA,  $P \leq 0.05$ )



would be highest where rainfall inputs were adequate to allow both nitrification and denitrification conditions to co-occur within the ecosystem. Under such circumstances, electron acceptors ( $\text{NO}_3^-$ ) produced by nitrification in localized oxic areas would be in close spatial or temporal proximity to more anoxic conditions and electron donors (DOC) where N could be rapidly denitrified. Our results generally support this prediction, with maximum trace gas fluxes occurring at the 2200 mm year<sup>-1</sup> MAP (Site 1) and dropping as soils become wetter. However, our gradient extends only from mesic to very wet conditions; if expanded to include dry end member sites with similarly low seasonal variation (no such forested sites exist in this region), we expect there would be relatively low trace gas flux due to water limitation of microbial processes. Indeed, in dry tropical forests of Mexico receiving less than 1000 mm MAP, fluxes of NO and  $\text{N}_2\text{O}$  were typically less than our observed fluxes at Site 1 both during their wet ( $\text{NO} = 1.27 \pm 0.11 \text{ ng cm}^{-2} \text{ h}^{-1}$ ,  $\text{N}_2\text{O} = 0.82 \pm 0.05 \text{ ng cm}^{-2} \text{ h}^{-1}$ ) and dry seasons ( $\text{NO} = 0.52 \pm 0.22 \text{ ng cm}^{-2} \text{ h}^{-1}$ ,  $\text{N}_2\text{O} = 0.29 \pm 0.07 \text{ ng cm}^{-2} \text{ h}^{-1}$ ; Davidson 1993). In humid forests and pastures of Rondônia, Brazil with equivalent MAP as our Site 1, Neill et al. (2005) observed similar patterns to ours with generally large trace gas emissions and NO fluxes substantially greater than  $\text{N}_2\text{O}$ .

With increased MAP, there is a clear shift in the relative importance of nitrification and denitrification for N trace gas production. Although  $\text{N}_2\text{O}$  flux was highest at Site 1 compared to Sites 4 and 5, NO dominated trace gas losses by roughly eightfold suggesting that nitrification is the primary contributor to N trace gas flux at the mesic, more oxic end of the gradient (Table 2, Fig. 2). Conversely, the increase in  $\text{N}_2\text{O}/\text{NO}$

suggests denitrification is likely the overriding process at Sites 4 and 5, although it is possible that some NO produced via nitrification was reduced to  $\text{N}_2\text{O}$  prior to leaving the soil.

This inferred change in trace gas source with increased MAP was substantiated through the isotopic labeling of soil inorganic pools. Results from the  $^{15}\text{N}$  tracer studies are presented as the ratio of  $^{15}\text{N}_2\text{O}-\text{N}$  from ( $^{15}\text{NH}_4$ )<sub>2</sub>SO<sub>4</sub> labeled plots relative to  $\text{K}^{15}\text{NO}_3$  labeled plots with each block. This ratio describes the relative contribution of  $^{15}\text{N}_2\text{O}$  evolved from nitrification [( $^{15}\text{NH}_4$ )<sub>2</sub>SO<sub>4</sub> labeled plots] to denitrification ( $\text{K}^{15}\text{NO}_3$  labeled plots) corrected for differences in enrichment of soil pools. Ratios greater than one indicate nitrification as the predominant source of  $\text{N}_2\text{O}$ , while ratios below one indicated the dominance of denitrification. Ratios ranged from less than 0.1 to 34.1, shifting from high to low with increased MAP (Fig. 3). Comparisons among sites showed MAP to have a significant effect for the July sampling ( $H = 5.9$ , d.f. = 2,  $P = 0.05$ ), but not for December because of low statistical power.

The labeling experiment confirms that at the mesic site  $\text{N}_2\text{O}$  was primarily produced by nitrification, while at the wettest site, denitrification dominated. It is important to note, however, that some labeled  $^{15}\text{N}_2\text{O}$  was detected from both  $^{15}\text{NH}_4$  and  $^{15}\text{NO}_3$  treatments at all sites, and thus both nitrification and denitrification are co-occurring at some level due to microsite heterogeneity.

