

Responses of nitrous oxide fluxes and soil nitrogen cycling to nutrient additions in montane forests along an elevation gradient in southern Ecuador

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Abstract Tropical montane forests are commonly limited by N or co-limited by N and P. Projected increases in N deposition in tropical montane regions are thought to be insufficient for vegetation demand and are not therefore expected to affect soil N availability and N₂O emissions. We established a factorial N- and P-addition experiment (i.e., N, P, N + P, and control) across an elevation gradient of montane forests in Ecuador to test these hypotheses: (1) moderate rates of N and P additions are able to stimulate soil-N cycling rates and N₂O fluxes, and (2) the magnitude and timing of soil N₂O-flux responses depend on the initial nutrient status of the forest soils. Moderate rates of nutrients were added: 50 kg N ha⁻¹ year⁻¹ (in the form of urea) and 10 kg P ha⁻¹ year⁻¹ (in the form of NaH₂PO₄·2H₂O) split in two equal applications. We tested the hypotheses by measuring changes in net rates of soil-N

cycling and N₂O fluxes during the first 2 years (2008–2009) of nutrient manipulation in an old-growth premontane forest at 1,000 m, growing on a Cambisol soil with no organic layer, in an old-growth lower montane forest at 2,000 m, growing on a Cambisol soil with an organic layer, and an old-growth upper montane rainforest at 3,000 m, growing on a Histosol soil with a thick organic layer. Among the control plots, net nitrification rates were largest at the 1,000-m site whereas net nitrification was not detectable at the 2,000- and 3,000-m sites. The already large net nitrification at the 1,000-m site was not affected by nutrient additions, but net nitrification became detectable at the 2,000- and 3,000-m sites after the second year of N and N + P additions. N₂O emissions increased rapidly following N and N + P additions at the 1,000-m site whereas only smaller increases occurred at the 2,000- and 3,000-m sites during the second year of N and N + P additions. Addition of P alone had no effect on net rates of soil N cycling and N₂O fluxes at any elevation. Our results showed that the initial soil N status, which may also be influenced by presence or absence of organic layer, soil moisture and temperature as encompassed by the elevation gradient, is a good indicator of how soil N cycling and N₂O fluxes may respond to future increases in nutrient additions.

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Introduction

One third of terrestrial net primary production (NPP) is generated by tropical forests (Field et al. 1998; Malhi and Phillips 2004) and their soils are the largest natural source of nitrous oxide (N_2O) globally (approximately $4.4 \text{ Tg N year}^{-1}$), produced by the microbial processes of nitrification and denitrification (IPCC 2007). Net primary production and soil microbial processes strongly depend on nutrients, as trees and microbes may need up to 25 chemical elements to grow and reproduce (Sterner and Elser 2002). Nutrient limitation to an ecosystem process is identified by nutrient manipulation experiments; if the rate of an ecosystem process is increased by an addition of a nutrient, then this process is considered to be limited by that nutrient (Tanner et al. 1998). The conceptual framework of nutrient limitation for nitrogen (N) and phosphorus (P), as the two most common limiting nutrients in terrestrial ecosystems, suggests that during soil development N is accumulated from the atmosphere whereas P, which is mainly derived from geological substrate, becomes progressively unavailable during pedogenesis (Walker and Syers 1976). As many tropical lowland forests grow on heavily weathered soils they are thought to be P limited. In contrast, most tropical montane forests grow on younger soils due to recurrence of substrate addition and removal (e.g. erosion and slope processes) and are thought to be N limited or simultaneously N and P limited (Tanner et al. 1998). This conceptual soil–age nutrient limitation model was validated by field studies where increased NPP was observed following N and in some cases P additions to tropical montane forests (Adamek et al. 2009; Tanner et al. 1990, 1992; Vitousek et al. 1993). In addition, soil N availability (measured as gross rates of soil-N cycling) increases across an increasing soil–age gradient in Hawaiian montane forests (Hall and Matson 2003) and decreases across an increasing elevation gradient in Ecuadorian forests, which depicts a decreasing degree of soil development and decreasing temperatures (Arnold et al. 2009).

Tropical montane forest soils are typically covered by thick organic layers, in which considerable amounts of nutrients are stored but not actively cycling (Grubb 1977). Organic layer thickness tends to increase with elevation which is related to interaction among differences in plant species composition,

soils and related nutrient availability, temperature, and soil moisture, affecting quality of organic matter and rates of nutrient turnover (Arnold et al. 2009; Burghouts et al. 1998; Trumbore 2009; Wilcke et al. 2002). Several studies suggest that in these ecosystems nutrient limitations may control decomposition and ultimately N cycling rates. Nutrient limitation to decomposition and N cycling is often different from nutrient limitation to NPP. In tropical montane forests in Hawaii, leaf litter decomposition was N and P limited whereas stem diameter growth and leaf litterfall was only N limited (Hobbie and Vitousek 2000). Furthermore, N addition to a montane forest in Panama increased not only stem diameter growth, fine litter production (Adamek et al. 2009) and soil N-cycling rates but also losses via nitrate leaching and N-oxide emissions (Corre et al. 2010). These reactions of vegetation growth, nutrient cycling and N losses to nutrient additions suggest that vegetation does not act as the dominant sink of added nutrient but that simultaneous increases in soil N cycling rates can lead to concurrent increases in N sink (e.g. N incorporation into soil and biomass sinks) and losses. Most nutrient manipulation experiments in tropical montane forests have only focused on NPP responses (Tanner et al. 1998) and only very few have investigated the effects on soil N cycling and the associated emissions of N_2O which is a potent greenhouse gas (Hall and Matson 1999; Koehler et al. 2009; Corre et al. 2010).

