

Immediate and long-term nitrogen oxide emissions from tropical forest soils exposed to elevated nitrogen input

BIRGIT KOEHLER*, MARIFE D. CORRE*, EDZO VELDKAMP*, HANS WULLAERT*¹ and S. JOSEPH WRIGHT†

*Buesgen Institute – Soil Science of Tropical and Subtropical Ecosystems, Georg-August-University of Goettingen, Goettingen, Germany, †Smithsonian Tropical Research Institute, Box 0843-03092, Balboa, Republic of Panama

Abstract

Tropical nitrogen (N) deposition is projected to increase substantially within the coming decades. Increases in soil emissions of the climate-relevant trace gases NO and N₂O are expected, but few studies address this possibility. We used N addition experiments to achieve N-enriched conditions in contrasting montane and lowland forests and assessed changes in the timing and magnitude of soil N-oxide emissions. We evaluated transitory effects, which occurred immediately after N addition, and long-term effects measured at least 6 weeks after N addition. In the montane forest where stem growth was N limited, the first-time N additions caused rapid increases in soil N-oxide emissions. During the first 2 years of N addition, annual N-oxide emissions were five times (transitory effect) and two times (long-term effect) larger than controls. This contradicts the current assumption that N-limited tropical montane forests will respond to N additions with only small and delayed increases in soil N-oxide emissions. We attribute this fast and large response of soil N-oxide emissions to the presence of an organic layer (a characteristic feature of this forest type) in which nitrification increased substantially following N addition. In the lowland forest where stem growth was neither N nor phosphorus (P) limited, the first-time N additions caused only gradual and minimal increases in soil N-oxide emissions. These first N additions were completed at the beginning of the wet season, and low soil water content may have limited nitrification. In contrast, the 9- and 10-year N-addition plots displayed instantaneous and large soil N-oxide emissions. Annual N-oxide emissions under chronic N addition were seven times (transitory effect) and four times (long-term effect) larger than controls. Seasonal changes in soil water content also caused seasonal changes in soil N-oxide emissions from the 9- and 10-year N-addition plots. This suggests that climate change scenarios, where rainfall quantity and seasonality change, will alter the relative importance of soil NO and N₂O emissions from tropical forests exposed to elevated N deposition.

Keywords: climate change, deposition, fertilization, nitric oxide, nitrification, nitrogen, nitrous oxide, organic layer, trace gases, tropical forest

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Introduction

Humans have more than doubled the rate of nitrogen (N) entering the land-based N cycle worldwide, thereby enhancing the mobility of 'reactive' nitrogen (N_r) within and between ecosystems (Vitousek *et al.*, 1997). The

three main causes of anthropogenic increases of N_r are N fertilizers used in agriculture, fossil fuel combustion and cultivation of N-fixing plants. Until recently, enhanced inputs of N were concentrated in economically developed regions of the temperate zone, but for the coming decades deposition of N_r is projected to increase substantially in economically emerging tropical regions such as Southeast Asia and Latin America due to demands for food and energy by growing populations with increasing per capita use of N (Galloway *et al.*, 2003, 2004, 2008).

Correspondence: Marife D. Corre, fax +49 551 393310, e-mail: mcorre@gwdg.de

¹Present address: Geographic Institute, Johannes Gutenberg University Mainz, 55099 Mainz, Germany.

The increase in tropical N deposition is projected to stimulate soil nitrous oxide (N₂O) and nitric oxide (NO) emissions, increase nitrate (NO₃⁻) leaching with accompanying base cation losses, and enhance soil acidification (Matson *et al.*, 1999). On a global basis, atmospheric transport and subsequent deposition has become the dominant N_r distribution process and it is critical to better understand gaseous N emission rates (Galloway *et al.*, 2008). Tropical rain forests are already the largest natural source of terrestrial N₂O emissions (Bouwman *et al.*, 1995) and the third most important biome for NO emissions (Yienger & Levy, 1995). N₂O is a long-lived greenhouse gas with an atmospheric lifetime of 114 years and a 100-year global warming potential of 298 relative to CO₂. N₂O also contributes to the depletion of stratospheric ozone. The atmospheric concentration of N₂O has increased approximately linearly by about 0.26% yr⁻¹ over the past few decades. The dominant impact of NO emissions on climate is through the photochemical formation of tropospheric ozone, the third largest contributor to positive radiative forcing (IPCC, 2007). NO further generates indirect negative radiative forcing by shortening the atmospheric lifetime of methane and regulates the production of nitric acid and organic nitrates, both acid rain precursors (Crutzen, 1979).

Commonly, tropical forests growing on heavily weathered soils exhibit a conservative phosphorus (P) cycle, while tropical forests growing on younger soils exhibit a conservative N cycle, much like undisturbed temperate forest ecosystems (Walker & Syers, 1976; Vitousek, 1984). This relationship has been shown along a soil chronosequence in Hawaii where the supply of available N is low in the younger volcanic soils and increases with soil age. This is in contrast to rock-derived P, which is relatively abundant in young soils but becomes increasingly bound in unavailable forms in older heavily weathered soils (Hedin *et al.*, 2003). ¹⁵N signatures and N:P ratios in leaves are consistent with the hypothesis that P conservation increases and N conservation decreases with soil weathering stage on a global scale (Martinelli *et al.*, 1999; McGroddy *et al.*, 2004). A conservative N cycle is implied when the biological N demand (primarily vegetation growth) exceeds N supply (primarily N fixation, deposition and mineralization). Forest ecosystems with a conservative N cycle are characterized by small rates of soil N cycling and N losses (N leaching and gaseous N emissions; Davidson *et al.*, 2000). In contrast, the N cycle of forest ecosystems where N supply exceeds biological N demand has been termed 'open' or 'leaky' because N losses are large relative to the amount of soil N cycling.

N-oxides are produced in soils largely by nitrification and denitrification. A large fraction of the observed variation in soil N-oxide emissions can be explained by the conceptual 'hole-in-the-pipe' (HIP) model, which is based on their biogeochemical controls (Firestone & Davidson, 1989; Davidson *et al.*, 2000). The HIP model proposes that the total N-oxide gas flux (NO + N₂O) is proportional to the rates of nitrification and denitrification while the relative proportion of each gas emitted from the soil is controlled by the soil aeration status. Thus, the HIP model predicts that forest ecosystems with a conservative N cycle will have smaller soil N-oxide emissions while forest ecosystems with a leaky N cycle will have larger soil N-oxide emissions.

One way to evaluate potential future effects of N deposition on tropical forests is to create N-enriched conditions through N addition. Just six N-addition experiments have evaluated N-oxide emissions from tropical forest soils. These include four one-time pulse N additions and two chronic N additions. The one-time pulse N additions caused transitory (days to 2 weeks) increases of soil N-oxide emissions (Kaplan & Wofsy, 1988; Keller *et al.*, 1988; Bakwin *et al.*, 1990; Steudler *et al.*, 2002) as well as intermediate-term (sustained elevated emissions 6 months after N addition) increases of soil NO emissions (Steudler *et al.*, 2002). The two chronic N additions took place in Puerto Rican and Hawaiian montane forests dominated by single-tree species. In Puerto Rico, chronic N addition doubled soil N₂O emissions and quadrupled soil NO emissions compared with the largest mean emissions from a fertile midsuccessional control site (Erickson *et al.*, 2001). In an N-limited Hawaiian forest with a conservative soil N cycle, N-oxide emissions did not increase significantly after first-time N addition but did increase significantly after chronic N addition. In contrast, in a P-limited Hawaiian forest with a leaky soil N cycle, N-oxide emissions increased rapidly and by much larger amounts than in the N-limited forest after both first-time and chronic N additions. Thus, the timing and magnitude of fertilizer-induced soil N-oxide emissions were influenced by the N status (i.e. N supply and demand) of the Hawaiian forest ecosystem (Hall & Matson, 1999, 2003). Chronic N additions are lacking for species-rich tropical forests, and these missing experiments are needed to provide broader insight into the consequences of future N deposition on soil N-oxide emissions from the tropical mainland.

Apart from soil age/weathering stage, the N status of old-growth tropical forests is also influenced by altitude (lowland vs. montane) and presence of an organic layer. Lowland forests generally have larger N concentrations in leaf and litterfall (Tanner *et al.*, 1998), NO₃⁻ leaching losses (Hedin *et al.*, 2003; Klinge *et al.*, 2004; Dechert

et al., 2005; Schwendenmann & Veldkamp, 2005), soil N-oxide emissions (Keller & Reiners, 1994; Davidson *et al.*, 2000; Purbopuspito *et al.*, 2006), and $\delta^{15}\text{N}$ signatures in leaves and soils (Martinelli *et al.*, 1999) than montane forests. This suggests that lowland forests are characterized by more leaky soil N cycling. On the other hand, thick and densely rooted organic layers are common in tropical montane forests (Edwards & Grubb, 1977) and may be important nutrient sources (Wilcke *et al.*, 2002; Röderstein *et al.*, 2005). N concentrations and cycling rates on a mass basis are larger in organic layers than in mineral soils of tropical forests (Livingston *et al.*, 1988; Vitousek & Matson, 1988; Wilcke *et al.*, 2002), but due to the small mass of the organic layer (or low bulk density) its large N-cycling rates may be unimportant on an areal basis (Livingston *et al.*, 1988).