#### Soil inorganic N and N transformations

Mineralization, nitrification, and denitrification rates are primarily determined by availability of substrate (DON,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ) and redox status of soils (Chapin

**Table 2** Soil N pools and rates of N transformation across mesic to wet precipitation gradient

Site	Mean annual precipitation mm yr <sup>-1</sup>	NH <sub>4</sub> <sup>+</sup> -N		NO <sub>3</sub> <sup>-</sup> -N		Net mineralization		Net nitrification	
		mg m <sup>-2</sup> n=4	mg kg <sup>-1</sup> n=4	mg m <sup>-2</sup> n=4	mg kg <sup>-1</sup> n=4	mg m <sup>-2</sup> day <sup>-1</sup> n=3	mg kg <sup>-1</sup> day <sup>-1</sup> n=3	mg m <sup>-2</sup> day <sup>-1</sup> n=3	mg kg <sup>-1</sup> day <sup>-1</sup> n=3
1	2200	543 ± 197 <sup>A</sup>	19 ± 7 <sup>A</sup>	1004 ± 414 <sup>A</sup>	36 ± 15 <sup>A</sup>	252 ± 67 <sup>A</sup>	9 ± 2 <sup>A</sup>	230 ± 64 <sup>A</sup>	8 ± 2 <sup>A</sup>
4	3350	300 ± 40 <sup>A</sup>	18 ± 2 <sup>A</sup>	192 ± 97 <sup>B</sup>	11 ± 3 <sup>A</sup>	185 ± 38 <sup>AB</sup>	11 ± 1 <sup>AB</sup>	193 ± 25 <sup>AB</sup>	11 ± 1 <sup>AB</sup>
5	4050	431 ± 160 <sup>A</sup>	41 ± 15 <sup>A</sup>	20 ± 10 <sup>C</sup>	2 ± 0.5 <sup>B</sup>	14 ± 34 <sup>B</sup>	1 ± 2 <sup>B</sup>	25 ± 5 <sup>B</sup>	2 ± 0 <sup>B</sup>

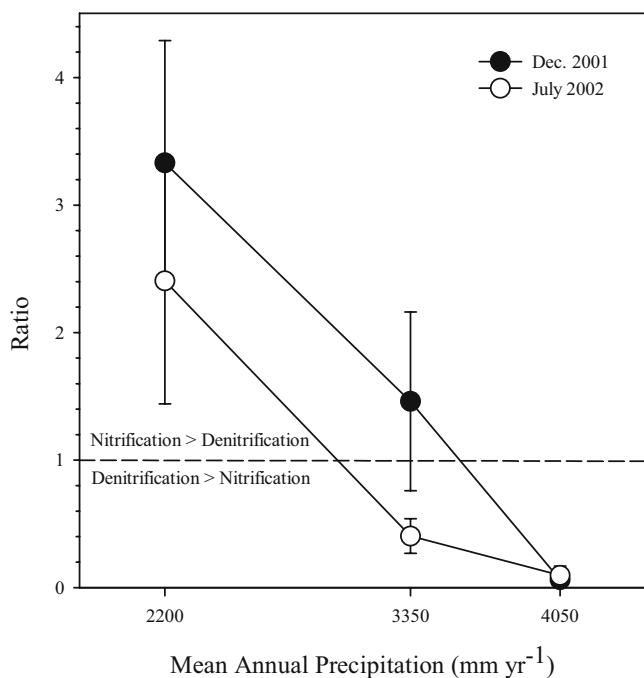
Values are mean ± 1 SE from sampling dates in 6/2000, 11/2000 (twice), and 7/2001 with five to eight replicates per date. Letters indicate significant differences among treatments to  $P \leq 0.05$  (Tukey-Kramer HSD). MAP interpreted from Giambelluca et al. (1986) as described in Schuur et al. (2001).

et al. 2002 and others). As expected, MAP had a significant effect on net rates of mineralization ( $F_{2,6} = 7.0$ ,  $P < 0.05$ ) and nitrification ( $F_{2,6} = 8.3$ ,  $P < 0.05$ ) (Table 2). For most individual chambers, net mineralization and nitrification were approximately equal ( $\rho = 0.88$ ,  $P < 0.001$ ,  $n = 72$ ) suggesting that, at least in the mesic and intermediate sites, most mineralized N is rapidly nitrified to NO<sub>3</sub><sup>-</sup>. Substantial decline in N transformation rates was not observed until MAP was 4050 mm year<sup>-1</sup> (Site 5) indicating that N availability is substantially lower at the wettest site (Table 2). Lab incubations are also likely to overestimate in situ

mineralization and nitrification, particularly at Sites 4 and 5 where mixing and aeration of soils could stimulate microbial activity, and therefore, underestimate the degree to which N availability decreases with increasing precipitation across the sites.