Here we evaluate how moderate rates of N and P additions ($50 \text{ kg N ha}^{-1} \text{ year}^{-1}$ and $10 \text{ kg P ha}^{-1} \text{ year}^{-1}$, respectively) influence soil N cycling and the associated N_2O fluxes along an elevation gradient of tropical montane forests. The elevation gradient depicted increasing soil moisture and organic layer thickness, decreasing soil temperature, and changes in forest structures with increasing elevation. We tested the following hypotheses: (1) moderate rates of N and P additions are able to stimulate soil–N cycling rates and N_2O fluxes in tropical montane forests, and (2) the magnitude and timing of soil N_2O -flux responses depend on the initial nutrient status of the forest soil. We tested these hypotheses along a 1,000–3,000-m elevation gradient of montane forests in southern Ecuador by measuring net rates of soil–N cycling, N_2O fluxes, soil mineral N, temperature and moisture during the first 2 years of N-, P-, and N + P-addition experiment.

Materials and methods

Study area

Our research was conducted within and adjacent to the Podocarpus National Park in the Cordillera del Consuelo, a part of the eastern chain of the Andes in southern Ecuador. Three study sites were selected along an altitudinal gradient: 990–1,100 m above sea level (Bombuscaro, 4.115° S, 78.968° W; hereafter called the 1,000-m site), 1,950–2,100 m (San Francisco, 3.982° S, 79.083° W; hereafter called the 2,000-m site) and 2,900–3,050 m (Cajanuma, 4.110° S, 79.178° W; hereafter called the 3,000-m site; Table 1). Along this altitudinal gradient, mean annual temperature decreased whereas mean annual rainfall increased (Moser et al. 2007). Rainfall showed little seasonality (Emck 2008) and ambient N and P deposition from bulk precipitation estimated in the area near the 2000-m site ranged from 9.5 to 10 kg N ha⁻¹ year⁻¹ and 0.6–1.1 kg P ha⁻¹ year⁻¹ (Boy et al. 2008). Soil characteristics (see below for the sampling and analytical methods) and forest structure description are given in Tables 1 and 2. In short, the 1,000-m site consists of an old-growth premontane rain forest over deeply weathered granitic rock; the 2,000-m site consists of an old-growth lower montane rain forest over metamorphosed schist; and the 3,000-m site consists of an old-growth upper montane rain forest over metamorphosed schist (Homeier et al. 2008, 2010). The mineral soil at the 1,000-m site was covered by a thin layer of decomposing leaves (Ol layer) whereas a thick organic layer, consisting of an Ol layer, a fermentation layer (Of layer) and a humus layer (Oh layer), covered the mineral soils at the 2,000- and 3,000-m sites. At all sites, soil characteristics were determined in November 2007 prior to first nutrient application (Table 1). In each three replicate strata per site (see Experimental design), we took samples from the top 5 cm of the organic layer (except for the 1,000-m site, where no organic layer was present) and the first 5 cm of the mineral soil. These samples were air-dried and shipped to the laboratory of the Soil Science of Tropical and Subtropical Ecosystems (SSTSE, University of Göttingen, Germany). Sample preparation and analytical methods are the same as those described in detail by Corre et al. (2010).

Experimental design

At each site (1,000, 2,000, and 3,000 m), we established a factorial N- and P-addition experiment (i.e. N, P, N + P, and control) in a stratified random design, in which treatments were assigned randomly in each of the three replicate strata or blocks. Each treatment plot in a block had an area of 20 m × 20 m and was separated by 10-m distance. The three replicate strata covered short topographic gradients and were treated as statistical blocks, as nutrient availability and other soil factors may vary over relatively small topographic gradients in these forests (Wolf et al. 2011). Fertilizers were applied by hand at a rate of 50 kg N ha⁻¹ year⁻¹ (in the form of urea) and 10 kg P ha⁻¹ year⁻¹ (in the form of NaH₂PO₄·2H₂O with analytical grade quality) split in two equal applications per year (February and August 2008 and 2009 at the 1,000-m site; January and July 2008 and February and July 2009 at the 2,000-m site; February and September 2008 and February and August 2009). Our rates of N and P addition are moderate compared to higher addition rates in other nutrient addition experiments in tropical montane forests (Hall and Matson 2003; Koehler et al. 2009). During fertilizer application, chamber bases for gas flux measurements were covered and received the exact amount of fertilizer relative to their area afterwards.

N₂O flux measurements

We measured N₂O fluxes monthly from January 2008 to September 2009 using static vented chambers (12 N₂O flux measurements per treatment, elevation and month). In each replicate plot, two round chamber bases (area 0.04 m², height 0.25 m, ~0.02 m inserted into the soil) were permanently installed along two randomly selected perpendicular lines (in total four chamber bases per plot), ensuring that distance to the border of the plot was at least 2 m to avoid edge effects. On each sampling day, a chamber hood (approx. 0.12 m height) was attached tightly to a chamber base, and four gas samples were taken at 2, 14, 26, and 38 min of chamber closure and stored in pre-evacuated glass containers (60 mL) with stop-cocks. One day after the field sampling, gas samples were transported to our laboratory located in the nearby Universidad Técnica Particular de Loja and were analyzed using a gas chromatograph (Shimadzu

Table 1 Site and soil characteristics (mean \pm SE, $n = 3$) of the forest sites across the elevation gradient, determined in November 2007 before the first nutrient application

