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Impact of elevated N input on soil N cycling and losses in old-growth lowland and montane forests in Panama

Marife D. Corre,[1,3] Edzo Veldkamp,[1] Julia Arnold,[1] and S. Joseph Wright[2]

[1]*Buesgen Institute, Soil Science of Tropical and Subtropical Ecosystems, Georg-August University of Goettingen, Buesgenweg 2, Goettingen 37077 Germany*
[2]*Smithsonian Tropical Research Institute, Box 0843-03092, Balboa, Republic of Panama*

Abstract. Nitrogen deposition is projected to increase rapidly in tropical ecosystems, but changes in soil-N-cycling processes in tropical ecosystems under elevated N input are less well understood. We used N-addition experiments to achieve N-enriched conditions in mixed-species, lowland and montane forests in Panama. Our objectives were to (1) assess changes in soil mineral N production (gross rates of N mineralization and nitrification) and retention (microbial immobilization and rapid reactions to organic N) during 1- and 9-yr N additions in the lowland forest and during 1-yr N addition in the montane forest and (2) relate these changes to N leaching and N-oxide emissions.

In the old-growth lowland forest located on an Inceptisol, with high base saturation and net primary production not limited by N, there was no immediate effect of first-year N addition on gross rates of mineral-N production and N-oxide emissions. Changes in soil-N processes were only apparent in chronic (9 yr) N-addition plots: gross N mineralization and nitrification rates, NO_3^- leaching, and N-oxide emissions increased, while microbial biomass and NH_4^+ immobilization rates decreased compared to the control. Increased mineral-N production under chronic N addition was paralleled by increased substrate quality (e.g., reduced C:N ratios of litterfall), while the decrease in microbial biomass was possibly due to an increase in soil acidity. An increase in N losses was reflected in the increase in ^{15}N signatures of litterfall under chronic N addition.

In contrast, the old-growth montane forest located on an Andisol, with low base saturation and aboveground net primary production limited by N, reacted to first-year N addition with increases in gross rates of mineral-N production, microbial biomass, NO_3^- leaching, and N-oxide emissions compared to the control. The increased N-oxide emissions were attributed to increased nitrification activity in the organic layer, and the high NO_3^- availability combined with the high rainfall on this sandy loam soil facilitated the instantaneous increase in NO_3^- leaching. These results suggest that soil type, presence of an organic layer, changes in soil-N cycling, and hydrological properties are more important indicators than vegetation as an N sink on how tropical forests respond to elevated N input.

Key words: abiotic N immobilization; gross nitrification; gross N mineralization; litterfall ^{15}N signature; long-term nutrient manipulation experiment; montane forest organic horizon; nitrogen deposition; N leaching; NH_4^+ immobilization; NO_3^- immobilization; N-oxide emissions; tropical lowland forest.

Introduction

Nitrogen deposition in the tropics is projected to increase rapidly in the next decades due to continued increases in N fertilizer use, cultivation of N-fixing plants, fossil fuel consumption, industrial use of N (Galloway et al. 1994, 2008), and biomass burning (Crutzen and Andreae 1990, Cochrane 2003). In the past three decades, studies on the impact of high N deposition on ecosystem function have been focused on temperate ecosystems. Temperate forest soils under long-term N addition (e.g., Venterea et al. 2004) or extremely high N deposition (Corre et al. 2007) show that microbial immobilization of mineral N is lower than or does not keep pace with the increases in gross N mineralization and nitrification rates, resulting in increased NO (important in tropospheric ozone and acid rain formation) and N_2O emissions (a potent greenhouse gas) and NO_3^- leaching. From the studies conducted in old-growth forests on tropical soils, Hall and Matson (1999, 2003) reported that a forest on an Inceptisol, with N-limited net primary production and small gross N mineralization rate, does not exhibit an increase in N-oxide emission after first-time N addition but only after chronic N addition. In contrast, a forest on an Oxisol, with net primary production not limited by N and large rates of gross N mineralization and nitrification, shows rapid and larger increases in N-oxide emissions than in a N-limited forest after both first-time and chronic N additions. These studies in Hawaii and in

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[3] E-mail: mcorre@gwdg.de

temperate forests shows that changes in soil mineral N production and retention processes with increased N input clearly influence N losses, and that the timing and magnitude of losses depend on the N status (i.e., low or high N availability) of the ecosystem.

In old-growth tropical forests, some of the factors influencing N status are altitude (lowland vs. montane), soil age or degree of development, substrate type, temperature, rainfall, soil texture, and presence of an organic layer. Lowland forests show larger N contents in leaf and litterfall (Tanner et al. 1998), larger NO_3^- leaching losses (Hedin et al. 2003, Klinge et al. 2004, Dechert et al. 2005, Schwendenmann and Veldkamp 2005), larger N-oxide emissions (Keller and Reiners 1994, Davidson et al. 2000, Purbopuspito et al. 2006, Koehler et al. 2009*a*), and larger ^{15}N signatures in leaves and soils (Martinelli et al. 1999) than montane forests. These observations support the speculation that N is in relative excess in old-growth lowland forests. Gross rates of soil-N cycling increase across an increasing soil-age gradient in Hawaiian montane forests (Hall and Matson 2003) and decrease across an increasing elevation gradient in Ecuadorian forests, which depicts a decreasing degree of soil development and decreasing temperatures (Arnold et al. 2009). N-oxide emissions also decrease across a toposequence in Malaysian forests and are larger from sedimentary-derived soils than from ultrabasic-derived soils (Hall et al. 2004). These studies support that soil age or development, substrate type, and temperature influence soil-N status of tropical forests. Net primary production and N availability also decrease across a Hawaiian precipitation gradient on soils of similar elevation and age (Schuur and Matson 2001). The increased intensity and duration of anaerobic conditions with increased rainfall could slow decomposition rates and decrease N mineralization. High rainfall can also increase soil leaching rates (Radulovich and Sollins 1991), and persistent N losses that are not subject to direct biotic retention (e.g., humic complexes of dissolved organic N) can sustain N limitation of an ecosystem (Hedin et al. 1995). Soil texture also influences soil N availability and NO_3^--leaching response to N addition. Clay Oxisol soils have larger rates of soil-N transformation than sandy Oxisols in Brazilian Amazon forests (Silver et al. 2000, Sotta et al. 2008). In Hawaiian montane forests, coarse-textured young Andisol reacts to N addition with immediate NO_3^- losses while the fine-textured old Oxisol shows a delayed reaction (Lohse and Matson 2005). Finally, many tropical montane forest soils have an organic layer in which roots proliferate (Edwards and Grubb 1977, Grieve et al. 1990, Purbopuspito et al. 2006, Leuschner et al. 2007), and both organic layer (Wilcke et al. 2002) and root turnover (Röderstein et al. 2005) are suggested as important nutrient sources and sinks for these forests.

Our present study reports the impact of elevated N input on soil mineral N production and retention processes on two mixed-species, old-growth tropical forests: a lowland forest on an Inceptisol and a montane forest on an Andisol. We had the following hypotheses.