Silver et al. (2001) have shown that dissimilatory reduction of NO<sub>3</sub><sup>-</sup> to NH<sub>4</sub><sup>+</sup> (DNRA) can be a significant N transformation pathway in upland tropical forests. At sites in Puerto Rico similar in rainfall to our Site 5, DNRA was three times greater than N oxide flux and 75% of the total NO<sub>3</sub><sup>-</sup> pool turnover. We found only minimal evidence of DNRA during our tracer experiment (July 2002) with 0.2–0.5% of the labeled <sup>15</sup>NO<sub>3</sub><sup>-</sup> recovered as <sup>15</sup>NH<sub>4</sub><sup>+</sup> after the 2 h incubations with no apparent pattern among sites. In contrast, we recovered 0.8–12% of the labeled <sup>15</sup>NH<sub>4</sub><sup>+</sup> as <sup>15</sup>NO<sub>3</sub><sup>-</sup> with highest recovery at Site 1 and lowest at Site 5. Based on these data, we conclude nitrification and denitrification, not DNRA, are the dominant processes affecting emissions of N trace gases from these sites.

With increasing MAP, the relative amounts of extractable N shifted from the more oxidized form (NO<sub>3</sub><sup>-</sup>) to the more reduced form (NH<sub>4</sub><sup>+</sup>) (Table 2). MAP had no effect on soil NH<sub>4</sub><sup>+</sup> ( $F_{2,9} = 1.9$ ,  $P > 0.2$ ), but a highly significant effect on NO<sub>3</sub><sup>-</sup> ( $F_{2,9} = 17.8$ ,  $P < 0.001$ ). Site 1 had the highest total inorganic N concentration and nearly twice as much NO<sub>3</sub><sup>-</sup> as NH<sub>4</sub><sup>+</sup>. In contrast, Site 5 inorganic soil N concentrations were primarily NH<sub>4</sub><sup>+</sup> with very little extractable NO<sub>3</sub><sup>-</sup> (42 vs. 2 mg N kg<sup>-1</sup> of dry soil).



**Fig. 3** N<sub>2</sub>O sources. Result of <sup>15</sup>N tracer experiment to partition sources of N<sub>2</sub>O. Data presented is the ratio of  $\mu\text{mol } ^{15}\text{N}_2\text{O-N}$  collected in chamber headspace of <sup>15</sup>NH<sub>4</sub><sup>+</sup> labeled plots to <sup>15</sup>NO<sub>3</sub><sup>-</sup> labeled plots, following correction for differences in source pool enrichment. This unitless value describes the relative contribution of <sup>15</sup>N<sub>2</sub>O evolved from nitrification (<sup>15</sup>NH<sub>4</sub><sup>+</sup> labeled plots) to denitrification (<sup>15</sup>NO<sub>3</sub><sup>-</sup> labeled plots). Ratios greater than 1 indicate nitrification as the predominant contributor to N<sub>2</sub>O flux while ratios below one indicate denitrification. Values are means ± 1 SE of the three to four replicate blocks within each site for two repeat sampling dates (12/2001 and 7/2002). MAP had a significant effect of the relative source of N<sub>2</sub>O emission for the July 2002 sampling only (ANOVA,  $P = 0.05$ )

#### Nitrification potential and DEA

Nitrification potential and DEA assays were performed on soils from control plots during both tracer experiments (Fig. 4). Measured nitrification potential ranged from 0.2 to 233.4 mg N m<sup>-2</sup> h<sup>-1</sup>. For both December 2001 and July 2002 sampling dates, nitrification potential decreased significantly with increasing MAP (December:  $F_{2,9} = 31.1$ ,  $P < 0.001$ ; July:  $F_{2,9} = 20.5$ ,  $P < 0.001$ ; Fig. 4a). DEA ranged from 1.7 to 60.1 mg N m<sup>-2</sup> h<sup>-1</sup>. There was no overall treatment effect of MAP on DEA in December 2001 ( $F_{2,9} = 1.6$ ,  $P > 0.05$ ), but contrary to what we expected, DEA significantly decreased as MAP increased in July 2002 ( $F_{2,9} = 9.6$ ,  $P < 0.01$ ; Fig. 4b).