Our present study reports the impact of first-time and chronic N additions on soil N-oxide emissions from two species-rich, old-growth tropical forests in the Republic of Panama: a lowland forest on a deeply weathered soil and a montane forest on a less-developed volcanic soil with an organic layer. We hypothesized the following:

1. The lowland forest, where stem diameter growth and annual fine litterfall mass were not N limited (S.J. Wright, unpublished results; Kaspari *et al.*, 2008), should exhibit relatively large soil N-cycling rates and thus a rather leaky N cycle. This forest will react to first-time N addition with immediate increases in soil N-oxide emissions, and chronic N addition will lead to sustained larger N-oxide emissions.
2. The montane forest, with N-limited stem diameter growth and fine litterfall mass (M. Adamek, unpublished results), should exhibit relatively small soil N-cycling rates and thus a conservative N cycle. However, a substantial organic layer covers the mineral soil, and we expect that first-time N addition will immediately increase soil N-cycling rates in this organic layer and consequently cause immediate increases in N-oxide emissions. Chronic N addition will lead to sustained larger N-oxide emissions.

We tested these hypotheses by intensive measurements of soil N-oxide emissions supported by measurements of soil factors known to influence gaseous N losses including temperature, moisture, extractable mineral N, and N cycling rates. This is the first study to evaluate (1) transitory and long-term soil N-oxide emissions in response to chronic N input in species-rich tropical forests and (2) the effects of elevated N input on the organic layer of a species-rich tropical montane forest.

Materials and methods

Approach

N-addition experiments differ from atmospheric N deposition in mode and amount in which N is added to the ecosystem. N deposition enters the ecosystem at the canopy level and through frequent inputs at relatively low concentrations, whereas fertilizer is typically applied to the soil and in one or a few large doses of high concentration. Furthermore, atmospheric N deposition normally enters an ecosystem as a combination of NH_4^+ , NO_3^- and organic N, whereas we applied fertilizer N in the form of urea. We chose urea for a practical reason: NH_4NO_3 is not sold in Panama due to security concerns.

We did not intend to simulate the mode of atmospheric deposition, but rather we intended to create an N-enriched condition, which is ultimately the result of chronic atmospheric N deposition. One 'artifact' of N addition is the occurrence of pronounced 'peaks' in soil mineral N concentrations and N-oxide emissions, which is typically a transitory effect occurring within a month following N addition (Keller *et al.*, 1988; Veldkamp *et al.*, 1998; Steudler *et al.*, 2002). We therefore differentiate the impact of N additions on soil N-oxide emissions between 'transitory' and 'long-term' effects measured within 42 days and at least 6 weeks following N addition, respectively. Long-term effects should be less sensitive to the type of N fertilizer because all urea-N will be hydrolyzed and processed in the soil N cycle within 6 weeks of N addition.

Study area

The lowland study site (between 25 and 61 m elevation) consists of an old-growth (>300 years) semideciduous tropical forest (Leigh *et al.*, 1996) and is located on Gigante Peninsula (9°06'N, 79°50'W) which is part of the Barro Colorado Nature Monument, Republic of Panama. On nearby Barro Colorado Island, annual rainfall (1995–2007) averages 2650 ± 146 mm with a dry season from January to mid-May during which 297 ± 40 mm of rainfall is recorded. Ambient N deposition from rainfall was $9 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, measured bi-weekly in 2006–2007 at the shore of Gigante Peninsula near the study site. The mean annual air temperature is 27.4 ± 0.1 °C. Litter mass on nearby Barro Colorado Island has a rapid turnover time of 210 days (Yavitt *et al.*, 2004). Stem diameter growth (S.J. Wright, unpublished results) and annual fine litterfall mass (Kaspari *et al.*, 2008) were not effected after 5 and 6 years of N addition, respectively.

The montane study site (between 1200 and 1300 m elevation) consists of an old-growth lower montane rainforest (Grubb, 1977) and is located in the Fortuna Forest Reserve in the Cordillera Central (8°45'N, 82°15'W), Chiriquí province, Republic of Panama. Mean annual rainfall is 5532 ± 322 mm (1997–2007), and rainfall distribution exhibits only a weak seasonality (11-year average of $244\text{--}288$ mm month⁻¹ from February to April and $403\text{--}683$ mm month⁻¹ from March to January). Ambient N deposition from rainfall was 5 kg N ha⁻¹ yr⁻¹, measured biweekly in 2006–2007 at a forest clearing near the study site. The average monthly air temperature is 19 °C from December to March and 21 °C for all other months [annual mean (1999–2007) of 20.1 ± 0.1 °C]. Stem diameter growth and fine litterfall mass increased compared with the control during the first 2 years of N addition (M. Adamek, unpublished results). See Tables 1 and 2 for information on soil characteristics/classification and forest structure, respectively, of both sites.

Experimental design

In the lowland, our study was conducted in the only ongoing large-scale chronic nutrient addition experiment in old-growth species rich tropical forest. The site covers a gentle slope from the northeast corner to the southwest and lacks pronounced ridges, slopes, valleys and bottomlands. The experiment includes N-addition and control plots, among other treatments, laid out in four replicates across a 26.6 ha area in a stratified random design. Each treatment plot is 40 m × 40 m and the distance between adjacent plots is at least 40 m. N addition started in June 1998. Just outside the long-term experimental plots, we set up four 20 m × 20 m plots (at least 40 m apart) for first-time N addition in May 2006. In the montane forest, the experiment was set up in a paired-plots design with four replicates. Each plot is 40 m × 40 m, and plots are separated by at least 40 m. Plots lack streams, swampy areas, gaps or clearings, and slopes > 15°. Plot size is corrected for inclination (Condit, 1998). Control and N-addition treatments were randomly assigned to each pair of plots. N addition started in February 2006.

The N-addition plots received 125 kg urea-N ha⁻¹ yr⁻¹, which was split in four equal applications. In the lowland site, fertilizer was applied during the wet season with 6–8 weeks between applications (May 15–30, July 1–15, September 1–15, October 15–30). In the montane site, the four N additions were spread during the year with at least 7 weeks between applications. Urea was applied manually, walking back and forth across 10 m × 10 m subplots and changing directions (east-to-west and north-to-south) in subsequent N ad-

ditions. Gas flux measurement chambers were covered during plot N addition and received the exact amount of fertilizer for their area afterwards.

We measured soil N-oxide fluxes, air and soil temperatures (at 0.05 m depth), soil moisture and soil extractable mineral N. Measurements were conducted every 6 weeks on all plots (specifically before N additions), and intensively (two to five times) following fertilizations on the N-addition plots. Two pretreatment measurements in the montane site and one pretreatment measurement in the first-time N-addition lowland site indicated that initial N-oxide fluxes did not differ between control and N-addition plots before manipulation. We do not have pretreatment measurements of N-oxide fluxes from the 9- and 10-year N-addition lowland plots. All measurements took place within 10 m of the center of each plot so that all sampling points were surrounded (buffered) by at least 10 m of forest receiving the same treatment (in case of the first-time N-addition lowland plots, the buffer zone was 5 m).

Soil characteristics

Soil characteristics were determined in January 2006 before first treatment in the montane site. Organic layer samples were air-dried and ground before analyses. Mineral soil samples (from 0 to 0.05, 0.05 to 0.10, 0.10 to 0.25 and 0.25 to 0.50 m depth with one profile per plot) were air-dried, sieved (2 mm) and ground for analysis of total organic C, N, $\delta^{15}\text{N}$ and total P. Total C and N were measured by a CNS Elemental Analyzer (Elementar Vario EL, Hanau, Germany), $\delta^{15}\text{N}$ by isotope ratio mass spectrometry (Finigan MAT, Bremen, Germany), and total P and total base cations (only for the organic layer samples) by pressure digestion in concentrated HNO₃ (König & Fortmann, 1996) followed by analysis of the digests using inductively coupled plasma-atomic emission spectrometer (ICP-AES; Spectro Analytical Instruments, Kleve, Germany). Effective cation exchange capacity (ECEC) of the mineral soil was determined from sieved samples by percolating with unbuffered 1 M NH₄Cl (König & Fortmann, 1996) and measuring cations in percolates using ICP-AES. Base saturation was calculated as percentage base cations of the ECEC. Soil pH was measured from a saturated paste mixture (1 : 1 and 1 : 10 ratio of soil to H₂O for mineral soil and organic layer, respectively). Soil bulk density was determined by the soil core method (Blake & Hartge, 1986).