1) The lowland site, where stem diameter growth, leaf litterfall, and fine-root mass were not N limited (Appendix A), should exhibit large soil-N cycling rates exemplifying a system with high N availability. First-year N addition may not immediately change the soil-N cycling rates in a system with ample N availability, but the added N will increase N losses. Long-term N addition may increase soil mineral N production by improving quality of plant-derived substrates, decrease N immobilization by decreasing microbial biomass, and further increase N losses.

2) The montane site, where stem diameter growth and leaf litterfall were N limited (Appendix A), should exhibit small soil-N cycling rates typifying a system with low N availability. However, a substantial organic layer covers the mineral soil, and first-year N addition may immediately increase soil-N cycling rates by enhancing microbial activity in the organic layer, and thus may instantaneously increase N losses.

We tested these hypotheses by measuring gross rates of soil-N cycling and losses during 1- and 9-yr N additions in the lowland forest and during a 1-yr N addition in the montane forest. Our objectives were (1) to assess the changes in soil mineral N production (gross rates of N mineralization and nitrification) and retention (microbial immobilization and by rapid reactions to organic N) and (2) to relate these changes to N leaching at 1.5-m depth and N-oxide emissions.

Methods

Site description, experimental design, and soil characteristics

The lowland site (between 25 and 61 m elevation and ~7% mean slope) is an old-growth (>300 years) semi-deciduous forest and is located on Gigante Peninsula (9°06′ N, 79°50′ W), which is part of the Barro Colorado Nature Monument, Republic of Panama (see Plate 1). At the nearby Barro Colorado Island (BCI; 5 km from the study site), annual rainfall from 1995 to 2007 was 2650 ± 146 mm (mean ± SE for all data presented), with a pronounced dry season from January to mid-May when 11% of the annual rainfall occurred, and annual air temperature is 27.4° ± 0.1°C. Total N deposition from rainfall was 9 kg $N{\cdot}ha^{-1}{\cdot}yr^{-1}$, measured every other week in 2006–2007. Rain from an open area was sampled over a 2–3 day period using rain collectors (acid-washed, dark bottles encased in PVC tubes and covered with lids to which funnels were attached) that were installed 3 m above the surface at the shore of Gigante Peninsula near the study site (see *Methods: Gross N mineralization, gross nitrification, and NH_4^+ and NO_3^- consumption rates*). For trees ≥ 10 cm in diameter at breast height (dbh), *Alseis blackiana*, *Dialium guianense*, *Heisteria concinna*, and *Tetragastris panamensis* constitute 19% while *Leguminosae* make up 15%

of the 384 trees/ha in the study site. Stem-diameter growth, leaf litterfall, and fine-root mass were not affected by 3, 9, and 11 years of N addition, respectively (Appendix A; Kaspari et al. 2008; S. J. Wright, *unpublished data*).

The montane site (between 1200 and 1300 m elevation and ~15% mean slope) is an old-growth lower montane forest and is located in Quebrada Honda area (8°45′ N, 82°15′ W), which is part of the Fortuna Forest Reserve, Chiriquí province, Republic of Panama (see Plate 1). Annual rainfall from 1997 to 2007 was 5532 ± 322 mm (mean ± SE for all data shown; no month with mean monthly rainfall <200 mm) and mean annual air temperature was 20.0° ± 0.1°C. Total N deposition from rainfall was 5 kg $N \cdot ha^{-1} \cdot yr^{-1}$, measured biweekly from 2006 to 2007 at a clearing near the study site using the same methods. For trees ≥ 10 cm dbh, *Oreomunnea mexicana*, *Eschweilera panamensis*, *Vochysia guatemalensis*, and *Cassipourea elliptica* constitute 38% while *Leguminosae* represents 4% of the 1039 trees/ha in the study site. Stem diameter growth and leaf litterfall increased while fine-root biomass and production were unaffected by 1–2 yr N addition (Appendix A; Adamek 2009, Adamek et al. 2009).

In the lowland site, our study was conducted on the only ongoing long-term nutrient manipulation experiment in old-growth tropical forest. The experiment includes control and N-addition plots laid out across a 26.6-ha area in a stratified random design with four replicates. N addition started in June 1998. Just outside these long-term manipulation plots, we established four additional plots in 2006 to represent the first-year N-addition treatment. In the montane site, the experiment was set up in a paired-plots design with four replicates. Control and N-addition treatments were randomly assigned to each pair of plots. N addition started in February 2006. At both sites, each treatment plot was 40 × 40 m, and plots were separated by at least 40 m. The N-addition plots received 125 kg urea-$N \cdot ha^{-1} \cdot yr^{-1}$ split in four equal applications.

Our experimental approach simulates N-enriched conditions through large doses of fertilizer additions, exposing the existing microbial community to abrupt environmental changes as opposed to gradual changes that may arise from increases in atmospheric N depositions to natural ecosystems. However, similar trends in changes of soil-N cycling and losses are observed across a gradient of increasing atmospheric-N depositions (Corre et al. 2007) and N-fertilization experiments (e.g., Hall and Matson 1999, 2003, Venterea et al. 2004). Thus, the main difference between N deposition and N fertilization is the timing of changes but not the direction of changes.

Soil characteristics were determined in January 2006, and all analyses were conducted at the Soil Science of Tropical and Subtropical Ecosystems (SSTSE, Göttingen, Germany); part of the data (Table 1) was reported by Koehler et al. (2009*a*). Leaf-litter layer (Oi) was randomly sampled in the inner 20 × 20 m area of each plot. Organic layer (Oe + Oa; only present in the montane site) and mineral soil (0–5, 5–10, 10–25, and 25–50 cm depths) were sampled from one profile per plot. Air-dried and ground samples were analyzed for total C and N by a CNS Elemental Analyzer (CN Elementar Analyzer Vario EL, Hanau, Germany), natural abundance ^{15}N by isotope ratio mass spectrometry (IRMS; Delta Plus, Finigan MAT, Bremen, Germany), and total P by pressure digestion in concentrated HNO_3 followed by analysis of the digests using inductively coupled plasma-atomic emission spectrometer (ICP-AES; Spectroflame, Spectro Analytical Instruments, Kleve, Germany). Effective cation exchange capacity (ECEC) of the mineral soil was determined from air-dried and 2-mm sieved samples by percolating with unbuffered 1 mol/L NH_4Cl and measuring cations in percolates using ICP-AES. Base and Al saturation were calculated as percentage exchangeable base cations and Al of the ECEC, respectively. Soil pH was measured from a saturated paste mixture (1:1 and 1:10 ratio of soil to H_2O for mineral soil and organic layer, respectively). Soil bulk density was determined by the soil core method.

Gross N mineralization, gross nitrification, and NH_4^+ and NO_3^- consumption rates

We measured gross rates of soil-N cycling in the lowland site at the beginning of the dry season (mid-January 2006, only from the control and 9-yr N-addition plots) and in the middle of the wet season (1 September 2006, including the 1-yr N-addition plots). No N was applied during the dry season, and the wet-season sampling was carried out 3 weeks after the third N application of the year. In the montane site, measurements were conducted on 5 September 2006, which was 6 weeks after the third N application of the first-year N addition. These sampling periods avoided the transitory effects of pulse N application than showed peak of soil mineral N concentrations within 2 weeks after an N application (Koehler et al. 2009*a*). In the inner 10 × 10 m area of each plot, five intact soil cores were taken within a 0.06-m^2 area using stainless steel cores of 5 cm height and 8 cm diameter. Soil cores were taken after removing the thin leaf-litter layer, which included mainly the mineral soil for the lowland site. In the montane site, five soil cores were taken separately from the organic layer (Oe + Oa) and from the 0–5 cm mineral soil.