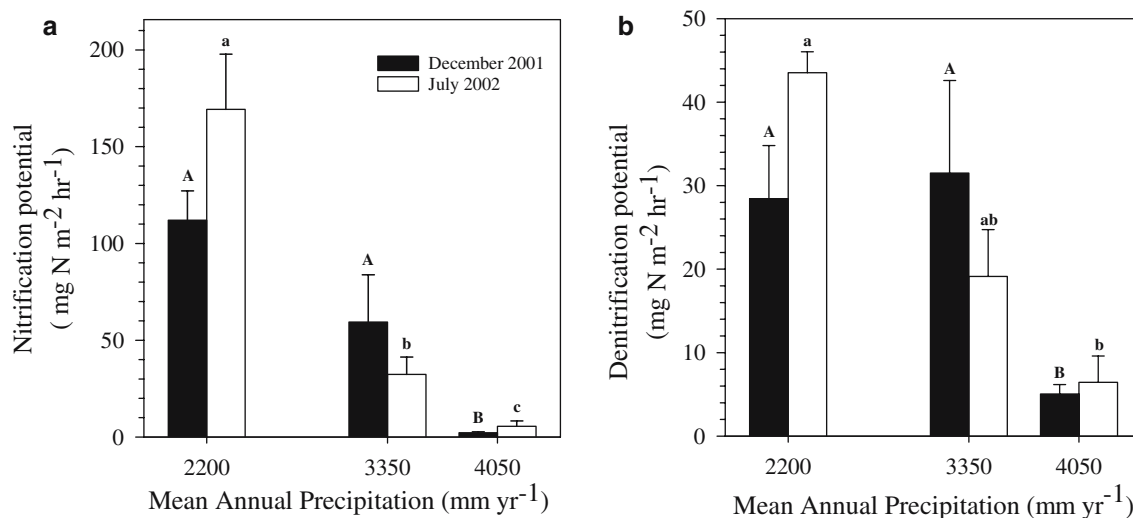
Indicators of potential nitrification and denitrification reflect the measured soil fluxes and N turnover rates. As expected, nitrification potential generally decreased with increased MAP due to changes in soil redox conditions and the inability of autotrophic nitrifiers to undergo aerobic respiration (Fig. 4a). DEA also varied across the gradient (Fig. 4b) and was correlated with soil  $\text{NO}_3^-$  concentrations (Spearman's  $\rho = 0.66$ ,  $P < 0.001$ ,  $n = 24$ ). Denitrification enzymes are thought to be relatively long-lived in soils, persisting for several months, and thus integrative of conditions over time (Smith and Parsons 1985; Veldkamp et al. 1999). DEA values were quite high in Site 1, with rates roughly equivalent to highly fertile, 20,000-year-old sites in Hawai'i (Hall and Matson 2003), higher than previous studies for tropical soils (Silver et al. 2000; Veldkamp et al. 1999), and comparable to those of urban riparian zones (Groffman et al. 2002; Groffman and Crawford 2003). DEA remains high in Site 4, which suggests that this site experiences pronounced variation in nitrification and denitrification processes and the conditions that regulate them. The observed high DEA, available  $\text{NO}_3^-$  substrate, wet soils (soil moisture greater than 60%), and  $\text{N}_2\text{O}$  from denitrification indicate that Sites 1 and 4 could have substantial  $\text{N}_2$  flux, although we did not measure this directly.