N-oxide flux measurements

Four permanent chamber bases (area 0.04 m², height 0.25 m, ~ 0.02 m inserted into the soil) were installed

Table 1 Soil characteristics of the forest sites determined in January 2006, after 8 years of nitrogen (N) addition in the lowland site and before first N addition in the montane site

Characteristics/depth	Lowland*	Montane†		
Parent material	Basalt	Volcanic ash deposits		
Texture	Heavy clay	Sandy loam		
Soil type (FAO)	Endogleyic Cambisol in the upper part to Acric Nitisol in the lower part of the landscape	Aluandic Andosols		
Soil type (USDA)	Dystrudepts	Hapludands		
Organic layer (median thickness, cm)	None	8 (25% quantile of 5 cm and 75% quantile of 12 cm, $n = 64$)		
		Control	8-year N addition	All plots
Organic layer				
Bulk density (g cm^{-3})		–	–	0.07 ± 0.01
pH (1:10 H_2O)		–	–	4.1 ± 0.1
Total carbon (C) (mg g^{-1})		–	–	443.0 ± 18.7
Total nitrogen (N) (mg g^{-1})		–	–	22.4 ± 1.1
C/N ratio		–	–	19.9 ± 0.4
$\delta^{15}\text{N}$ (‰)		–	–	0.92 ± 0.15
Total phosphorus (P) (mg kg^{-1})		–	–	0.72 ± 0.07
Total base cations [$\text{mg (K + Na + Ca + Mg) g}^{-1}$]		–	–	5.8 ± 0.6
Mineral topsoil (0–0.05 m)				
Bulk density (g cm^{-3})		0.62 ± 0.02	0.62 ± 0.02	0.47 ± 0.02
pH (1:1 H_2O)		5.3 ± 0.2 a	4.5 ± 0.1 b	4.1 ± 0.1
Total C (mg g^{-1})		51.0 ± 5.3	47.2 ± 3.8	94.5 ± 11.4
Total N (mg g^{-1})		3.9 ± 0.4	3.6 ± 0.2	6.2 ± 1.0
C/N ratio		13.3 ± 0.5	13.3 ± 0.4	15.7 ± 0.6
$\delta^{15}\text{N}$ (‰)		4.86 ± 0.52	5.56 ± 0.17	3.81 ± 0.42
Total P (mg g^{-1})		0.55 ± 0.08	0.50 ± 0.02	0.56 ± 0.05
Effective cation exchange capacity (ECEC) [mmol(+) kg^{-1}]		205 ± 44	116 ± 8	132 ± 25
Base saturation (%)		91.8 ± 3.6 a	61.8 ± 8.6 b	20.9 ± 3.6
Mineral soil (0.05–0.5 m)				
pH (1:1 H_2O)		5.1 ± 0.1 a	4.9 ± 0.1 b	4.5 ± 0.1
Total C (mg g^{-1})		15.2 ± 1.1	14.6 ± 0.9	30.7 ± 5.8
Total N (mg g^{-1})		1.4 ± 0.1	1.3 ± 0.1	1.8 ± 0.3
C/N ratio		10.2 ± 0.7	11.0 ± 0.2	16.5 ± 0.8
$\delta^{15}\text{N}$ (‰)		7.33 ± 0.79	8.30 ± 0.38	5.95 ± 0.23
Total P (mg g^{-1})		0.40 ± 0.07	0.36 ± 0.02	0.29 ± 0.04
ECEC [mmol(+) kg^{-1}]		149 ± 48	110 ± 22	71 ± 19
Base saturation (%)		55.7 ± 7.0 a	41.4 ± 5.3 b	11.2 ± 4.6

*Means (\pm SE, $n = 4$) with different letters indicate differences between treatments (independent t -test at $P \leq 0.05$).

†Means (\pm SE, $n = 8$) did not differ between plots which were later randomly assigned to control and N addition treatment.

on each plot in a stratified random design along two perpendicular 20 m long transects that crossed in the plot center. Soil N_2O fluxes were measured using vented static chambers. Four gas samples (100 mL each) were removed at 2, 12, 22 and 32 min after chamber closure and stored in pre-evacuated glass containers with a teflon-coated stopcock. Gas samples were analyzed using a gas chromatograph (Shimadzu GC-14B, Columbia, MD, USA) equipped with an electron capture detector and an autosampler (Loftfield *et al.*, 1997).

Gas concentrations were determined by comparison of integrated peak areas of samples and three to four standard gases (317, 503, 1000 and 2992 ppb N_2O ; Deuste Steinger GmbH, Mühlhausen, Germany). Soil NO fluxes were measured on-site using open dynamic chambers which were placed for 5–7 min on the same chamber bases used for N_2O sampling. Flow rate through the chamber was between 450 and 750 mL min^{-1} . Dilution of the NO concentration in the chamber by outside air flow through the chamber is

Table 2 Forest characteristics of the lowland and montane sites

Forest structure (based on trees ≥ 0.1 m in diameter at breast height)					
Site	Tree height (m)	Mean basal area ($\text{m}^2 \text{ha}^{-1}$)	Tree density (trees ha^{-1})	Most abundant tree species	Trees belonging to the family <i>Fabaceae</i> (%)
Lowland	30–35	20.3	384	<i>Oenocarpus mapora</i> , <i>Dialium guianense</i> , <i>Heisteria concinna</i> , <i>Tetragastris panamensis</i> ; together contributing 28%	15
Montane	~ 20 (some emergent trees up to 40 m, mainly <i>Oreomunnea mexicana</i>)	46.1	1039	<i>Oreomunnea mexicana</i> , <i>Eschweilera panamensis</i> , <i>Vochysia guatemalensis</i> , <i>Cassipourea elliptica</i> ; together contributing 38%*	4†

*The palm *Colpotherinax aphanopetala* and vascular epiphytes (especially the genus *Anthurium* and *Monstera*) are also abundant.

†All belonging to the genus *Inga*, which is the only *Fabaceae* known from the site (J. Dalling, personal communication).

negligible during the initial linear part of the concentration increase (Bakwin *et al.*, 1990). NO was analyzed with a Scintrex LMA-3 chemiluminescence detector (Scintrex Unisearch, ON, Canada) after oxidation to NO₂ by a CrO₃ catalyst. The reaction vessel in the detector has a fabric wick saturated with luminol II solution, which contains additives to enhance reaction and reduce interference from other gases including ozone. Because the catalyst is sensitive to relative humidity, a known flux of ambient air dried by silica gel was mixed to the chamber air to reach a humidity of ~ 50%. In order to minimize potential changes in catalyst efficiency caused by variations of air humidity between calibration and measurements (Williams & Davidson, 1993), we calibrated the detector in the field before and after the four chamber flux measurements per plot using a standard gas (3400 ppb NO; Deuste Steininger GmbH).

Gas fluxes were calculated from the linear increase of gas concentration in the chamber vs. time, and were adjusted for air temperature and atmospheric pressure measured at the time of sampling:

$$\Phi = \frac{V}{A} \left(\frac{P}{R \times T} \right) Mf \frac{\partial c}{\partial t}, \quad (1)$$

where Φ is the flux ($\text{g N m}^{-2} \text{h}^{-1}$), V the chamber volume (L), A the chamber area (m^2), P the atmospheric pressure (Pa), R the ideal gas constant ($8.315 \text{ Pa m}^3 \text{ mol}^{-1} \text{ K}^{-1}$), T the temperature (K), M the molar mass of NO-N or N₂O-N (g mol^{-1}), $\delta c / \delta t$ the rate of gas concentration change within the chamber ($\text{ppm h}^{-1} = \mu\text{L L}^{-1} \text{h}^{-1}$) and f a conversion factor ($10^{-9} \text{ m}^3 \mu\text{L}^{-1}$). Positive gas fluxes indicate emission from the soil; negative fluxes indicate consumption of

the gas by the soil. Zero fluxes were included. NO fluxes from the open dynamic chambers were calculated using 3 min of continuous data with values logged every 5 s. In contrast, N₂O fluxes from the vented static chambers were calculated using four points in time. N₂O fluxes might be significantly underestimated if a linear model was uncritically applied, ignoring potential chamber feedbacks (especially decreasing diffusion gradient over time; Livingston *et al.*, 2006). If N₂O concentration increased asymptotically with time, both a linear and a quadratic regression model were fitted (Wagner *et al.*, 1997) and the more adequate model was chosen using the Akaike Information Criterion (AIC). The quadratic model was used in about 25% of the N₂O flux calculations. The annual N-oxide losses were approximated by applying the trapezoid rule on time intervals between measured flux rates, assuming constant flux rates per day.