We conducted the soil N-cycling measurements in situ, including ^{15}N injection, incubation, and mineral-N extraction. We used the ^{15}N-pool dilution technique as described in detail by Davidson et al. (1991) and Hart et al. (1994). Of the four soil cores at each plot and sampling depth, two were injected with $(^{15}NH_4)_2SO_4$ solution (for gross N mineralization and NH_4^+ consumption rates), while the other two were injected with $K^{15}NO_3$ solution (for gross nitrification and NO_3^-

TABLE 1. Soil characteristics determined in January 2006, at lowland control, after eight years of N addition in the lowland site on the Gigante Peninsula (Barro Colorado Nature Monument, Panama), and prior to first N addition in the montane site in the Quebrada Honda area (Fortuna Forest Reserve, Panama).

Characteristic	Lowland		Montane†
	Control	After 8-yr N addition	
Parent material	basalt flow	basalt flow	volcanic ash
Soil texture	heavy clay	heavy clay	sandy loam
Decomposing leaf litter (Oi)‡			
Total C (g C/kg)	451 (10)	454 (5)	489 (3)
Total N (g N/kg)	12.4 (0.7)	12.4 (0.5)	12.4 (0.5)
C:N ratio	36.6 (1.9)	36.6 (1.4)	40.2 (1.9)
$\delta^{15}N$ (‰)	0.05 (0.25)	0.90 (0.35)	−1.65 (0.22)
Organic layer (Oe + Oa)	absent	absent	8 cm (median depth)
Bulk density (g/cm^3)			0.07 (0.01)
pH (1:10 H_2O)			4.1 (0.1)
Total C (g C/kg)			443 (19)
Total N (g N/kg)			22.4 (1.1)
C:N ratio			19.9 (0.4)
$\delta^{15}N$ (‰)			0.92 (0.15)
Total P (g P/kg)			0.59 (0.03)
0–5 cm mineral soil			
Bulk density (g/cm^3)	0.62 (0.02)	0.62 (0.02)	0.51 (0.06)
pH (1:1 H_2O)	5.3^a (0.2)	4.5^b (0.1)	4.1 (0.2)
Total C (g C/kg)	51 (5)	47 (4)	73 (8)
Total N (g N/kg)	3.8 (0.4)	3.6 (0.2)	5.0 (0.6)
C:N ratio	13.3 (0.5)	13.3 (0.4)	14.5 (0.5)
$\delta^{15}N$ (‰)	4.86 (0.52)	5.56 (0.17)	3.81 (0.42)
Total P (g P/kg)	0.55 (0.08)	0.50 (0.02)	0.56 (0.05)
Effective CEC (mmol$_c$/kg)	205 (44)	116 (8)	132 (25)
Base saturation (%)	92^a (4)	62^b (9)	21 (4)
Al saturation (%)‡	3^b (3)	27^a (9)	72 (6)
5–50 cm mineral soil			
pH (1:1 H_2O)	5.1^a (0.1)	4.9^b (0.1)	4.6 (0.1)
Total C (g C/kg)	15 (1)	15 (1)	31 (5)
Total N (g N/kg)	1.4 (0.0)	1.3 (0.1)	1.8 (0.2)
C:N ratio	10.2 (0.6)	11.0 (0.1)	16.5 (0.6)
$\delta^{15}N$ (‰)	7.33 (0.79)	8.30 (0.38)	5.95 (0.23)
Total P (g P/kg)	0.40 (0.07)	0.36 (0.02)	0.29 (0.04)
Effective CEC (mmol$_c$/kg)	149 (48)	110 (22)	71 (18)
Base saturation (%)	56^a (7)	41^b (5)	11 (4)
Al saturation (%)‡	29^b (9)	53^a (7)	86 (4)

Notes: Values are means, with SE in parentheses. For the lowland site ($n = 4$ plots), different lowercase superscript letters indicate significant differences between treatments (independent t test at $P \leq 0.05$). CEC is cation exchange capacity. The lowland site soils are classified by FAO as Endogleyic Cambisol to Acric Nitisol (lower to upper landscape positions); soil of the montane site is Aluandic Andisol; lowland soils are classified as Dystrudepts by USDA; the montane soil is Hapludands.

† For the montane site ($n = 4$ plots), characteristics did not differ between plots, which were later randomly assigned as control and N addition.

‡ These soil data were not reported in the earlier publication where all other soil characteristics were reported (Koehler et al. 2009*a*).

consumption rates). Each soil core received five 1-mL injections containing 25 μg N/mL with 95% ^{15}N enrichment. This was equivalent to the rates of 1.1 ± 0.1 μg N/g for the mineral soils of the lowland and montane sites and 6.2 ± 0.5 μg N/g for the organic layer of the montane site. The injected NH_4^+ was on average 5% (mineral soil of the lowland site) and 7–9% (organic layer and mineral soil of the montane site) of the initial soil NH_4^+ concentrations. The injected NO_3^- was 17% (9-yr N-addition plots of the lowland site), 69% (control and 1-yr N-addition plots of the lowland site), and 85–96% (organic layer and mineral soil of the montane site) of the initial soil NO_3^- concentrations. One soil core of each labeled pair was broken up, mixed well in a plastic bag, and subsampled for 0.5 mol/L K_2SO_4 extraction 10 minutes after ^{15}N injection (T_0 cores). Prepared extraction bottles containing 150 mL K_2SO_4 solution were brought in the field and soil samples were added to get an approximate solution to fresh soil ratio of 3:1. Based on the measured gravimetric moisture content of each soil core (see next paragraph), the mean K_2SO_4 volume to dry soil mass ratios was 4:1 for the mineral soils of the lowland and montane sites and 20:1 for the organic layer of the montane site. The T_0 cores were used to correct

for the reactions that occur immediately after addition of $^{15}NH_4^+$ and $^{15}NO_3^-$. The other soil core of the labeled pair was put in a plastic bag, inserted back into the soil to incubate for 1 day (T_1 cores), and extracted with K_2SO_4.

The soil-K_2SO_4 bottles and the rest of the soil from the cores were brought to the laboratory within 6 hours, where extraction was continued by shaking the soil-K_2SO_4 bottles for 1 hour and filtering them through K_2SO_4-prewashed filter papers (4 μm nominal pore size). Extracts were frozen immediately. Gravimetric moisture content was measured from each soil sample by oven drying at 105°C for 1 day. The remaining soil of the T_0 cores was frozen upon arrival in the laboratory until analysis for total ^{15}N recovery (described later in this section).

For the lowland site, we also measured microbial immobilization of NH_4^+ by $CHCl_3$ fumigation upon arrival in the laboratory. Approximately 25 g of the T_1 $^{15}NH_4^+$-labeled soils were fumigated with $CHCl_3$ for 5 days, extracted with 0.5 mol/L K_2SO_4 (~5:1 solution to dry soil mass ratio), and the extracts were frozen immediately. NH_4^+ immobilization rates were calculated using the nonlinear model described by Davidson et al. (1991). For the montane site, we were unable to conduct immediate $CHCl_3$ fumigation because we did not have the equipment in our field station at the time of sampling.