Characteristics	Elevation (m) site		
	1,000 (Bombuscaro)	2,000 (San Francisco)	3,000 (Cajanuma)
Height above sea level (m)	990–1,100	1,950–2,100	2,900–3,050
Annual mean air temperature ($^{\circ}\text{C}$) ^a	19.4	15.7	9.4
Annual mean soil temperature ($^{\circ}\text{C}$) ^b	16.8 (0.3) ^{2008A} 16.8 (0.2) ^{2009A}	12.5 (0.7) ^{2008B} 13.0 (0.5) ^{2009B}	4.4 (0.4) ^{2008C} 3.9 (0.4) ^{2009C}
Annual mean gravimetric moisture (g g^{-1}) ^b	0.6 (0.0) ^{2008C} 0.7 (0.0) ^{2009C}	4.2 (0.1) ^{2008B} 4.1 (0.1) ^{2009B}	5.4 (0.1) ^{2008A} 5.1 (0.2) ^{2009A}
Precipitation (mm year^{-1}) ^a	2,230	1,950	4,500
Soil type (FAO)	Dystric Cambisol	Stagnic Cambisol	Stagnic Histosol
Bulk density (from the surface down to 5-cm depth, g cm^{-3}) ^c	0.84 (0.1) ^a	0.18 (0.05) ^b	0.11 (0.01) ^b
Organic layer (top 5 cm)			
Thickness (cm)	0	10–30	10–40
pH–H ₂ O	–	3.9 (0.03)	3.9 (0.06)
C (mg C g^{-1})	–	487.1 (19.7)	516.4 (3.4)
N (mg N g^{-1})	–	20.8 (0.7)	20.1 (0.6)
C/N	–	23.5 (1.1)	25.8 (0.7)
Total P (mg P g^{-1}) ^c	–	0.5 (0.02) ^b	0.7 (0.03) ^a
Mineral soil layer (top 5 cm)			
pH–H ₂ O ^c	4.6 (0.06) ^a	3.8 (0.03) ^b	4.7 (0.03) ^a
Base saturation(%) ^c	16.5 (1.1) ^a	4.0 (1.5) ^b	12.0 (1.1) ^a
C (mg C g^{-1}) ^c	6.0 (1.2) ^b	10.6 (1.1) ^a	9.6 (1.4) ^{ab}
N (mg N g^{-1})	0.6 (0.1)	0.7 (0.1)	0.7 (0.1)
C/N ^c	9.3 (0.4) ^b	17.2 (2.2) ^a	14.2 (0.5) ^{ab}
Total P (mg P g^{-1}) ^c	0.14 (0.00) ^b	0.04 (0.01) ^c	0.51 (0.04) ^a
Texture	Sandy loam	Medium loam	Medium loam

^a Moser et al. (2007)

^b Means of each year with different capital letters indicate significant differences among sites (linear mixed effects model with ANOVA at $P \leq 0.05$)

^c Means with different lowercase letters indicate significant differences among sites (one-way ANOVA with Tukey HSD test at $P \leq 0.05$)

GC-14B, Duisburg, Germany) equipped with an electron capture detector (ECD) and an autosampler (Loftfield et al. 1997). Gas concentrations were determined by comparison of integrated peak areas of samples to standard gases (320, 501, 1,001, and 3,003 ppb N₂O; Deuste Steininger GmbH, Mühlhausen, Germany). Gas fluxes were calculated from the linear increase of gas concentration in the chamber versus time and were adjusted for the field-measured air temperature and atmospheric pressure (Koehler et al. 2009). Our reported data included all flux measurements. As we were primarily interested in the mid- to long-term effects of nutrient enrichment on

soil mineral N, net rates of soil N cycling and soil N₂O fluxes, only measurements conducted 4 weeks after nutrient application were included in our analysis. One pre-treatment measurement at each site indicated that initial N₂O fluxes did not differ between the control and other treatment plots in January 2008 prior to the first nutrient application.

Soil mineral N, soil moisture, and net rates of soil N cycling

Parallel to the gas sampling, four subsamples of the top 5-cm depth were collected within one meter

Table 2 Forest stand characteristics of the forest sites across the elevation gradient (Homeier et al. unpublished data)

Elevation (m) site	Forest structure characteristics (trees ≤ 0.1 m in diameter at breast height were excluded)				
	Stand height (m)	Mean basal area ($\text{m}^2 \text{ha}^{-1}$)	Tree density (trees ha^{-1})	Abundant tree families	Common tree species
1,000 (Bombuscaro)	20–25	33.4	747.5	<i>Moraceae</i> , <i>Sapotaceae</i>	<i>Pouteria torta</i> , <i>Clarisia racemosa</i>
2,000 (San Francisco)	10–14	22.8	1142.5	<i>Melastomataceae</i> , <i>Euphorbiaceae</i>	<i>Graffenrieda emarginata</i> , <i>Myrcia sp.</i>
3,000 (Cajanuma)	6–8	25.5	1305.0	<i>Cunoniaceae</i> , <i>Clusiaceae</i>	<i>Weinmannia elliptica</i> , <i>Weinmannia loxensis</i>

distance from the four chamber bases in each replicate plot. The four subsamples were pooled to represent each plot after leaves, roots, and stones had been removed. In the field, part of the composite sample was added to a prepared extraction bottle containing 150 mL of 0.5 M K_2SO_4 solution while the rest of the sample was stored in plastic bags for gravimetric moisture determination (drying for 24 h at 105 °C) in the field station. Samples were brought to the field station and extracted within 24 h after field sampling. After shaking the soil- K_2SO_4 bottles for 1 h, the extracts were filtered through filter papers (4 μm nominal pore size), frozen immediately and remained frozen during transport by air to SSTSE, where analysis was conducted. Ammonium (NH_4^+) and nitrate (NO_3^-) contents of the extracts were analyzed using continuous flow injection colorimetry (Cenco/Skalar Instruments, Breda, Netherlands). Soil moisture was expressed as gravimetric moisture content.