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

Parallel to gas sampling, four samples of 0–0.05 m mineral soil (for the montane site, organic layer and mineral soil were sampled separately) were collected within the central 10 m × 10 m of each plot. While in the field, samples were pooled for each plot, leaves and roots were manually removed, and a subsample (50–60 g fresh weight) was added to a prepared extraction bottle containing 150 mL of 0.5 mol L⁻¹ K₂SO₄ solution. The rest of the sample was stored in plastic bags for gravimetric moisture determination in the laboratory. Subsamples (40–100 g fresh weight) were dried for 24 h at 105 °C. Moisture content is expressed as the percen-

tage of water-filled pore space (WFPS), assuming a particle density of 2.65 g cm^{-3} for mineral soil (Linn & Doran, 1984) and of 1.4 g cm^{-3} for organic layer (Breuer *et al.*, 2002). The soil- K_2SO_4 bottles were transported to the laboratory and stored in a refrigerator (4°C) until extractions were performed. Extraction was done by shaking the soil- K_2SO_4 bottles for 1 h and filtering them through K_2SO_4 -prewashed filter papers ($4\ \mu\text{m}$ nominal pore size). Extracts were frozen immediately and remained frozen during transport by air to Germany, where analysis was conducted. NH_4^+ and NO_3^- contents of the extracts were analyzed using continuous flow injection colorimetry (Cenco/Skalar Instruments, Breda, the Netherlands), in which NH_4^+ was determined using the Berthelot reaction method (Skalar Method 155-000) and NO_3^- was measured using the copper-cadmium reduction method (NH_4Cl buffer but without ethylenediamine tetraacetic acid; Skalar Method 461-000). In the first 6 months of the study, mineral N extraction of the soil samples was conducted within 3 days for the lowland site and 8 days for the montane site. In June 2007, we brought equipment to the field stations so that soil- K_2SO_4 extraction proceeded within 12 h after field sampling. We compared the NO_3^- concentrations of the soil samples before and after June 2007, and these were not statistically different. Also, statistical analyses of treatment effects and site differences were conducted separately on mineral N concentrations before and after June 2007, to verify that observed differences were not influenced by the change in methodology.

Net rates of soil N cycling were measured in the first week of September 2006. In the lowland site, this was in the middle of the wet season and 3 weeks after the third N application of the year for the N-addition plots. In the montane site, this was 1.5 months after the third N application of the first-year N addition plots. Two intact soil cores were taken from the mineral soil (0–0.05 m depth) and, for the montane site, also from the organic layer within the central $10 \text{ m} \times 10 \text{ m}$ of each plot. One soil core was extracted immediately in the field with $0.5 \text{ mol L}^{-1} \text{ K}_2\text{SO}_4$ to determine initial NH_4^+ and NO_3^- levels (T_0). The other intact soil core was put in a plastic bag, inserted back into the soil for 7 days of incubation, and afterwards extracted in the field with K_2SO_4 (T_1). Soil- K_2SO_4 extractions proceeded within 12 h after field sampling. Net N mineralization and nitrification rates were calculated as the difference between T_1 - and T_0 - NH_4^+ and NO_3^- concentrations, respectively.

Statistical analyses

Statistical analysis was carried out on the plot mean NO and N_2O fluxes (average of four chambers). If datasets were rightly skewed, we applied either a square root or

a logarithmic transformation before analysis (after adding a constant value if the dataset included negative values). For analysis of time-series data (i.e. repeated measurements of the response variables N-oxide fluxes, soil mineral N, soil temperature and WFPS) we used linear mixed effects models, where explanatory variables are included as either fixed (influencing the mean of the response variable) or random (influencing its variance) effect (Crawley, 2002). Linear mixed effects models are advantageous over repeated-measures analysis of variance because these can properly account for correlation between repeated measurements, allow to specify the within-group variance of a stratification variable and are unaffected by randomly missing data (Gueorguieva & Krystal, 2004; Pinheiro & Bates, 2004). Our basic model included treatment (if testing for an effect of N addition) or site (if testing for differences between lowland and montane forest) as fixed effect and the spatial replication (experimental plots) nested in time (temporal sampling scheme) as random effects. We extended the model to include (1) a variance function which allows different variances of the response variable per level of the fixed effect, and/or (2) a first-order temporal autoregressive process, which assumes that correlation between measurements decreases with increasing time distance, if this improved the relative goodness of the model fit based on the AIC. We then checked the model using diagnostic residual plots and assessed the significance of the fixed effect based on analysis of variance (Crawley, 2002). For linear regression, we tested whether the slope is different from zero using analysis of variance in regression. For soil characteristics and net N-cycling rates, treatment effects and differences between sites were assessed using independent *t*-tests. Mean values in the text are given with ± 1 standard error. Missing values were excluded from analyses and effects were accepted as statistically significant if *P*-value ≤ 0.05 . All analyses were conducted using R 2.6.0 (R Development Core Team, 2008).

Results

Soil conditions, N-cycling rates and N-oxide fluxes from control forest soils

WFPS followed a clear seasonal pattern in the lowland forest but not in the montane forest, and was much higher in the mineral soil than in the organic layer of the montane forest (Fig. 1a and b). Soil temperature at 0.05 m depth varied seasonally by 1.6°C (annual mean of 25.4°C) in the lowland forest and by 3.9°C (annual mean of 18.1°C) in the montane forest (Fig. 1c and d). Linear regressions between N-oxide fluxes and WFPS or soil temperature were not significant. WFPS and soil

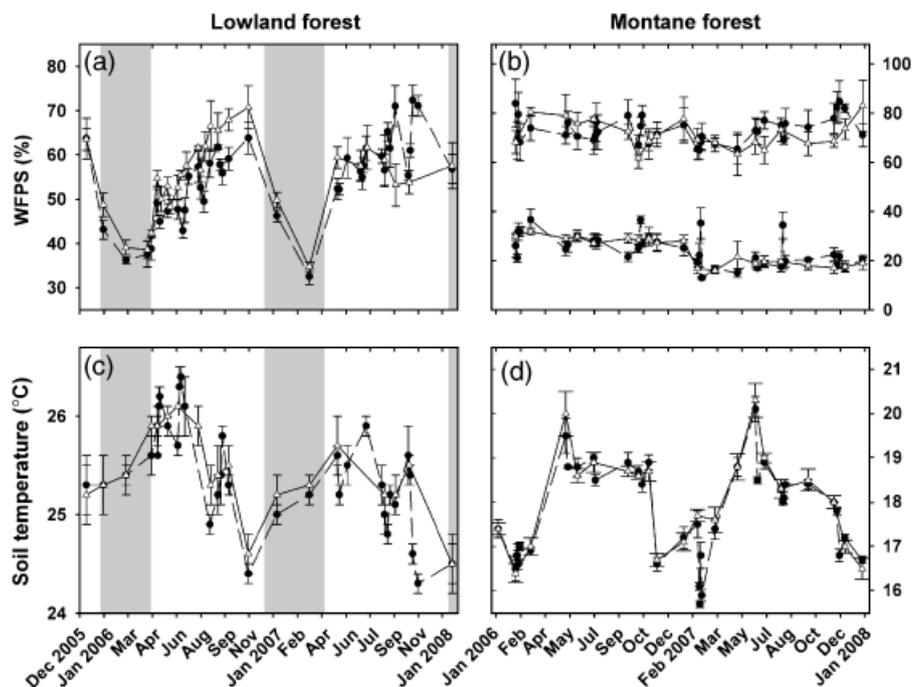


Fig. 1 Mean (\pm SE, $n = 4$) water-filled pore space (WFPS) and soil temperature at 0.05 m depth in control (Δ) and N-addition (\bullet) lowland (a, c) and montane (b, d) forests. For WFPS in the montane forest, the upper and lower values are for 0–0.05 m mineral soil and the organic layer, respectively. Grey shading marks the dry season in the lowland forest.

temperature did not differ between control and N-addition plots.

Ammonium (NH_4^+) was the dominant form of soil mineral N at both sites. In the lowland forest, NH_4^+ concentrations increased throughout the wet season until the dry season began and then decreased towards the end of the dry season, whereas nitrate (NO_3^-) concentrations were always small in the wet season and increased in the dry season (Fig. 2a–d). Soil mineral N did not show a seasonal pattern in the montane forest (Fig. 3). NH_4^+ concentrations were larger in the organic layer of the montane forest than in the mineral soil of either forest (both $P < 0.010$) but did not differ between forests in the mineral soil. NO_3^- concentrations were statistically indistinguishable between the organic layer of the montane forest and the mineral soil of both forests, as well as between the mineral soils of both forests.

Net N mineralization, which is often used as an index of plant available mineral N, was larger in the organic layer of the montane forest than in the mineral soil of either forest (both $P \leq 0.001$), and did not differ between forests in the mineral soil. Net nitrification was larger in the mineral soil of the lowland forest than in the organic layer ($P = 0.001$) and mineral soil of the montane forest ($P = 0.050$, Table 3).

Across the 2-year measurement period, the lowland forest soil had larger mean NO ($P = 0.024$), N_2O ($P = 0.032$) and total N-oxide emissions ($P = 0.043$) than

the montane forest soil (Tables 4 and 5). Soil N-oxide emissions were dominated by N_2O at both sites and the ratio of annual $\text{N}_2\text{O}/\text{NO}$ fluxes was 5.6 in 2006 and 3.2 in 2007 in the lowland forest and 8.2 in 2006 and 8.0 in 2007 in the montane forest (Table 6).