All extracts and T_0 soil samples were transported frozen and analyzed at SSTSE. NH_4^+ and NO_3^- contents of the extracts were analyzed using continuous-flow injection colorimetry (CFIC; Skalar, Cenco Instruments, Breda, The Netherlands), in which NH_4^+ was determined by Berthelot reaction method (Skalar Method 155-000) and NO_3^- by copper-cadmium reduction method (Skalar Method 461-000). The organic-N content of the extracts was determined by persulfate digestion (Corre et al. 2007), followed by colorimetric analysis of NO_3^-. Extractable organic N is the difference between persulfate-N and $NH_4^+ + NO_3^-$ concentrations. For ^{15}N analysis of the extracts, the same diffusion procedures and blank correction were followed as described previously (Corre et al. 2003, 2007, Corre and Lamersdorf 2004), and ^{15}N was analyzed using IRMS.

Fate of added ^{15}N 10 minutes, T_0, after its injection to intact soil cores

We identified the fates of added ^{15}N at T_0 by measuring ^{15}N recoveries in K_2SO_4-extractable N (NH_4^+, NO_3^-, and organic N) and non-extractable (hereafter insoluble) organic N pools. Part of the T_0 extracts was used for serial diffusion of NH_4^+ and NO_3^- and part for persulfate digestion with subsequent diffusion for ^{15}N analysis; ^{15}N enrichment in the extractable organic N pool was calculated based on an isotope mixing equation. Calculation of ^{15}N recoveries in different N pools follows that of Hart et al. (1994), requiring the natural abundance ^{15}N of different N pools, which were measured from separate soil cores (see *Methods: Statistical analyses*). ^{15}N recovery in the insoluble-organic-N pool was calculated as the difference between ^{15}N recoveries in total-N pool (analyzed from freeze-dried T_0 soil samples) and in K_2SO_4-extractable N pools.

Previous reports of evidence of fast NO_3^- reaction to extractable organic N was disputed by Colman et al. (2007) to be due to analytical artifact, caused by interference of soluble iron in NO_3^- quantification that resulted in underestimation of NO_3^- and consequently overestimation of organic N. We addressed this concern in three ways. First, we compared our standard CFIC method with NH_4Cl buffer (but without ethylenediamine tetraacetic acid) against CFIC with imidazole buffer (claimed to be unaffected by iron interference; Colman et al. 2007) for NO_3^- determination of our soil extracts and of standard (0.5 mol/L K_2SO_4) solutions containing 4.9 mg NO_3-N/L with increasing Fe^{2+} levels (Fe is dissolved in a 2% HCl solution to maintain the soluble Fe(II) oxidation state): 0, 5, 10, 25, and 30 mg Fe/L. Second, we measured iron concentrations in our soil extracts using ICP-AES to determine if they were high enough to be worrisome. Third, we added a known amount of NO_3^- (1 mg N in 20 μL water) to our soil extracts to determine if the native iron concentrations of the extracts cause analytical error.

Other supporting parameters

From the fifth soil core, initial levels of extractable N and natural abundance ^{15}N of different N pools were determined by immediately extracting the soil in the field with 0.5 mol/L K_2SO_4. Microbial biomass C and N were also determined from the same soil core 6 hours after sampling from the lowland site and 2 days after sampling from the montane site, during which time the samples were stored at 4°C. We conducted an ancillary test to compare microbial biomass in the montane site measured after 6 hours and after 2-day cold storage of the same soil samples. Our results showed no significant difference between these measurement periods. We used the $CHCl_3$ fumigation-extraction method. Organic C in the extracts was analyzed by UV-enhanced persulfate oxidation using a Dohrmann DC-80 Carbon Analyzer with an infrared detector (Rosemount Analytical Division, Irvine, California, USA) and organic N by persulfate digestion. Microbial biomass C and N are calculated as the difference in extractable organic C and persulfate-N between the fumigated and unfumigated soils divided by $k_C = 0.45$ and $k_N = 0.68$ for 5-day fumigated samples (Brookes et al. 1985), where k is the conversion constant.

In the lowland site, we expected that the long-term impact of N addition on soil-N-cycling rates and losses are reflected in the natural abundance ^{15}N of fresh leaves, fine litterfall, and soil. Foliar ^{15}N was measured from the already-mentioned four most common tree species (*Methods: Site description, experimental design,*

TABLE 2. Gross rates of soil-N transformations (mg $N \cdot m^{-2} \cdot d^{-1}$) in the lowland and montane forests exposed to elevated N input, measured in the dry (January 2006) and wet (September 2006) seasons.

Site, sampling depth, and treatment	N mineralization		NH_4^+ immobilization§		NH_4^+ consumption	
	Dry	Wet	Dry	Wet	Dry	Wet
Lowland, 0–5 cm mineral soil						
Control	1108[b] (47)	894[b] (58)	837[a] (127)	819 (67)	1020[b] (59)	962 (178)
1-yr N addition†		1028[ab] (172)		950 (211)		1051 (216)
9-yr N addition	1437[a] (102)	1158[a] (99)	541[b] (86)	682 (40)	1361[a] (112)	1195 (125)
Montane, organic layer (Oe + Oa)‡						
Control		532[b] (115)				658 (186)
1-yr N addition		817[a] (106)				606 (195)
Montane, 0–5 cm mineral soil						
Control		570 (84)				571 (68)
1-yr N addition		726 (139)				750 (167)

Notes: For each site and sampling depth, values are means with SE in parentheses ($n = 4$ plots). Different lowercase superscript letters indicate significant differences among treatments (lowland dry season and montane, Mann-Whitney U test at $P \leq 0.05$; lowland wet season, Kruskal-Wallis H test with multiple comparison extension at $P \leq 0.05$).

† The lowland 1-yr N-fertilized plots were established only at the beginning of the wet season (May 2006) when N addition began.

‡ The montane site has no dry season, and the wet-season measurements were conducted in the same week as the lowland site.

§ NH_4^+ immobilization in the lowland site was measured by 5-d $CHCl_3$ fumigation-extraction method (Davidson et al. 1991). This was not conducted for the montane site because we did not have the equipment in our field station at the time of sampling.

and soil characteristics); sunlit leaves were shot from the very top of the trees (≥10-cm dbh) in December 2007 and March 2008 (after 10-yr N addition). Fine litterfall ^{15}N was measured from samples collected in September of each year from 1-yr (1998) to 10-yr (2007) N addition.