Net rates of soil N cycling were measured once a year (1 month after the second nutrient application in September 2008 and 1 month after the fourth nutrient application in September 2009) using the buried bag method (Hart et al. 1994). It was not possible to take intact soil cores because of too many roots. Thus, a composite soil sample of the top 5-cm depth was taken in each replicate plot as described above. One subsample was extracted immediately in the field with 0.5 mol L^{-1} K_2SO_4 to determine the initial NH_4^+ and NO_3^- levels (T_0). The other subsample was put into a plastic bag, reburied in the soil, incubated for 10 days and afterwards extracted with 0.5 mol L^{-1} K_2SO_4 (T_1). The plastic bag was closed with a rubber band to prevent rain coming in but not too tight to permit air exchange. Net N mineralization and nitrification rates were calculated as the difference

between T_1 and T_0 of $\text{NH}_4^+ + \text{NO}_3^-$ concentrations and NO_3^- concentrations, respectively.

Statistical analysis

Statistical analyses were carried out on the plot-mean N_2O fluxes (average of four chambers per replicate plot) for 2008 (including ten monthly measurements) covering first and second nutrient applications and for 2009 (including seven monthly measurements) covering third and fourth nutrient applications. Logarithmic transformation was applied to correct for non-normal distribution of the dataset before analysis. We used linear mixed effects models for analysis of time-series data (N_2O fluxes, soil mineral N, soil temperature, and gravimetric moisture content) (Crawley 2010). When testing for differences among N-, P-, N + P-addition, and control plots, treatment is included in the model as the fixed effect whereas replicate plots and time are included as random effects. We included a first-order temporal autoregressive process in the model, which assumes that correlation between measurements decreases with increasing time distance, if this improved the relative goodness of the model fit. The significance of the fixed effect was determined based on analysis of variance (Crawley 2010). For net rates of soil N cycling, site differences (i.e. comparison among control plots of the three elevation sites) or treatment effects at each site were assessed using one-way ANOVA followed by a Tukey HSD test. Differences between the two sampling campaigns (September 2008 and September 2009) in soil N-cycling rates of the control plots at each site was assessed using paired *T* test. All levels of significance were defined at $P \leq 0.05$. Analyses were conducted using R 2.10.1 (R Development Core Team 2009).

Results

Soil temperature, moisture, mineral N, N-cycling rates, and N₂O fluxes from control forest soils

Annual mean soil temperature decreased and annual mean gravimetric moisture contents at the top 5-cm depth increased with increasing elevation (Table 1). There were no clear seasonal patterns of soil temperature and moisture contents at all elevations. NH₄⁺ contents varied between 22.5 and 57.0 μg N cm⁻² at the 1,000-m site (Fig. 1a), between 22.1 and 159.6 μg N cm⁻² at the 2,000-m site (Fig. 1b), and between 11.6 and 82.0 μg N cm⁻² at the 3,000-m site (Fig. 1c), all without any clear seasonal pattern. Substantial amounts of NO₃⁻ were only detected at the 1,000-m site with a maximum of 5.9 μg N cm⁻² soil (Fig. 2a). NO₃⁻ was often below the analytical detection limit (0.10 mg NO₃-N L⁻¹) at the 2,000- and 3,000-m sites (Fig. 2b, c).

At each site, net N mineralization and net nitrification rates of the control plots did not differ between 2008 and 2009. Furthermore, net N mineralization rates did not differ across the elevation gradient and

net nitrification rates were only detectable at the 1,000-m site (Table 3). Soil N₂O fluxes were higher at the 1,000-m site than at the 2,000- and 3000-m sites ($P < 0.05$) and did not differ between the 2000- and 3000-m sites (Fig. 3). At each elevation, soil N₂O fluxes showed no seasonal pattern over the whole study period (Fig. 3) and were not correlated with soil mineral N, soil temperature, or gravimetric moisture content. Average N₂O emissions were highest at the 1,000-m site whereas a net N₂O uptake was observed at the 3,000-m site (Table 4).

Nutrient addition effects on soil mineral N, N-cycling rates, and N₂O fluxes

At the 1,000-m site, following the first and second nutrient addition in 2008, there were no differences in soil mineral N among treatments. Following the third and fourth nutrient addition in 2009, NH₄⁺ contents did not differ between the control and the nutrient-addition plots (Fig. 1a); NO₃⁻ contents in the N- and P-addition plots were higher compared to the control plots ($P < 0.05$; Fig. 2a) but did not differ among the nutrient-addition plots. Net rates of N cycling did not

Fig. 1 Mean (\pm SE, $n = 3$) soil extractable NH₄⁺ of the top 5-cm soil depth at the **a** 1,000-m, **b** 2,000-m, and **c** 3,000-m sites in the control (open diamond), N-addition (filled square), P-addition (filled circle), and N + P-addition (open triangle) plots. Red vertical lines indicate nutrient applications. (Color figure online)