Transitory N addition effects ('fertilization peaks')

After each addition of $31.25 \text{ kg N ha}^{-1}$, a distinctive 'peak' was observed in soil N-oxide emissions (with the exception of the first-time N addition in the lowland forest) and in soil mineral N concentrations. We call these 'fertilization peaks' and refer to them as 'transitory' effects. Maximum NH_4^+ and NO_3^- concentrations (Figs. 2a, b and 3) and maximum NO emissions were measured within the first 2 weeks while N_2O emissions peaked between the first and third week following N additions (Figs. 4a, b and 5a, b). In the lowland forest, fertilization peaks of soil N-oxide emissions increased as the wet season progressed (Fig. 4a and b). Twice in the montane forest, a second maximum of NO emissions occurred within 5 weeks after N addition (Fig. 5a in March 2006 and March 2007). In the montane forest, transitory N-oxide emissions reached higher levels in the second year than in the first year of N addition (Fig. 5a and b), and the second year fertilization peaks were in the same order of magnitude as those from the low-

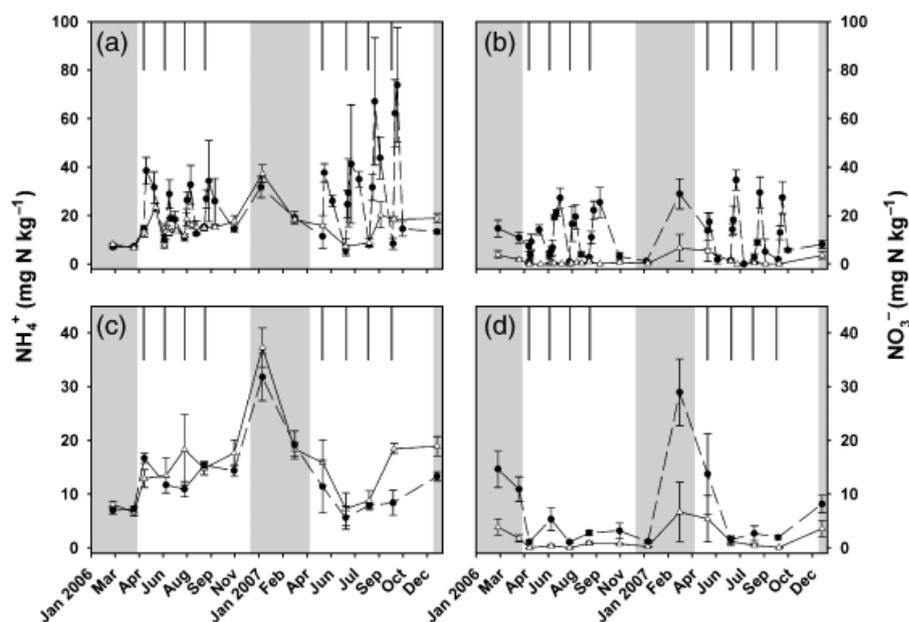


Fig. 2 Mean (\pm SE, $n = 4$) soil extractable NH_4^+ (left side) and NO_3^- (right side) in control (Δ) and 9- and 10-year N-addition (\bullet) lowland forest. Black vertical lines indicate dates of N addition. Grey shading marks the dry season. Upper panels (a, b) include the transitory 'fertilization peaks', and lower panels (c, d) show only the 'background concentrations' at least 6 weeks after an N addition.

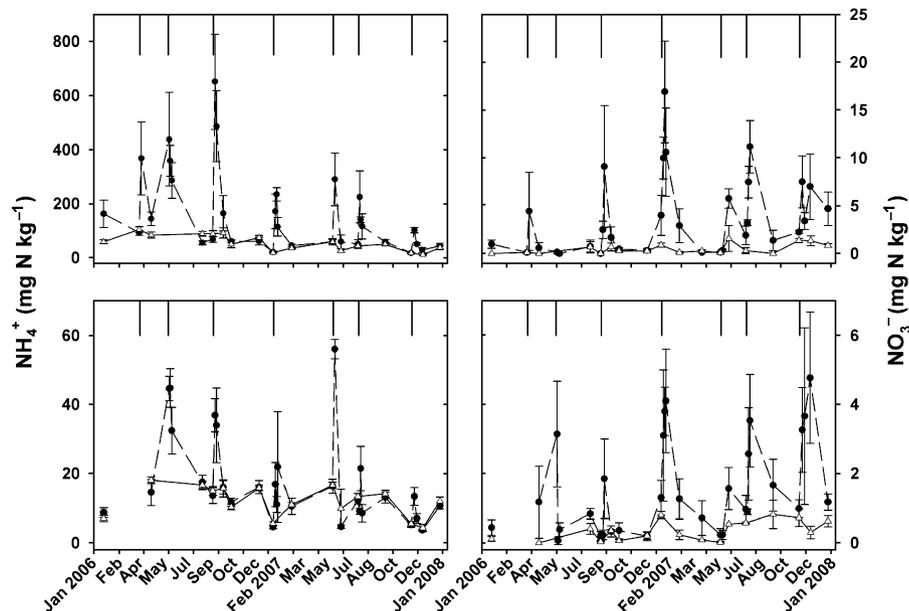


Fig. 3 Mean (\pm SE, $n = 4$) soil extractable NH_4^+ (left side) and NO_3^- (right side) in control (Δ) and 1- and 2-year N-addition (\bullet) montane forest. Black vertical lines indicate dates of N addition. Upper and lower panels present the organic layer and 0–0.05 m mineral soil, respectively.

land forest at the height of the wet season (Fig. 4a and b). Maximum NO emissions reached $15.41 \pm 4.49 \text{ ng N cm}^{-2} \text{ h}^{-1}$ in the montane and $20.52 \pm 6.51 \text{ ng N cm}^{-2} \text{ h}^{-1}$ in the lowland forest. Maximum N_2O emissions were $29.33 \pm 11.95 \text{ ng N cm}^{-2} \text{ h}^{-1}$ in the montane and $42.19 \pm 5.25 \text{ ng N cm}^{-2} \text{ h}^{-1}$ in the lowland forest.

Long-term effects of N enrichment

We assessed the effects of N enrichment by considering only the data at least 6 weeks after an N addition to eliminate the transitory fertilization peaks. We call these 'background' soil N-oxide fluxes and 'background' soil

Table 3 Mean (\pm SE; $n = 4$) net rates of soil nitrogen (N) cycling ($\text{ng N cm}^{-2} \text{h}^{-1}$) measured in September 2006 (wet season in the lowland forest)

Depth/N-cycling processes	Lowland			Montane	
	Control	1-year N-addition	9-year N-addition	Control	1-year N-addition
Organic layer					
Net mineralization	–	–	–	258.77 \pm 32.90 b	534.33 \pm 74.20 a
Net nitrification	–	–	–	15.17 \pm 9.10 b	221.43 \pm 80.97 a
Mineral soil (0–0.05 m depth)					
Net mineralization	21.96 \pm 103.33	12.92 \pm 64.58	9.04 \pm 102.04	41.44 \pm 26.56	49.94 \pm 5.31
Net nitrification	140.79 \pm 80.08	193.75 \pm 25.83	178.25 \pm 114.96	–3.19 \pm 3.19 b	20.19 \pm 9.56 a

Means with different letters indicate differences between treatments (independent *t*-test at $P \leq 0.05$).

Table 4 Mean (\pm SE) soil NO and N₂O emissions from old-growth tropical lowland forests*

Location	Mean annual temperature (°C)	Mean annual precipitation (mm)	Soil type (USDA classification)	N ₂ O ($\text{ng N cm}^{-2} \text{h}^{-1}$)	NO ($\text{ng N cm}^{-2} \text{h}^{-1}$)	Reference
Tapajos National Forest, Pará, Brazil (3.04°S, 54.95°W)	25	2000	Sandy loam Ultisol	1.6 \pm 0.3	8.8 \pm 5.0	Keller <i>et al.</i> (2005)
Tapajos National Forest, Pará, Brazil (3.04°S, 54.95°W)	25	2000	Clay Oxisol	7.0 \pm 0.6	9.0 \pm 2.8	Keller <i>et al.</i> (2005)
Paragominas, Pará, Brazil (2°59S, 47°31W)		1850	Haplustox	2.1	1.3	Verchot <i>et al.</i> (1999)
La Selva, Heredia, Costa Rica (10°26N, 84°0W)	25.8	3962	Inceptisols and Ultisols	6.7 \pm 3.0	1.0 \pm 0.3	Keller & Reiners (1994)
Gigante Peninsula, Republic of Panama (9°06N, 79°50W)	27.4	2650	Clay Dystrudepts	1.6 \pm 0.1	0.3 \pm 0.03	This study

*Only studies with at least 1 year of measurements for which averages are provided by the authors are included. The listing is not meant to be complete but to give an overview of the range of published data.

mineral N concentrations and refer to them as 'long-term effects'.

In the lowland forest, background NH₄⁺ concentrations did not differ between N-addition and control plots (Fig. 2c). Background NO₃[–] concentrations were larger and showed a more pronounced seasonal pattern in the N-addition than the control plots ($P = 0.001$; Fig. 2d). As a result, the ratio of NH₄⁺/NO₃[–] decreased with N enrichment. Net rates of soil N cycling in the lowland forest did not differ between treatments during the sampling in wet season 2006 (Table 3).

In the montane forest, N addition did not change background NH₄⁺ concentrations relative to the control. Background NO₃[–] concentrations increased in the organic layer of the N-addition plots ($P = 0.022$) but not in the mineral soil. Hence, the ratio of NH₄⁺/NO₃[–] in the organic layer decreased with N addition. Net N mineralization and nitrification rates increased with N enrichment (Table 3).