Dissolved organic C (DOC) and N (DON) in soil solution and N-oxide fluxes were measured intensively throughout 2006 at both sites; an exception to this was the 1-yr N-addition treatment in the lowland site, where N-oxide emissions were measured only until July 2006, and leaching losses were not quantified. For soil solution sampling, suction cup lysimeters (P80 ceramic, maximum pore size 1 μm; CeramTec AG, Marktredwitz, Germany) were installed at 1.5 m depth 4 months prior to the first sampling. Three lysimeters spaced 2 m apart were installed within the inner 20 × 20 m area of each plot. Soil solution was sampled every other week and collected over a 2–3 day period by applying 40 kPa vacuum to acid-washed, dark glass bottles. Samples from three lysimeters were pooled for each plot, filtered through pre-washed filter papers within 6 hours of field collection, frozen, and transported to SSTSE, where analyses were conducted. These samples were analyzed for NH_4^+, NO_3^- (as described earlier in this section), dissolved total N (UV-persulfate oxidation followed by hydrazine sulfate reduction; Skalar Method 473-000), and DOC (Pt-catalyzed high-temperature combustion followed by infrared detection of CO_2; Shimadzu TOC-5050, Duisburg, Germany). For N-oxide-flux measurement, four permanent chamber bases were installed in each plot in a stratified random design along two perpendicular 20 m long transects that cross the plot's central point. N_2O fluxes were measured using vented static chambers (area 0.04 m^2, height 0.25 m). Four gas samples (100 mL each) were removed at 2, 12, 22, and 32 minutes after chamber closure, stored in pre-evacuated glass containers, and analyzed using a gas chromatograph (Shimadzu GC-14B, Columbia, Maryland, USA) equipped with an electron capture detector and an autosampler. Nitric oxide fluxes were measured on-site using open dynamic chambers (area 0.04 m^2, height 0.25 m) during 5–7 minutes of closure. Nitric oxide was analyzed with a Scintrex LMA-3 chemiluminescence detector (Scintrex Unisearch, Ontario, Canada) after oxidation to NO_2 by a CrO_3 catalyst. Details on flow rates, maintenance of low relative humidity of air samples, on-site calibration, standard gases, and flux calculation are described by Koehler et al. (2009*a*). Only fluxes measured at least 6 weeks after a N application were reported here to exclude the transitory, fertilizer-induced peaks of N-oxide fluxes.

Statistical analyses

We expressed soil-N-transformation rates on an area basis because of the differences in soil bulk densities between the lowland and montane sites (Table 1). For each parameter, we conducted tests for normality (Kolmogorov-Smirnov D statistic) and equality of variance (Levene statistic; Sokal and Rohlf 1981). Nonparametric tests were used for parameters that showed non-normal distribution and heterogeneous variance: Mann-Whitney U test for assessing differences between sites (lowland and montane control plots) or between two treatments in the montane site, and Kruskal-Wallis H test with multiple comparison extension for testing differences among three treatments in the lowland site. Parameters that showed normal distribution and homogenous variance were tested using independent t test (two treatments) or one-way analysis of variance with Tukey hsd test (three treatments). For

TABLE 2. Extended.

Nitrification		NO_3^- consumption	
Dry	Wet	Dry	Wet
7 (4)	26[b] (1)	3 (3)	53[b] (22)
	29[b] (20)		9[b] (7)
95 (64)	223[a] (129)	64 (37)	257[a] (177)
	39[b] (7)		50 (20)
	70[a] (9)		57 (17)
	2 (2)		4 (4)
	9 (8)		3 (3)

time-series data (i.e., yearly analyses of natural abundance ^{15}N, N concentrations and C:N ratios of fine litterfall, and repeated measurements of dissolved C and N and N-oxide emissions), we used linear mixed-effects models (Crawley 2002) in which treatment (if testing for effects of N addition) or site (if testing for differences between lowland and montane control plots) is considered as fixed effect and the spatial replication (experimental plots) nested in time (temporal sampling scheme) as a random effect. Details are described in our earlier study (Koehler et al. 2009*a*). In short, the model includes (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 the correlation between measurements decreases with increasing time difference. Pearson's and Spearman's rank correlation tests were carried for parameters that showed normal and non-normal distributions, respectively.

RESULTS

Lowland vs. montane control plots

The Inceptisol soil in the lowland site had higher pH ($P = 0.01$), base saturation ($P = 0.00$), lower C:N ratio ($P = 0.03$), and Al saturation ($P = 0.00$) in the 0–5 cm and 5–50 cm mineral soil than the Andisol soil in the montane site (Table 1). Gross N-mineralization rates in the lowland mineral soil were higher than in the montane organic layer and mineral soil ($P = 0.05$; Table 2). Gross nitrification rates were comparable in the lowland mineral soil and montane organic layer, while these rates were higher than in the montane mineral soil ($P = 0.03$; Table 2). Microbial biomass C and N in the lowland mineral soil were higher than in the montane organic layer, while the montane mineral soil showed intermediate levels ($P = 0.00$; Table 3). Microbial C:N ratios were comparable in the lowland and montane mineral soils, while these ratios were smaller than in the montane organic layer ($P = 0.02$; Table 3). We expressed N-mineralization activity per unit microbial biomass (i.e., specific gross N-mineralization rate = gross N-mineralization rate ÷ microbial N; mg N·g microbial $N^{-1}\cdot d^{-1}$) to account for the differences in microbial biomass size and to get an index of the influence of substrate quantity and quality. Specific N-mineralization rates in the lowland mineral soil were smaller than in the montane organic layer ($P = 0.02$; Fig. 1). N-oxide emissions ($P = 0.05$; Table 4) and ^{15}N signatures of the litter layer ($P = 0.00$) and mineral soil ($P = 0.05$; Table 1) were larger in the lowland than in the montane site.

N addition to the lowland and montane sites

In the lowland site, 1-yr N addition did not affect gross N-cycling rates and microbial biomass. Chronic (9-yr) N addition, however, increased gross N-mineralization and nitrification rates and decreased NH_4^+ immobilization rates (i.e., dry season), microbial biomass, and microbial C:N ratio compared to the control (Tables 2 and 3). Specific gross N-mineralization rates also markedly increased under chronic N addition compared to the control (Fig. 1). In the chronic N-addition plots, specific NH_4^+ immobilization rates (i.e., NH_4^+ immobilization rates ÷ microbial N; 139 (±21) and 100 (±4) (±SE) mg N·g microbial $N^{-1}\cdot d^{-1}$ in the

TABLE 3. Soil microbial biomass measured in the wet season (September 2006).

Site, sampling depth, treatment	Microbial biomass C (g C/m^2)	Microbial biomass N (g N/m^2)	Microbial C:N ratio
Lowland, 0–5 cm mineral soil			
Control	61.45[a] (7.16)	9.66[a] (1.14)	6.4[a] (0.2)
1-yr N addition	56.32[a] (8.46)	8.89[a] (1.74)	6.5[a] (0.3)
9-yr N addition	33.39[b] (5.20)	6.85[b] (0.62)	4.9[b] (0.6)
Montane, organic layer (Oe + Oa)			
Control	14.30[b] (2.50)	1.42[b] (0.06)	11.6 (2.5)
1-yr N addition	24.57[a] (1.57)	2.74[a] (0.17)	9.1 (0.7)
Montane, 0–5 cm mineral soil			
Control	24.03 (4.25)	4.11 (0.64)	5.8 (0.4)
1-yr N addition	25.16 (3.94)	3.89 (0.53)	6.4 (0.2)

Note: For each site and sampling depth, values are means with SE in parentheses ($n = 4$ plots). Different lowercase superscript letters indicate significant differences among treatments (montane, independent t test at $P \leq 0.05$; lowland, one-way ANOVA with Tukey hsd test at $P \leq 0.05$).