DEA values at Site 5 were substantially lower than at the other two sites but indicate that denitrification may occur there. Results from our limited sampling show little in situ  $\text{NO}_3^-$  production (Table 2, Fig. 4) or oxidized N gas losses from soils at this site (Fig. 2); any potential gaseous losses are likely to be in the form of  $\text{N}_2$ . This is inconsistent with theoretical expectations for a shift in emissions ratios favoring  $\text{N}_2$  under very anaerobic conditions where nitrate levels are low relative to organic C (Chapin et al. 2002), and also with the findings of Houlton (2005), whose isotopic budgets suggest that

denitrification occurs and is relatively more important than  $\text{NO}_3^-$  leaching at Site 5.

## Conclusions

Other studies from Hawai'i have shown that, as MAP increases above 2000 mm, decomposition and nutrient availability decline, resulting in a progressively more closed N cycle and a decline of above-ground net primary productivity (Austin and Vitousek 2000; Schuur 2001; Vitousek 2004). Our study supports these findings and illustrates that MAP and the resulting oxygen status of soils within sites impart strong controls on N cycling and losses via trace gases through the activity of nitrifying and denitrifying bacteria and presence of electron donors and acceptors. Nitrous oxide fluxes in our mesic tropical forest appear to be largely a result of the nitrification process, with denitrification becoming a more important source in wetter sites. While most other studies of N gas fluxes in tropical forests have not directly separated nitrification from denitrification sources, the general pattern of NO flux greater than  $\text{N}_2\text{O}$  flux in seasonally dry tropical forests (Davidson et al. 1991, 1993; Neill et al. 2005) and savannah (Cardenas et al. 1993; Donoso et al. 1993; Johansson et al. 1988) suggests that nitrification may be more important there, while denitrification is typically thought to be more important in humid and wet forests (Davidson et al. 2000; Keller and Reiners 1994; Verchot et al. 1999). With MAP ranging from less than 1000 to over 5000 mm and with major variation in soils and parent materials, forests in the tropical latitudes experience substantial variation in N cycling processes and N gas fluxes (Matson and Vitousek 1987; Vitousek and Sanford 1986). This study illustrates that dramatic changes in soil moisture across space can create a wide range of thermodynamic



**Fig. 4** Nitrification potential (a) and denitrification potential (b) as measured by enzyme activity across mesic to wet precipitation gradient. Soils are from control plots of  $^{15}\text{N}$  tracer experiment.

Results are means  $\pm$  1 SE ( $n = 4$ , 2 duplicates). Letters indicate significantly different groups within each sampling date (Tukey-Kramer HSD,  $P \leq 0.05$ )

conditions even over small distances, resulting in large variation in N cycling processes, as well as shifts in source and magnitude of N trace gas emissions. In contrast to our sites, much of the tropics also experience pronounced seasonality in precipitation and therefore, are likely to see changes in N trace gas sources temporally, as well as spatially. Incorporating fine scale patterns of ecosystem state factors (precipitation, soils, and vegetation) in predictions of N oxide emissions at coarser spatial scales remains a significant challenge.

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## Appendix: N<sub>2</sub>O source study equations

1. Atom percent <sup>15</sup>N<sub>2</sub>O, <sup>15</sup>NH<sub>4</sub><sup>+</sup>, and <sup>15</sup>NO<sub>3</sub><sup>-</sup> of soil and gas samples were calculated from the 46/45 (N<sub>2</sub>O), 29/28 and 30/28 (NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>) mass ratios. Data were blank corrected and corrected for analytical drift between runs.
2. We determined the amount of <sup>15</sup>N<sub>2</sub>O-N accumulated in the chamber headspace (<sup>15</sup>N<sub>2</sub>O<sub>h</sub> in μmol) during incubation by the following equation:

$$^{15}\text{N}_2\text{O}_h = S_1 \left( \frac{\text{N}_2\text{O}_{\text{ap}}}{100} \right) - S_0(0.003663) \quad (1)$$

where N<sub>2</sub>O<sub>ap</sub> is the measured atom percent <sup>15</sup>N<sub>2</sub>O-N at the end of the incubation, S<sub>1</sub> is the highest observed headspace N<sub>2</sub>O concentration, and S<sub>0</sub> is the lowest. N<sub>2</sub>O concentration was converted to μmol using the Ideal Gas Law. <sup>15</sup>N natural abundance is assumed to be 0.3663%.