In the lowland forest, background soil NO ($0.80 \pm 0.14 \text{ ng N cm}^{-2} \text{h}^{-1}$) and N₂O ($5.32 \pm 0.90 \text{ ng N cm}^{-2} \text{h}^{-1}$) fluxes were larger from the N-addition than the control plots ($P = 0.027$ and 0.002 , respectively) and displayed a pronounced seasonal pattern. Background NO and N₂O fluxes from the N-addition plots were largest during the dry and wet seasons, respectively (Fig. 4c and d). In the N-addition plots, background NO fluxes were negatively correlated with WFPS (Fig. 6a) while the converse was true for the background N₂O fluxes (Fig. 6b). The logarithm of background N₂O/NO ratios was positively correlated with WFPS in the N-addition plots (Fig. 6c), but not in the control plots (Fig. 6d). Annual background soil N-oxide emissions increased by a factor of 2–3 for NO and a factor of 4 for N₂O (Table 6), such that the N₂O/NO ratio increased with N enrichment.

In the montane forest, background soil NO fluxes ($0.28 \pm 0.04 \text{ ng N cm}^{-2} \text{h}^{-1}$) from the N-addition plots

Table 5 Mean (\pm SE) soil NO and N₂O emissions from old-growth tropical montane forests*

Location	Elevation (m)	Mean annual temperature (°C)	Mean annual precipitation (mm)	Soil type (USDA classification)	N ₂ O (ng N cm ⁻² h ⁻¹)	NO (ng N cm ⁻² h ⁻¹)	Reference
Kohala, Hawaii (20°03, 155°41)	1122	16.1	2540	Typic Placandept	~ 0.5		Riley & Vitousek (1995) ^{†,§}
Wuasa, Central Sulawesi, Indonesia (120°17E, 01°25S)	1190	22.5	1590	Entisols and Inceptisols	1.1 \pm 0.1	0.5 \pm 0.2	Purbopuspito <i>et al.</i> (2006) [‡]
Rorekatimbu, Central Sulawesi, Indonesia (120°18E, 01°19S)	1800	18.3		Inceptisols	0.3 \pm 0.02	0.2 \pm 0.07	Purbopuspito <i>et al.</i> (2006) [‡]
Puncak Dingin, Central Sulawesi, Indonesia (120°18E, 01°16S)	2470	14.6		Inceptisols	1.0 \pm 0.2	0.2 \pm 0.03	Purbopuspito <i>et al.</i> (2006) [‡]
Maui, Hawaii (20°48, 156°15)	1300	16	4050	Inceptisols	\leq 0.2	\leq 0.2	Holtgrieve <i>et al.</i> (2006) ^{†,§}
Maui, Hawaii (20°48, 156°15)	1320	16	3350	Inceptisols	1.1 \pm 0.3	8.7 \pm 4.6	Holtgrieve <i>et al.</i> (2006) ^{†,§}
Fortuna, Republic of Panama (8°45'N, 82°15'W)	1200–	20	5532	Hapludands	1.3 \pm 0.1	0.1 \pm 0.02	This study [‡]

*The listing is not meant to be complete but to give an overview of the range of published data. For Purbopuspito *et al.* (2006) and this study, average N-oxide fluxes over 1–2 year measurements are provided by the authors. We included studies with less than 1 year of measurements as there are only few studies of soil N-oxide emissions from tropical montane forests.

[†]Average N₂O flux was estimated from a figure. No estimate is given for NO, but fluxes were often zero.

[‡]A substantial organic layer is mentioned by the authors.

[§]Forest dominated by a single species, *Metrosideros polymorpha*.

Table 6 Annual soil NO and N₂O emissions (kg N ha⁻¹, mean \pm SE, $n = 4$) for control and N-addition plots, separated into transitory effects (include transitory 'fertilization peaks') and long-term effects (include only fluxes measured at least 6 weeks after an N addition)

Site	Treatment	2006		2007	
		NO	N ₂ O	NO	N ₂ O
Montane	Control	0.13 \pm 0.02	1.06 \pm 0.12	0.13 \pm 0.02	1.26 \pm 0.16
	1- and 2-year N-addition, transitory effect	0.84 \pm 0.25*	4.49 \pm 1.35*	1.95 \pm 0.55 [†] (3.32 \pm 0.52)	6.51 \pm 2.65
	1- and 2-year N-addition, long-term effect	0.28 \pm 0.05 [‡]	2.24 \pm 0.82 [‡]	0.34 \pm 0.12	2.31 \pm 1.15
Lowland	Control	0.24 \pm 0.05	1.35 \pm 0.09	0.31 \pm 0.06	0.98 \pm 0.10
	9- and 10-year N-addition, transitory effect	2.06 \pm 0.39	6.93 \pm 1.82	1.44 \pm 0.33	8.36 \pm 1.38
	9- and 10-year N-addition, long-term effect	0.67 \pm 0.18	4.97 \pm 1.09	0.75 \pm 0.09	3.81 \pm 0.51

*Calculation includes the two pretreatment measurements (January and February 2006).

[†]Calculation excludes the exceptionally high flux in March 2007 (Fig. 5a), which we do not consider representative for the whole integration time (inclusion would change the estimate to the value given in parentheses).

[‡]Calculation excludes the two pretreatment measurements (January and February 2006) and starts with the first long-term effect measurement of May 2006 (Fig. 5c and d; 102 days after the first-time N addition).

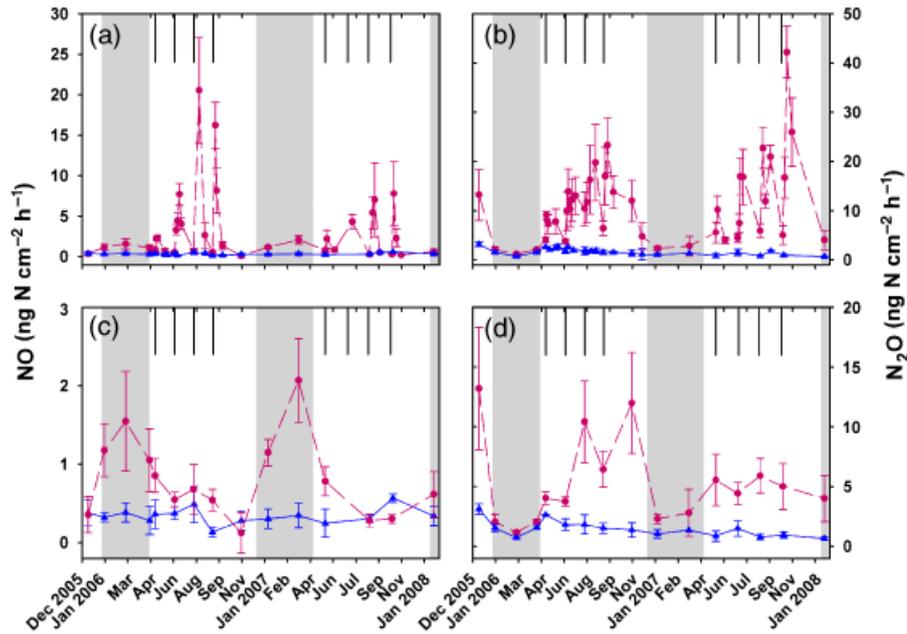


Fig. 4 Mean (\pm SE, $n = 4$) soil NO (left side) and N₂O emissions (right side) from control (\blacktriangle) and 9- and 10-year N-addition (\bullet) lowland forest. Black vertical lines indicate dates of N addition. Grey shading marks the dry season. Upper panels (a, b) include the transitory ‘fertilization peaks’, and lower panels (c, d) show only the ‘background fluxes’ at least 6 weeks after an N addition.

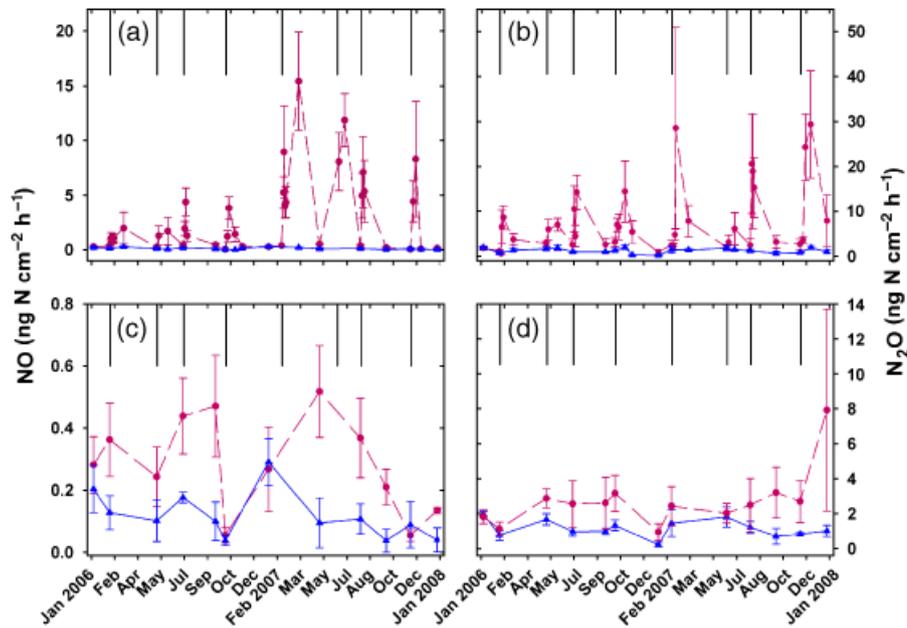


Fig. 5 Mean (\pm SE, $n = 4$) soil NO (left side) and N₂O emissions (right side) from control (\blacktriangle) and 1- and 2-year N-addition (\bullet) montane forest. Black vertical lines indicate dates of N addition. Upper panels (a, b) include the transitory ‘fertilization peaks’, and lower panels (c, d) show only the ‘background fluxes’ at least 6 weeks after an N addition. The first two fluxes are pretreatment measurements.