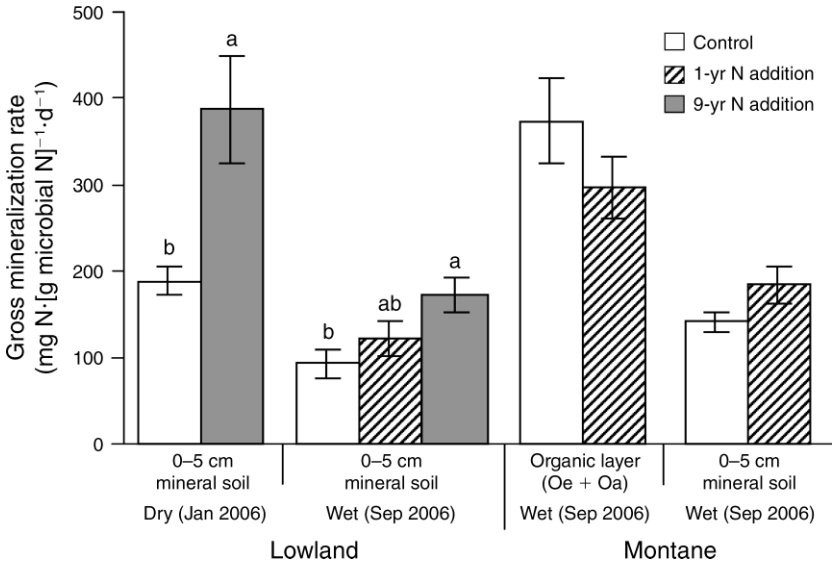

FIG. 1. Specific gross N mineralization rates (i.e., gross N mineralization rate ÷ microbial N) in the lowland site on the Gigante Peninsula (Barro Colorado National Monument) and in the montane site in the Quebrada Honda (Fortuna Forest Reserve), Panama. For the lowland site, means (±SE; $n = 4$ plots) with different letters indicate significant differences between treatments in the dry season (Mann-Whitney U test at $P \leq 0.05$) and among treatments in the wet season (Kruskal-Wallis H test with multiple comparison extension at $P \leq 0.05$). No treatment effect was detected in the montane site. Measurement was conducted in the first and ninth year of chronic N additions. See *Methods: Site description, experimental design, and soil characteristics* for a detailed description.

dry and wet seasons, respectively) were lower than specific N-mineralization rates (paired-sample t test at $P = 0.03$). Based on the control and 9-yr N-addition plots in both sampling seasons, NH_4^+ immobilization rates were correlated with microbial C ($R = 0.64$, $P = 0.01$, $n = 16$ plots) and microbial N ($R = 0.63$, $P = 0.01$, $n = 16$ plots). N-oxide emissions, NO_3^- concentrations in soil solution at 1.5 m depth (Table 4), litter layer ^{15}N ($P = 0.09$; Table 1), foliar ^{15}N of at least one tree species (Fig. 2A), fine-litterfall ^{15}N (Fig. 2B), and fine-litterfall-N concentration (Fig. 2C) increased while C:N ratios of fine litterfall (Fig. 2C) and of soil solution at 1.5 m depth (Table 4) decreased in chronic N addition plots compared to the control. Dissolved organic carbon (DOC) concentrations in soil solution at 1.5 m depth (Table 4), soil pH, and base saturation (Table 1) decreased while Al saturation (Table 1) increased in the chronic N-addition plots compared to the control.

In the montane site, 1-yr N addition increased gross N-mineralization rates, gross nitrification rates, and microbial biomass mainly in the organic layer compared to the control (Tables 2 and 3). Specific N-mineralization rates in 1-yr N-addition plots did not differ from the control (Fig. 1). N-oxide emissions and NO_3^- concentrations in soil solution at 1.5 m depth (Table 4) increased in the 1-yr N-addition plots compared to the control.

^{15}N recoveries 10 minutes, T_0, after ^{15}N injection to intact soil cores

In the lowland site, recoveries of injected $^{15}NH_4^+$ and $^{15}NO_3^-$ in different N pools did not differ between dry and wet seasons, and here we report the results from the

TABLE 4. Dissolved N and C concentrations in soil solution at 1.5-m depth sampled every other week during the rainy season of 2006 (mid-May to December) and N-oxide fluxes measured from January to December 2006.

Site, treatment	NO_3^- (mg N/L)	DON (mg N/L)	DOC (mg C/L)	DOC:DON ratio	NO flux (μg N·m^{-2}·d^{-1})†	N_2O flux (μg N·m^{-2}·d^{-1})†
Lowland, control	0.01^b (0.01)	0.15 (0.03)	6.55^a (0.43)	38.11^a (5.14)	70^b (7)	448^b (26)
Lowland, 9-yr N addition	0.93^a (0.14)	0.17 (0.02)	4.66^b (0.28)	4.50^b (0.24)	196^a (24)	1498^a (213)
Montane, control	0.03^b (0.01)	0.18 (0.05)	5.47 (0.32)	29.73^a (3.57)	28^b (4)	326^b (30)
Montane, 1-yr N addition	0.14^a (0.03)	0.16 (0.02)	4.99 (0.19)	21.18^b (2.59)	66^a (9)	685^a (123)

Notes: Values are means with SE in parentheses ($n = 4$ plots). Different lowercase superscript letters indicate significant differences between treatments at each site (linear mixed effects models at $P \leq 0.05$; $P = 0.06$ for N_2O flux from the montane site). DON is dissolved organic nitrogen; DOC is dissolved organic carbon.

† Only fluxes measured at least six weeks after a N application were reported here to exclude the transitory, fertilizer-induced peaks of N-oxide fluxes. These data were part of the two-year (2006–2007) measurements reported by Koehler et al. (2009*a*).