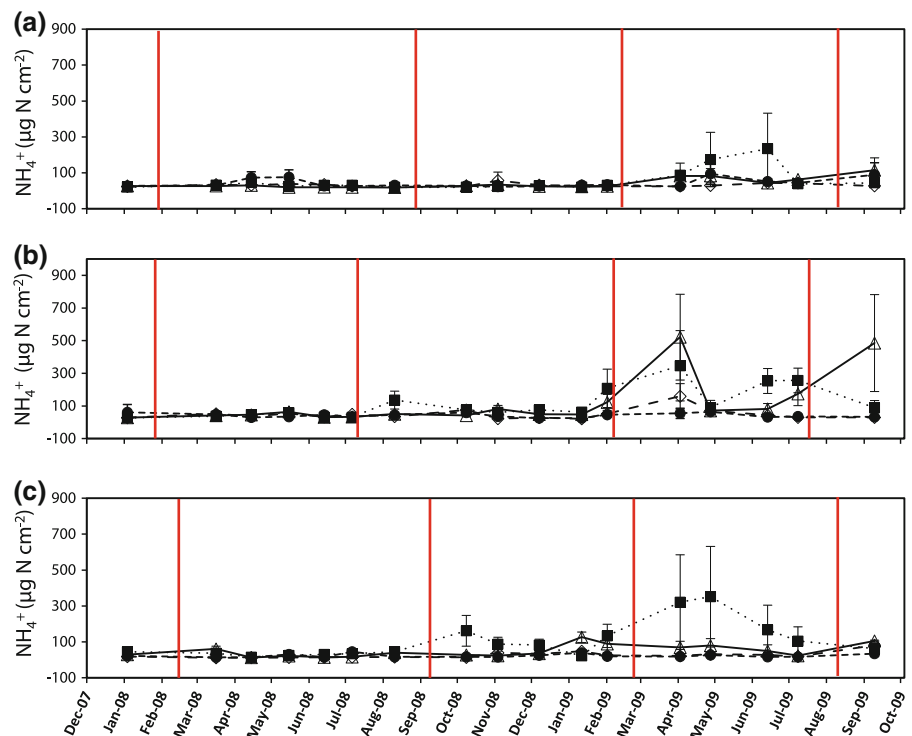
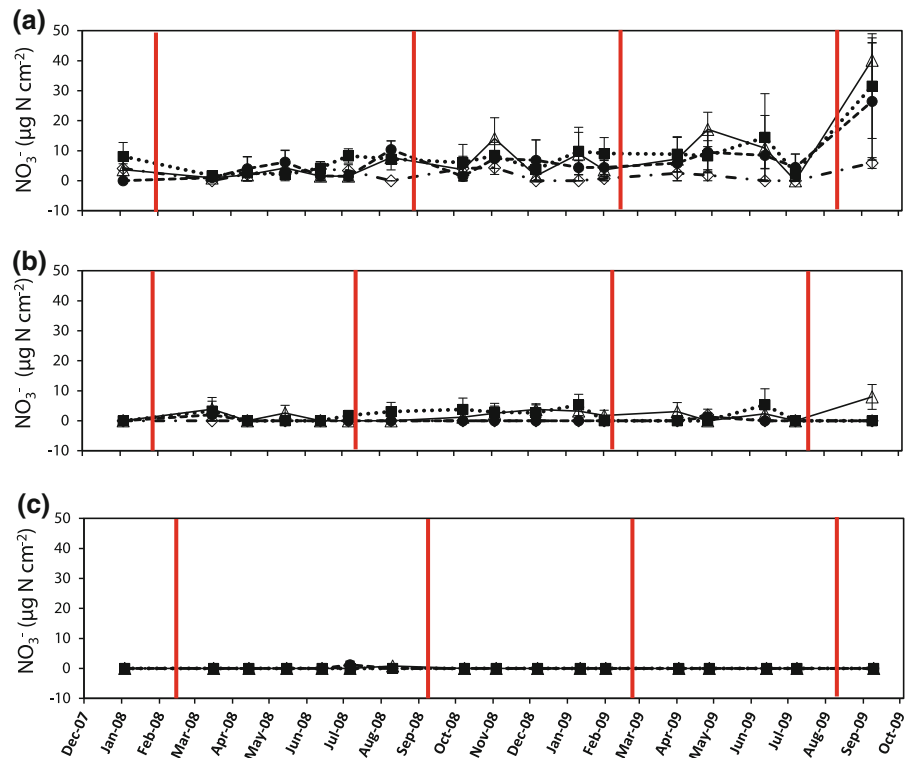


Fig. 2 Mean (\pm SE, $n = 3$) soil extractable NO_3^- of the top 5-cm soil depth at the **a** 1,000-m, **b** 2,000-m, and **c** 3,000-m sites in the control (open diamond), N-addition (filled square), P-addition (filled circle), and N + P-addition (open triangle) plots. Red vertical lines indicate nutrient applications. (Color figure online)



differ among treatments in 2008 and in 2009 (Table 3). At the 2000-m site, NH_4^+ contents in the N-addition plots increased already after the first and second nutrient addition in 2008 and remained elevated after the third and fourth nutrient addition in 2009 compared to the control ($P < 0.05$; Fig. 1b). Additionally, NH_4^+ contents in the N + P-addition plots increased after the third and fourth nutrient addition in 2009 ($P = 0.00$) and these did not differ from N-addition plots (Fig. 1b). Compared to the control plots, NO_3^- contents in the N- and N + P-addition plots increased after the first and second nutrient addition in 2008 ($P < 0.01$) and did not differ between each other (Fig. 2b). NO_3^- contents remained elevated only in the N + P-addition plots after the third and fourth nutrient addition in 2009 compared to the control ($P = 0.02$; Fig. 2b). Net rates of N cycling did not differ among treatments in 2008; only in 2009 that net nitrification rates increased in the N + P-addition plots which were also larger than in the N-addition plots (Table 3). At the 3,000-m site, NH_4^+ contents increased in the N- and N + P-addition plots after the first and second nutrient addition in 2008 ($P < 0.01$; Fig. 1c) and remained

elevated after the third and fourth nutrient addition in 2009 compared to the control plots ($P < 0.05$). Neither NO_3^- contents nor net N mineralization rates differed among treatments over the whole sampling period but net nitrification rates became detectable in N- and N + P-addition plots in 2009 (Fig. 2c; Table 3).