were larger than controls ($P = 0.025$; Fig. 5c) while background soil N₂O fluxes ($2.75 \pm 0.47 \text{ ng N cm}^{-2} \text{ h}^{-1}$) did not differ from controls (Fig. 5d). Background N-oxide fluxes did not show a pronounced temporal

pattern. Linear regressions between background N-oxide emissions and WFPS in organic layer or mineral soil were not significant. Annual background soil N-oxide emissions were two to three times larger for NO

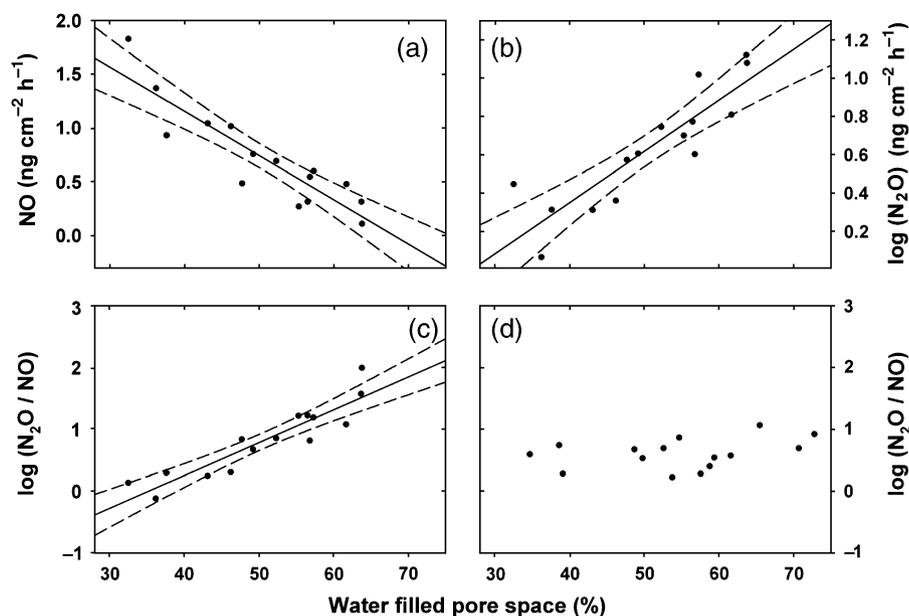


Fig. 6 Linear regressions (95% CI, $n = 15$) between mean water-filled pore space and (a) mean soil NO emissions [$y = -0.05 (\pm 0.01)x + 3.17 (\pm 0.71)$, $R^2 = 0.805$, $P < 0.001$], (b) mean soil log (N_2O) emissions [$y = 0.03 (\pm 0.01) \times -0.72 (\pm 0.45)$, $R^2 = 0.756$, $P < 0.001$] and (c) mean log (N_2O/NO) ratio [$y = 0.05 (\pm 0.01) \times -1.87 (\pm 0.72)$, $R^2 = 0.839$, $P < 0.001$] of four plots for the 9- and 10-year N-addition lowland forest. Similar regression for control (d) lowland forest was not significant ($R^2 = 0.116$, $P = 0.214$). Only N-oxide fluxes measured at least 6 weeks after an N addition were considered.

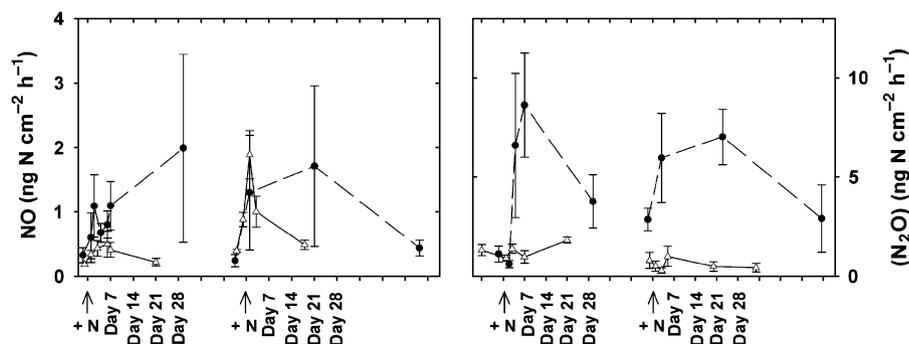


Fig. 7 Mean (\pm SE, $n = 4$) soil NO (left side) and N_2O emissions (right side) from the first-time N addition montane (\bullet) and lowland (Δ) forest following two subsequent N additions marked with '+ N' below the x-axis.

and two times larger for N_2O (Table 6), such that the N_2O/NO ratio decreased with N enrichment.

Background soil NO emissions from the N-addition plots were larger from the lowland than the montane forest ($P = 0.014$). Background soil N_2O and total background soil N-oxide emissions did not differ between forests, though total N-oxide emissions trended larger in the lowland forest ($P = 0.063$).

N-oxide fluxes following first-time N addition

In the lowland forest, first-time addition of $62.5 \text{ kg N ha}^{-1}$ (split in two equal parts and applied

early in the wet season) did not affect soil N_2O fluxes but increased soil NO emissions compared with the control ($P = 0.027$). However, over a period of 60 days, only $0.05 \pm 0.02\%$ of the applied N was lost as NO-N, and most of this loss occurred after the second N addition (Fig. 7). In contrast, the montane forest showed immediate increases in soil N-oxide emissions after the first-time N additions compared with the control ($P = 0.012$ for NO and $P = 0.037$ for N_2O ; Fig. 7). The losses of applied N were $0.76 \pm 0.43\%$ as NO-N and $3.09 \pm 0.84\%$ as N_2O -N within 31 days of the first N addition, and $0.48 \pm 0.33\%$ as NO-N and $1.65 \pm 0.71\%$ as N_2O -N within 25 days of the second N addition.

Discussion

Soil N cycle and N-oxide fluxes from control forests

Soil N cycles differed between the lowland and montane forests. In the lowland forest, most of the mineralized N was nitrified (Table 3). This suggests that mineral N production was more than sufficient for microbial immobilization, and the absence of competition from plant uptake during the 7-day incubation enabled nitrifiers to monopolize available substrate (NH_4^+ and organic N), resulting in accumulation of NO_3^- . In the montane forest, net nitrification rates were only 6% of net N mineralization rates in the organic layer and net NO_3^- immobilization occurred in the mineral soil. This indicates that a large fraction of mineral N was immobilized and the absence of plant competition for 7 days failed to allow nitrifiers to accumulate NO_3^- .

The soil N-oxide emissions from both forests were consistent with the expectations of the conceptual HIP model, which hypothesizes that the total N-oxide gas flux is proportional to the rates of nitrification and denitrification (first level of control) while the relative proportion of each gas is controlled by soil aeration status (second level of control; Firestone & Davidson, 1989). Differences in N-oxide emissions between forest soils are consistent with the hypothesized first level of control with larger emissions and larger net nitrification rates in the lowland compared with the montane forest (Table 3). Differences in $\text{N}_2\text{O}/\text{NO}$ ratios between forests are also consistent with the hypothesized second level of control with lower $\text{N}_2\text{O}/\text{NO}$ ratios and lower WFPS in the lowland compared with the montane mineral soil (Fig. 1a and b). Although WFPS in the montane organic layer stayed below 40%, which is favorable for NO production, WFPS in the montane mineral soil was $\geq 80\%$ (Fig. 1b), which is optimal for N_2O production (Bollmann & Conrad, 1998).

We bring net soil N-cycling rates and N-oxide losses together to evaluate the leakiness of soil N cycles. The average soil N-oxide emissions from the lowland forest (Table 4) were $\sim 1\%$ of the net N-cycling rates in the top soil (Table 3). This characterizes a rather conservative, or less leaky, soil N cycle than we hypothesized for this lowland site where stem diameter growth and annual fine litterfall were neither N nor P limited after 5 and 6 years, respectively (S.J. Wright, unpublished results; Kaspari *et al.*, 2008). Also, the average soil N-oxide emissions from our lowland forest site were at the low end for old-growth tropical lowland forests (Table 4). This further suggests that our lowland forest site has a less leaky soil N cycle than has been found for tropical lowland forests with strong P-limitation (Martinelli

et al., 1999; Hall & Matson, 2003; Davidson *et al.*, 2007). The average soil N-oxide emissions from our montane site, which were comparable with emissions reported from other old-growth montane forests (Table 5), were $\sim 0.4\%$ of the net N-cycling rates in the organic layer and top mineral soil (Table 3). This suggests a more conservative soil N cycle relative to the lowland forest, which supports our hypothesis for this montane forest where stem diameter growth and fine litterfall were N limited (M. Adamek, unpublished results). The soil N cycles of our forest sites thus differ in the efficiency of processing the available substrate for nitrification and in the degree of leakiness.