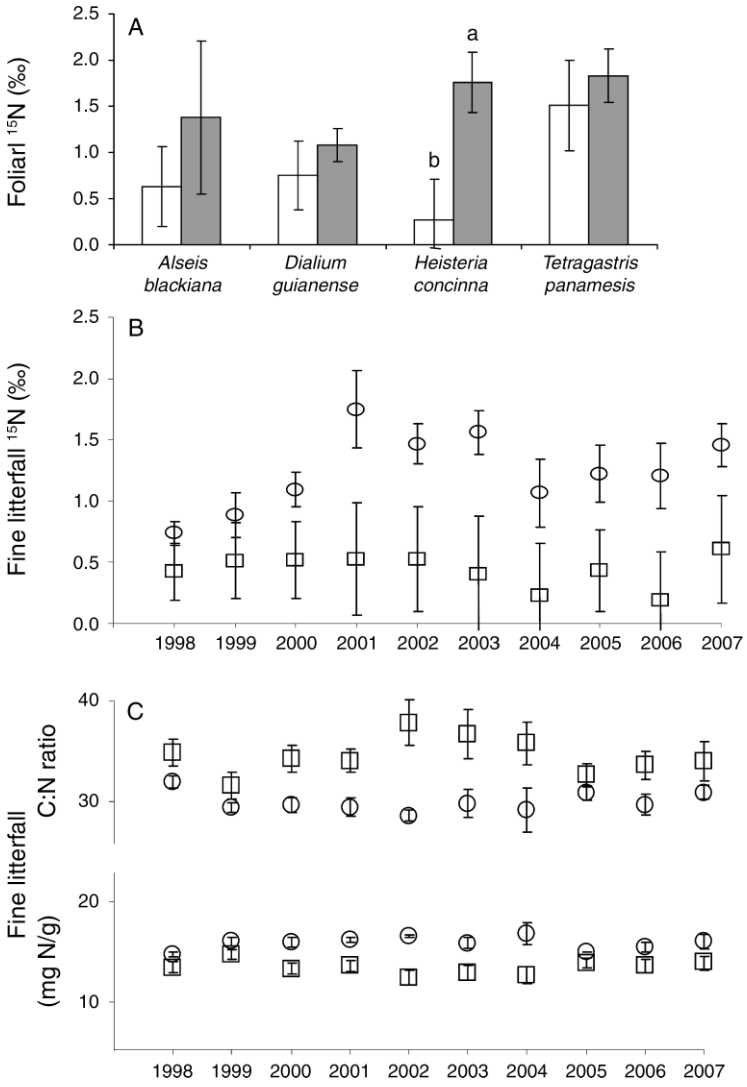

FIG. 2. (A) Foliar ^{15}N of the common tree species in the control (open bars) and after 10-yr N addition (solid bars); (B) fine litterfall ^{15}N and (C) N concentrations and C:N ratios of fine litterfall from the control (boxes) and across 10 years (yearly measurements each September) of N addition (ovals) in the lowland forest. N source (urea) had a ^{15}N signature of −2.22‰ (±0.03‰). For (A) foliar ^{15}N, means with different letters indicate significant differences between treatments (independent *t* test at $P \leq 0.05$). For fine litterfall, treatment effects were significant (linear mixed-effects models at $P = 0.00$) while year and treatment × year interactions were not significant. All data are shown as mean ± SE ($n = 4$ plots). For a detailed description, see *Methods: Other supporting parameters*.

wet season. From the $^{15}NH_4^+$-injected soil cores, ^{15}N recoveries in the NH_4^+ pool ranged from 91% (±10%) to 100% (±4%) (±SE) across sites and treatments, and no ^{15}N above the background level was detected in other N pools. From the $^{15}NO_3^-$-injected soil cores (Fig. 3), the lowland control plots showed higher ^{15}N recoveries in the NH_4^+ pool ($P = 0.00$) and lower ^{15}N recoveries in extractable organic N pool ($P = 0.00$) than the montane control plots. In the lowland site, 1-yr N addition did not differ in ^{15}N recoveries from the control while 9-yr N addition showed lower ^{15}N recoveries in the NH_4^+ pool and higher ^{15}N recoveries in the NO_3^- pool than the control. In the montane site, 1-yr N addition did not differ in ^{15}N recoveries from the control. ^{15}N recoveries in the total N pool showed complete recovery of the injected $^{15}NO_3^-$.

DISCUSSION

Soil-N cycling and losses in the lowland and montane control plots

Based on our previous study, which showed storage of soil samples can substantially change N-cycling rates (Arnold et al. 2008), we compared our measurements only with studies that employed in situ incubation and mineral-N extraction of intact soil cores. Gross NH_4^+-

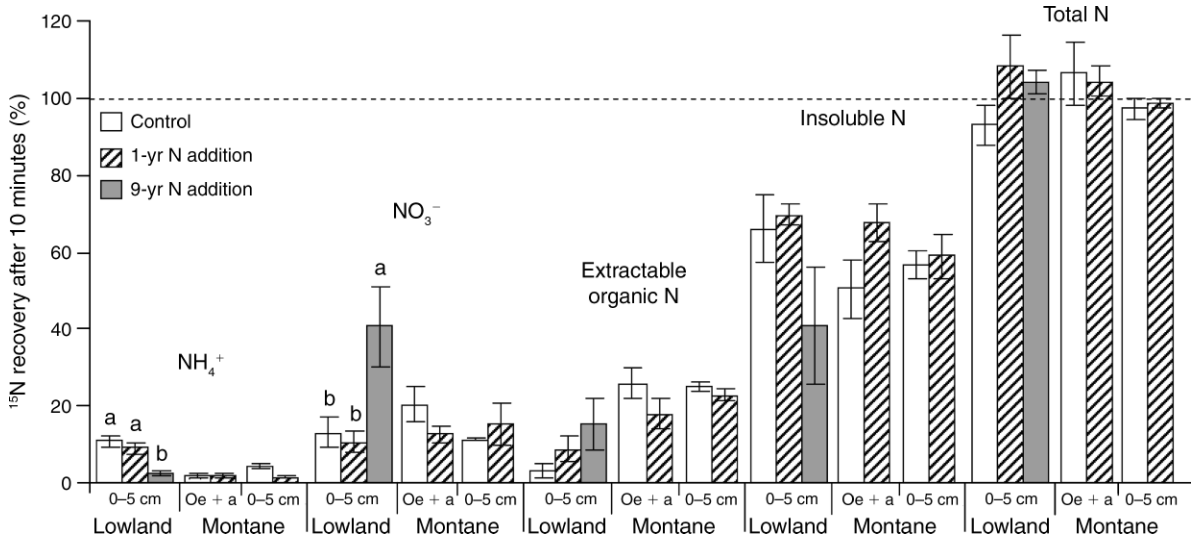

FIG. 3. Percentage recovery of injected $^{15}NO_3^-$ in different soil N pools after 10 minutes of ^{15}N injection to intact cores from 0–5 cm mineral soil and organic layer (Oe + Oa) during the wet-season sampling (September 2006). For the lowland site, means (±SE; $n = 4$ plots) with different letters indicate significant differences among treatments (one-way ANOVA with Tukey hsd at $P \leq 0.05$). The broken line shows 100% recovery. No treatment effect was detected in the montane site. Measurement was conducted in the first and ninth year of chronic N additions. See *Methods: Site description, experimental design, and soil characteristics* for a detailed description.

transformation rates from our lowland and montane forests were higher than those reported from an old-growth lower montane forest on an Ultisol in Puerto Rico (Silver et al. 2001) and an old-growth lowland forest on an Oxisol in Costa Rica (Silver et al. 2005). Aside from differences in soils and forest types, these studies also differ from ours in that they measured soil-N cycling in the top 10 cm depth, which may have lower microbial activity than the top 5 cm mineral soil and organic layer (for montane site only) from which we measured.