N_2O fluxes in the 1,000-m site started to increase following the first N addition in 2008 (Fig. 3a) and were larger in the N- and N + P-addition plots compared to the control after the first and second nutrient addition in 2008 and after the third and fourth nutrient addition in 2009 (Table 4). Between N- and N + P-addition plots, N_2O emissions did not differ. Addition of P alone did not affect N_2O fluxes (Table 4). Average soil N_2O emissions increased in 2009 relative to 2008 by a factor of 6 and 8 in the N- and N + P-addition plots, respectively (Table 4). At the 2,000-m site, N_2O fluxes did not differ among treatments after the first and second N addition in 2008 (Fig. 3b), but started to increase in 2009 and were larger in the N- and N + P-addition plots compared to the control after the third and fourth nutrient addition (Table 4). Similarly, N_2O emissions did not differ

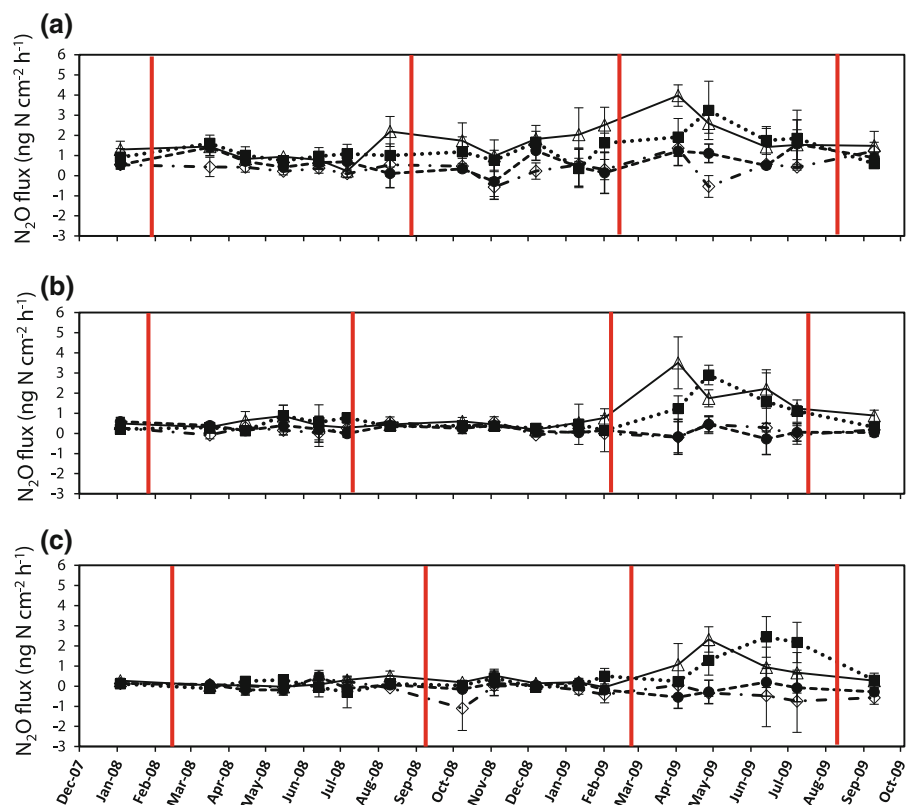
Table 3 Mean (\pm SE, $n = 3$) net rates of soil N cycling ($\text{ng N cm}^{-2} \text{h}^{-1}$) in the forest sites across the elevation gradient, measured in the top 0.05-m soil depth in September 2008 after two nutrient applications and in September 2009 after four nutrient applications

Treatment	1,000 m (Bombuscaro)		2,000 m (San Francisco)		3,000 m (Cajanuma)	
	Net N mineralization	Net nitrification	Net N mineralization	Net nitrification	Net N mineralization	Net nitrification
2008						
Control	17.89 (16.67)	19.47 (15.09)	23.44 (10.48)	ND	1.36 (1.44)	ND
Nitrogen	36.93 (13.48)	33.06 (15.05)	44.39 (15.3)	-1.68 (1.68)	10.99 (6.05)	ND
Phosphorus	21.95 (7.9)	23.78 (13.18)	11.17 (4.44)	ND	5.58 (4.78)	ND
Nitrogen + phosphorus	22.78 (13.57)	28.06 (12.16)	40.54 (8.01)	7.79 (4.32)	8.41 (3.96)	ND
2009						
Control	72.12 (62.49)	7.77 (6.17)	42.23 (21.11)	ND ^b	8.07 (3.83)	ND
Nitrogen	30.46 (22.03)	19.06 (13.87)	64.87 (14.76)	2.29 (1.65) ^b	49.65 (44.61)	2.19 (2.19)
Phosphorus	25.09 (16.20)	10.06 (7.56)	44.52 (32.79)	3.84 (1.94) ^{ab}	8.96 (8.44)	ND
Nitrogen + phosphorus	74.77 (68.45)	18.73 (12.98)	79.72 (3.19)	9.38 (1.6) ^a	8.8 (11.21)	1.02 (1.02)

For each year, means with different letters indicate significant differences among treatments at each site (one-way ANOVA with Tukey HSD at $P \leq 0.05$)