Response of soil N-oxide fluxes to N addition in the lowland forest

The small magnitude of soil N-oxide emissions following first-time N addition in the lowland forest is consistent with a relatively conservative soil N cycle. First-time N addition did not cause transitory increases in soil N-oxide emissions. Instead, there was a gradual and modest increase in soil N-oxide emissions following two subsequent N applications (Fig. 7). The strong dry season causes WFPS to drop below 40% (Fig. 1a). The first-time N addition was conducted during the transition between dry and wet season when WFPS was still between 45% and 50%. This is far below the optimum range for nitrification (Linn & Doran, 1984; Bollmann & Conrad, 1998) and might reduce nitrifying activity. Hence, water stress might also have contributed to the missing fertilization peaks of N-oxide emissions following the first-time N addition. Support for this explanation comes from the chronic N-addition plots where the fertilization peaks of N-oxide emissions at the beginning of the wet season although substantial were clearly smaller than subsequent N-oxide peaks later in the wet season (Fig. 4a and b).

The initially rather conservative soil N cycle became increasingly leaky with chronic N addition. In contrast to the first-time N addition, the 9- and 10-year N-addition plots had immediate and large transitory N-oxide emissions (Fig. 4a and b). Also, background soil N-oxide emissions (measured at least 6 weeks after N additions) were larger than controls (Fig. 4c and d; Table 6). N-oxide losses were $\sim 3.3\%$ of the net soil N-cycling rates in the chronic N-addition plots, which was also larger than controls (Tables 3 and 6). Additionally, background soil NO_3^- concentrations increased, and NO_3^- accumulated during the dry season when it reached larger concentrations than NH_4^+ (Fig. 2c and d). A dominance of NO_3^- relative to NH_4^+ suggests a leaky soil N cycle (Davidson *et al.*, 2000). The increase in

the background N_2O/NO ratio, which implies that the fluxes of the two gases are increasing unevenly, might reflect that a larger soil volume may contribute to the elevated N_2O emissions while due to its high reactivity the emitted NO should be produced within a smaller volume of the surface soil only. Supporting this assumption, N_2O concentrations in soil air are increasing down to at least 2 m depth which implies that although further reduction to N_2 will be occurring during upward diffusion, N_2O production is not restricted to the top soil (data not reported). There was a strong correlation between background soil N-oxide emissions and WFPS in the chronic N-addition but not in the control plots (Fig. 6c and d). As N availability increased due to N enrichment, an originally rather conservative soil N cycle, where N-oxide emissions were regulated by N availability (or the first level of control of the HIP model) was changed to an increasingly leaky soil N cycle where the second level of control (soil aeration status) caused pronounced seasonal changes in background soil N-oxide emissions.

Response of soil N-oxide fluxes to N addition in the montane forest

The magnitude of soil N-oxide emissions following first-time and chronic N additions in the montane forest tells us whether its response is regulated by the initial conservative soil N cycle or, as we hypothesized, by the presence of a substantial organic layer in which N mineralization and nitrification could increase substantially upon N addition. First-time N addition led to an immediate increase in soil N-oxide emissions (Figs. 5a, b and 7), and fertilization peaks of N-oxide emissions during the second-year N addition were similar to those from the chronic N-addition lowland plots. These results contrast with an N-limited Hawaiian montane forest, where first-time N addition did not cause increases in soil N-oxide emissions (Hall & Matson, 1999, 2003). Also, the background NO emissions were larger than controls and the background N_2O emissions remained elevated 2 months after the last N addition in November 2007 (Fig. 5c and d; Table 6).

We largely attribute the instantaneous and sustained increase in soil N-oxide emissions in the Panamanian montane forest to a substantial increase in net N-cycling rates in the organic layer in response to N addition. Net N mineralization and nitrification rates were two- and 15-fold increased, respectively, following the third N application during the first year of N addition (Table 3). The decrease in the background N_2O/NO ratio also suggests that the organic layer with its low WFPS (Fig. 1b) makes an important contribution to the soil N-oxide emissions in response to N addition. The soils in the

Hawaiian forest have a horizon of organic matter as well (Lohse & Matson, 2005), but the initial N-cycling rates (mineralization and nitrification) in the top 0.1 m of soil were small and not increased after 1 year of N addition (Hall & Matson, 2003). This differing response in soil N-cycling compared with our site and consequently the small response in N-oxide emissions might partly be related to the rapid drainage of these coarse-textured soils (i.e. >59% gravel in the mineral A horizon) causing immediate and substantial increases in N leaching losses (mainly NO_3^-) following N addition (Lohse & Matson, 2005). In our forest, background soil N-oxide emissions expressed as a percentage of soil N-cycling rates ($\sim 0.4\%$) were unchanged by N addition due to large offsetting increases in N-oxide emissions and N-cycling rates in the N-addition plots. Thus, this montane forest with N-limited primary production responded to N additions with a large increase in soil N cycling while the proportion of N-oxide emissions remained small as in the model of Aber *et al.* (1998). Whether the proportion of soil N-oxide emissions to N-cycling will increase progressively with further years of N addition remains to be seen. The presence of an organic layer where N cycling increased substantially with N addition (and not the initial degree of leakiness of the soil N cycle as in the lowland forest) was the main factor regulating the response of soil N-oxide emissions to N addition in this montane forest.

Factors influencing soil N-oxide emissions following anthropogenic N additions

The N status of the forest and its soil N-cycling properties allow us to deduce which factors govern soil N-oxide emission response to N addition. Biological demand for N (i.e. presence or absence of N limitation of vegetation growth) is clearly not the sole indicator of how N-oxide fluxes from tropical forest soils react to chronic N deposition. Our lowland forest, located on deeply weathered soil, is not N limited but displayed a rather conservative soil N cycle, which in combination with water stress caused a lack of fertilization peaks in N-oxide emissions following first-time N addition. Our montane forest, located on young volcanic soil, is N-limited and has a conservative soil N cycle, but the soil reacted with immediate and large N-oxide emissions following first N additions due to the large increase in nitrification activity in the organic layer. This contrasts with Hawaii, where the presence or absence of N limitation of tree growth reflected soil N-cycling characteristics across a soil chronosequence and predicted N-addition effects on soil N-oxide emissions (Hall & Matson, 1999, 2003). We therefore suggest that the status of the soil N cycle (conservative vs. leaky) as well as

absence/presence of an organic layer (where N-cycling rates may substantially increase) are important factors governing the response of soil N-oxide emissions from tropical forests following anthropogenic N input.

The status of the soil N cycle is likely to vary widely among lowland tropical forests (even though lowland tropical forests tend to have leakier N cycles than montane forests). Many tropical lowland forests occur on heavily weathered soils and have a relative excess of N; however, soil texture strongly affects N-cycling processes in heavily weathered soils across the Brazilian Amazon. Ultisols/Acrisols with a sandy loam or coarser texture tend to have more conservative soil N cycles while Oxisols/Ferralsols with a loamy or finer texture tend to have relatively leaky soil N cycles (Silver *et al.*, 2000; Sotta *et al.*, 2008). We expect that such variation in soil N cycles will influence N-oxide emissions in response to N addition.

On the other hand, many tropical montane forests occur on relatively young soils covered by thick and densely rooted organic layers (Edwards & Grubb, 1977), and their aboveground primary production is probably N limited (Tanner *et al.*, 1998). However, soil N-cycling rates can be large in the organic layer (Livingston *et al.*, 1988; Vitousek & Matson, 1988; Wilcke *et al.*, 2002). If the organic layer displays large nitrification rates (Wilcke *et al.*, 2002) or a large increase in nitrification with an increase in N availability (as was the case in our study), the soils will generally react to anthropogenic N addition with immediate increases in N-oxide emissions even if forest growth is N limited.

Consequences of chronic N deposition on soil N-oxide emissions from tropical lowland and montane forests

Substantial increases in the magnitude of soil N-oxide emissions can be expected from both lowland and montane tropical forests under chronic N deposition, independent of the transitory response to pulse N additions. Soil N-oxide emissions from N-limited montane forests will increase rapidly under elevated N deposition if these forests have an organic layer in which nitrification activity could substantially increase upon N addition. This contradicts the current assumption that N-limited tropical montane forests will respond to N additions with only small and delayed increases in soil N-oxide emissions. The relative contribution of N₂O to total N-oxide emissions from tropical forests on deeply weathered soils may increase. In tropical forests with a pronounced dry season, soil moisture might become the main factor regulating the type of N-oxide emitted from the soil surface as the ratio of N₂O/NO fluxes in the N-enriched lowland site strongly depended on soil water content. This suggests

that climate change scenarios, where rainfall quantity and seasonality change (IPCC, 2007), will also alter the relative importance of soil N₂O and NO emissions from tropical forests exposed to anthropogenic N addition.

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