3. Soil <sup>15</sup>N enrichment (N<sub>ap</sub> in atom percent) after labeling with <sup>15</sup>NH<sub>4</sub><sup>+</sup> or <sup>15</sup>NO<sub>3</sub><sup>-</sup> was estimated using a standard mixing equation (from Panek et al. 2000):

$$N_{\text{ap}} = \frac{N_{\text{native}} \times 0.3663 + N_{\text{added}} \times 99.0}{N_{\text{native}} + N_{\text{added}}} \quad (2)$$

where N<sub>native</sub> is the concentration of NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> of the soil prior to labeling (measured from control plots) and N<sub>added</sub> is the concentration of NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> added to the plots through injections. Soil <sup>15</sup>N natural abundance is assumed to be 0.3663% and label enrichment was 99.0 atom percent.

4. We calculated the amount of soil <sup>15</sup>N (<sup>15</sup>N<sub>NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup>, in μmol) using estimated soil enrichment and measured soil inorganic N concentrations.</sub>

$$^{15}\text{N}_{\text{NH}_4^+ \text{ or NO}_3^-} = N_{\text{NH}_4^+ \text{ or NO}_3^-} \times \frac{N_{\text{ap}}}{100} \times \text{BD} \times V \quad (3)$$

where N<sub>ap</sub> is atom percent <sup>15</sup>NH<sub>4</sub><sup>+</sup> or <sup>15</sup>NO<sub>3</sub><sup>-</sup> from Eq. 2, N<sub>NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup></sub> is the concentration of NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> at the end of the incubation (μmol N g<sup>-1</sup> dry soil), BD (g dry soil cm<sup>-3</sup>), and V is the volume of the experimental plot to 10 cm depth (cm<sup>-3</sup>).

For the July 2002 experiment, we were able to measure soil <sup>15</sup>N enrichment directly and compare with estimated values from Eq. 2. Measured were 75% (Site 1), 111% (Site 4), and 130% (Site 5) of estimated values and results presented in Fig. 3 were essentially unchanged with measured versus estimated enrichments. For consistency, we used estimated values for both experiments.

5. The amount of <sup>15</sup>N<sub>2</sub>O-N in the headspace derived from either <sup>15</sup>NH<sub>4</sub><sup>+</sup> or <sup>15</sup>NO<sub>3</sub><sup>-</sup> was corrected for differences in soil pool enrichment among chambers and sites. It is assumed the atom percent <sup>15</sup>N<sub>2</sub>O flux to the headspace is equal to that of the source pool (i.e. no fractionation).

$$^{15}\text{N}_2\text{O}_{\text{nitrification}} = \frac{^{15}\text{N}_2\text{O}_h}{^{15}\text{N}_{\text{NH}_4^+}} \quad (4)$$

(from <sup>15</sup>NH<sub>4</sub><sup>+</sup> labeled plots)

$$^{15}\text{N}_2\text{O}_{\text{denitrification}} = \frac{^{15}\text{N}_2\text{O}_h}{^{15}\text{N}_{\text{NO}_3^-}} \quad (4)$$

(from <sup>15</sup>NO<sub>3</sub><sup>-</sup> labeled plots)

where <sup>15</sup>N<sub>2</sub>O<sub>h</sub> is the result from Eq. 1, and <sup>15</sup>N<sub>NO<sub>3</sub><sup>-</sup></sub> and <sup>15</sup>N<sub>NH<sub>4</sub><sup>+</sup></sub> are results from Eq. 3 for the NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> pools, respectively.

6. The relative proportion of N<sub>2</sub>O derived from nitrification versus denitrification (R) was calculated on a block-by-block basis by comparing the <sup>15</sup>N<sub>2</sub>O-N recovered from the <sup>15</sup>NH<sub>4</sub><sup>+</sup> labeled plots to the <sup>15</sup>NO<sub>3</sub><sup>-</sup> labeled plots. Ratios greater than one indicate nitrification as the predominant source of N<sub>2</sub>O, while ratios below one indicate denitrification.

$$R = \frac{^{15}\text{N}_2\text{O}_{\text{nitrification}}}{^{15}\text{N}_2\text{O}_{\text{denitrification}}} \quad (5)$$

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