ND non-detectable (NO_3^- concentrations of soil extracts were below the detection limit of $0.10 \text{ mg NO}_3^- \text{ N L}^{-1}$)

Fig. 3 Mean (\pm SE, $n = 3$) N_2O fluxes at the **a** 1,000-m, **b** 2,000-m, and **c** 3,000-m sites in the control (*open diamond*), N-addition (*filled square*), P-addition (*filled circle*), and N + P-addition (*open triangle*) plots. Red vertical lines indicate nutrient applications. (Color figure online)



between N- and N + P-addition plots and addition of P alone did not affect N_2O fluxes (Table 4). Average soil N_2O emissions increased in 2009 relative to 2008 by a factor of 5 and 7 in the N- and N + P-addition

plots, respectively (Table 4). Also at the 3,000-m site, N_2O fluxes did not differ among treatments in 2008 but started to increase in 2009 and were larger in the N- and N + P-addition plots compared to the control

Table 4 Average soil N₂O fluxes (kg N ha⁻¹ year⁻¹, mean ± SE, *n* = 3 with 10 and 7 monthly measurements for 2008 and 2009, respectively) of the forest sites across the elevation gradient

Treatment ^a	Elevation (m)—site		
	1,000 (Bombuscaro)	2,000 (San Francisco)	3,000 (Cajanuma)
2008 ^{b,c}			
Control	0.24 (0.13) ^b	0.20 (0.03)	-0.03 (0.09)
Nitrogen	0.96 (0.18) ^a	0.37 (0.10)	0.06 (0.07)
Phosphorus	0.51 (0.18) ^b	0.25 (0.19)	0.03 (0.07)
Nitrogen + phosphorus	1.07 (0.17) ^a	0.41 (0.09)	0.18 (0.06)
2009 ^c			
Control	0.48 (0.14) ^b	0.09 (0.16) ^b	-0.34 (0.30) ^b
Nitrogen	1.41 (0.68) ^a	0.97 (0.16) ^a	0.87 (0.21) ^a
Phosphorus	0.73 (0.24) ^b	0.04 (0.21) ^b	-0.13 (0.31) ^b
Nitrogen + phosphorus	1.94 (0.23) ^a	1.36 (0.08) ^a	0.68 (0.35) ^a

^a For each year, means with different letters indicate significant differences among treatments at each site (linear mixed effects model with ANOVA at $P \leq 0.05$)

^b N₂O measurement from the pre-treatment in January 2008 was included

^c Only N₂O measurements conducted 1 month after the two nutrient applications in 2008 and 2009 are included

and P-addition plots after the third and fourth nutrient addition (Fig. 3c; Table 4). Additionally, average soil N₂O fluxes turned from a net uptake in the control and P-addition plots into a net source in the N- and N + P-addition plots in 2009 (Table 4).

Discussion

N cycling and N₂O fluxes from control forest soils

The pattern of N availability across the elevation gradient was best depicted by the net nitrification rates and NO₃⁻ contents as there was no significant trend of net N mineralization rates across sites. The 1,000-m site that showed measurable net nitrification rates also showed detectable NO₃⁻ levels. As the assay of net rates of soil N cycling basically meant exclusion of plant uptake during the 10-day incubation period, the appreciable net nitrification rates at 1,000 m suggests that the nitrifiers were able to compete for available N during this duration of plant-uptake exclusion and that the NO₃⁻ produced was not all consumed (e.g. by microbial immobilization) so as to be detectable. The latter further implies that mineral N production was larger than its consumption, depicting a less competitive condition for available N. On the contrary, neither net nitrification rate nor NO₃⁻ content was detectable at 2,000 and 3,000 m, which suggests low

nitrifying population and substrate availability, less favorable soil conditions (e.g. soil temperature, moisture, C:N ratio; Table 1), and/or rapid NO₃⁻ consumption such that it was undetectable within the same incubation period. The low soil temperatures and large C:N ratios in the organic layer at the 2,000- and 3,000-m sites (Table 1) may decelerate overall N-transformation processes. Additionally, the high moisture contents at the two higher-elevation sites (Table 1) may create anoxic microsites with low redox potentials (Conrad 1996), where nitrifier activity is suppressed but denitrification and dissimilatory nitrate reduction to ammonium are facilitated (Pett-Ridge et al. 2006). These possible causes imply an increasing competition for available N at higher elevations (Arnold et al. 2009). In sum, the net rates of soil N cycling showed that N availability decreased with increasing elevation, which corresponded to the sites with thick organic layers. Earlier studies have also shown decreasing rates of soil N cycling along elevation gradients although at lower elevation range than in our study (Arnold et al. 2009; Koehler et al. 2009; Marrs et al. 1988; Silver et al. 1994). In particular, net nitrification rates in forest soils in Northwestern Ecuador decreased across 300–1,500 m, with the nitrification activity at 1,500 m comparable to our measured rate at 1,000 m (Arnold et al. 2009). The low N availability at the 2,000- and 3,000-m sites is probably caused by incomplete decomposition of litter

and buildup of organic matter (Grubb 1977). In addition, the feedback between tree vegetation and soil conditions (e.g. low soil temperatures and high moisture contents and C:N ratios of organic matter, Table 1) may result in low litter quality and low microbial activity, contributing to the increased organic layer thickness with increasing elevation (Wolf et al. 2011).

Since soil N oxide emissions are primarily controlled by N availability (Davidson et al. 2000), average soil N₂O fluxes decreased with elevation as well (Table 4). However, their overall level was much lower than reported by other studies from tropical montane forest soils (Breuer et al. 2000; Holtgrieve et al. 2006; Koehler et al. 2009; Purbopuspito et al. 2006; Riley and Vitousek 1995). Low N availability in combination with strong competition for N should lead to high N-retention and efficient recycling of available N (Perakis et al. 2005). To evaluate the efficiency of soil N retention in all treatments across the elevation gradient, we used the ratio of N₂O fluxes (Table 4) to net N mineralization rates (Table 3). Among the control plots, average N₂O fluxes to net N mineralization ratios decreased with increasing elevation, with ~2 % at the 1,000-m site and ~1 % at the 2,000-m site. These ratios were higher than in a 1,200-m elevation forest (growing on Andosol soil with an organic layer) in Panama, where the ratio of N₂O emission to net N mineralization was 0.5 % (Koehler et al. 2009). At the 3,000-m site, average N₂O fluxes were not different from zero which leads to a ratio of also zero. The high soil N retention efficiency and low soil N availability at the 2,000- and 3,000-m sites are indicative of a conservative soil N cycle (Davidson et al. 2000). Although N availability was relatively high at the 1,000-m site, as shown by measurable nitrification activity, its N retention efficiency was still higher than the 7 % ratio of N₂O emission to net N mineralization in a lowland forest in Panama, growing on Cambisol and Nitisol soils without an organic layer (Koehler et al. 2009). Thus, in general the soil N cycling of these montane forests was more conservative than the lowland forest.

Nutrient addition effects on N₂O fluxes and soil N cycling across the elevation gradient

At the 1,000-m site, the soil N₂O-flux responses to N and N + P additions were more rapid compared to the

delayed response to N addition observed in other montane forests at comparable elevations with similar soil conditions, considering only the N₂O fluxes 1 month after N application, i.e. the Inceptisol soil (similar to a Cambisol soil as in our site) in Hawaii (Hall and Matson 2003) that received double the rate of N applied in our study. At our site, the ratios of N₂O fluxes to net N mineralization rates in the second year of nutrient addition were ~5 % and ~3 % in the N- and N + P-addition plots, respectively, indicating a decrease in N retention efficiency relative to that in the control. We attribute the rapid and sustained increases in N₂O emissions from the 1,000-m site to the high levels of net nitrification rates under N or N + P additions. In contrast, the Hawaiian site had low net nitrification rates in the top 10 cm of soil; its coarse-textured soil (i.e. > 59 % gravel in the mineral A horizon) also had a rapid drainage, causing immediate and substantial increases in NO₃⁻ leaching following N addition (Lohse and Matson 2005). P addition had no effect on rates of soil N cycling and N₂O emissions. Either the moderate amount of P we added was insufficient to influence net nitrification rates and N₂O production or these processes were not sensitive to P addition. Clearly, the moderate rates of N addition (N and combined N + P) to the Cambisol soil at the 1,000-m site that already had large rate of net nitrification brought immediate increases in N₂O emissions but did not affect stem diameter growth of trees at least during the first year of nutrient manipulation (Homeier et al. unpublished data).

At the 2,000- and 3,000-m sites, the N₂O-flux responses to N and N + P additions were delayed and were ~2 % of the net mineralization rates in the N- and N + P-addition plots at the 2,000-m site and ~2 % and ~9 %, respectively, at the 3,000-m site in the second year of nutrient addition. The responses of N₂O fluxes to N additions were similar to that from a Panamanian forest growing on an Andosol soil with an organic layer at ~1,200 m elevation (Koehler et al. 2009). We attribute the delayed N₂O-flux responses at the two higher elevation sites to an only small stimulation of net rates of nitrification in the organic layer during first-year N and N + P additions. In the second year, however, the increases of N₂O emissions and net rates of nitrification in the N- and N + P-addition plots at the two higher elevation sites corroborated the observations from the Panamanian forest site, where first-time N addition led to

immediate increases in soil N₂O emissions caused by increased rates of net nitrification in the organic layer (Koehler et al. 2009). The delayed responses of N₂O flux and soil N cycling at our sites may be due to the lower amount of N added and less favorable soil conditions (low soil temperatures, high soil moisture contents, and high C:N ratios at these high-elevation sites) that may slow down microbial activity. Although stem diameter growth did not respond to nutrient additions at the 2,000- and 3,000-m sites (Homeier unpublished data), moderate rate of N addition was sufficient to stimulate net rates of nitrification and N₂O production in the organic layer at both elevations. Hence, our results confirm our second hypothesis and support the assumption by Corre et al. (2010) that the initial N status of the forest soil, which may also be influenced by the presence or absence of an organic layer, hydrological properties and soil temperature, is a more important indicator than nutrient demand of vegetation of how soil N cycling and N₂O fluxes respond to elevated N additions. Projected increases in N deposition in neotropical montane forest regions amount to approximately 5–10 kg N ha⁻¹ year⁻¹ for the year 2030 (Reay et al. 2008) due to urban development and increasing agricultural land use (Galloway et al. 2008). The impacts of N addition in our nutrient manipulation experiment, albeit applied at a larger amount than this projected increase, on rates of soil N cycling and N₂O emissions and the rapidity with which these effects were manifested, support our first hypothesis. Moderate rates of N addition, as in our study, are able to stimulate microbial activity and increase N₂O emissions. Hence, we suggest that tropical montane forests, growing on soils that are covered by thick organic layers, are strongly susceptible to future increases in N deposition. Whether rates of soil N cycling and N₂O losses will progressively increase in the N- and N + P-addition plots or whether the nutrient limitation of vegetation or of microbial activity will change and P will become important in the long term and affect soil N cycling processes remain to be seen (Davidson and Howarth 2007; Elser et al. 2007).

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