The soil chemical characteristics attested that the Inceptisol in the lowland site was more fertile than the Andisol in the montane site. The larger N availability (i.e., larger gross N mineralization + nitrification rates), N-oxide emissions, and ^{15}N signatures of the litter layer and mineral soil in the lowland site than in the montane site followed the general pattern observed for old-growth tropical forests: forest ecosystems with large rates of soil-N cycling have large N-oxide emissions (Davidson et al. 2000) and large ^{15}N signatures (Purbopuspito et al. 2006, Sotta et al. 2008). The latter is due to the loss of isotopically depleted N from the ecosystem owing to fractionation during nitrification and denitrification, leaving isotopically enriched N behind (Amundson et al. 2003). We addressed the question of whether differences in gross N-mineralization rates between sites were due to differences in either microbial biomass size, substrate quantity/quality, or both. The small specific N mineralization in the lowland mineral soil suggests that large gross N-mineralization rates were mainly contributed by the modest activity of a large microbial biomass. Conversely, the large specific N mineralization in the montane organic layer suggests that its small microbial biomass was able to mineralize more than that in the lowland mineral soil, suggesting either high substrate quality, quantity, or both. Assuming that the quality of microbially labile substrate can be indicated by microbial C:N ratio (since microbial C:N ratio is stoichiometrically related to the substrate), substrate quality may not be the influencing factor, because the montane organic layer had a large microbial C:N ratio. Thus, substrate quantity (e.g., total N concentration; Table 1) may have been the driver for the high N-mineralization rate per unit microbial N in the montane organic layer.

Effects of N addition on soil-N cycling and losses in the lowland site

Our results differed from the findings from the Hawaiian studies that show N addition to a forest that is not limited by N results in large and immediate N losses (Hall and Matson 1999, 2003). Our lowland forest on an Inceptisol, with high base saturation and net primary production not limited by N, showed no immediate changes in soil-N-cycling rates and N losses (i.e., N-oxide emissions, reported by Koehler et al. [2009*a*]) in the 1-yr N addition but only in the 9-yr N addition. The increased mineral-N-production rates in the chronic N-addition plots were paralleled by reduced NH_4^+ immobilization rates and by increased N losses. The larger ^{15}N signatures of leaves, fine litterfall, and litter layer in the chronic N addition plots compared to the control also suggest an increasingly leaky N cycle

(Davidson et al. 2007). Gross nitrification rates increased only in the chronic N-addition plots that exhibited a decrease in NH_4^+ immobilization rates. This pattern suggests that the increased gross N-mineralization rates and decreased sink by microbial assimilation enabled the nitrifiers to have more access to the available NH_4^+. Our findings suggest that reactions of tropical forests to future increases in N deposition cannot be predicted from whether or not plant growth is N limited but instead from the changes in mineral N production and retention in the soil.

We looked into possible causes of increased N-mineralization activity after chronic N addition. The increased specific N mineralization in chronic N-addition plots compared to the control suggests either enhanced substrate quality, quantity, or both. Substrate quantity (e.g., leaf-litter and fine-root mass; Appendix A) was unaffected by chronic N addition. On the other hand, there were three lines of evidence suggesting enhanced substrate quality under chronic N addition: (1) increased N concentrations and diminished C:N ratios of fine litterfall, (2) decreased microbial C:N ratio, and (3) decreased C:N ratio of the soil solution, which in part may reflect the product of microbial activity.

Reduced microbial assimilation of NH_4^+ in the chronic N-addition plots was related to the decrease in microbial biomass. Similar findings were reported from N-enriched temperate forest soils (either by long-term N addition or extremely high N deposition): reduced microbial biomass (Corre et al. 2003, 2007, Compton et al. 2004, DeForest et al. 2004), decreased microbial C:N ratios (Corre et al. 2007), and reduced NH_4^+ immobilization relative to gross N mineralization (Corre et al. 2003, 2007, Corre and Lamersdorf 2004, Venterea et al. 2004). Soil microbial communities are altered by N addition directly or indirectly through a decrease in soil pH. N additions reduce fruiting body abundance, hyphal networks, and formation of mycorrhizal fungi in some systems (Wallenda and Kottke 1998, Frey et al. 2004, Treseder 2004). It is possible that a reduction in fungal biomass contributed to the decreased microbial biomass in the chronic N-addition plots, as this was accompanied by a decrease in microbial C:N ratio, which suggests a shift toward a bacterial-dominated microbial community. Also, reduced microbial biomass and altered microbial community structure are attributed to increased acidity (Compton et al. 2004, Frey et al. 2004). It remains to be seen whether the pattern observed in the lowland site will follow the trend observed in temperate forests: initial increase in mineral N production followed by a downturn at excessively N-enriched sites (Venterea et al. 2004, Corre et al. 2007) due to reduced microbial biomass, changed microbial community structure, and reduced enzyme activity (e.g., cellulose- and lignin-degrading enzymes; Carreiro et al. 2000, Sinsabaugh et al. 2002).

Furthermore, dissolved organic carbon (DOC) concentrations in the soil solution may be influenced by changes in soil chemical properties due to chronic N addition. The reduced DOC concentrations in soil solution under chronic N addition suggest altered production, sorption, or heterotrophic use of DOC in the soil. We speculate that DOC production might not have changed with chronic N addition because fine litterfall and decomposition (Kaspari et al. 2008), fine-root mass (Appendix A), and CO_2 efflux (Koehler et al. 2009*b*) are not affected by 6, 11, and 9–11 yr N additions, respectively. The reduced DOC concentrations under chronic N addition could be due to increased sorption of negatively charged organic solute, owing to decrease in soil pH, which presumably leads to an increase in (pH-dependent) positively charged surface. Chronic N addition resulted in decreased base saturation and mobilization of Al; Al hydrous oxides possess a net positive charge at acidic soil pH and provide a strong sorption for DOC, which could be irreversible (Kaiser et al. 1997, Kaiser and Zech 1999, Kaiser and Guggenberger 2000). Also, increased NO_3^- concentrations in the soil solution under chronic N addition may have increased heterotrophic use of DOC through denitrification; support for this was the larger soil air N_2O

PLATE 1. (Upper) Lowland forest in Gigante Peninsula (Barro Colorado National Monument) and (lower) montane forest in Quebrada Honda (Fortuna Forest Reserve), Panama. Photo credit: J. Loss.

concentrations down to at least 2 m depth in the chronic N addition plots than the control (M. D. Corre, E. Veldkamp, and B. Koehler, *unpublished data*). Taken together, it may not only be elevated N input but also N-induced change in soil acidity that affect DOC mobility.

Effects of N addition on soil-N cycling and losses in the montane site

Our results also contrasted the findings from the Hawaiian studies that show N addition to an N-limited forest results in small and delayed N losses (Hall and Matson 1999, 2003). In our montane forest on an Andisol, with low base saturation and aboveground net primary production limited by N, the 1-yr N addition showed immediate increases in mineral-N production rates in the organic layer and increases in N losses. The instantaneous increase in NO_3^- concentrations in soil solution could have also been facilitated by the sandy loam soil texture, which may offer less hydraulic resistance to the high drainage flux in this site (as was shown by the commonly high soil moisture contents in this site with 5.5 m annual rainfall; Koehler et al. 2009*a*). A similar result was reported from a N-limited montane forest in Hawaii (Lohse and Matson 2005), where immediate NO_3^- leaching occurs following first-time N addition to a poorly developed volcanic soil. Our findings suggest that changes in soil-N cycling as well as soil hydrological properties, rather than tree responses, govern how tropical forest soils will react to future increases in N deposition.

We attributed the immediate increases in mineral-N-production rates in the organic layer during the 1-yr N addition to the increase in microbial biomass, since specific N mineralization (as an index for effects of substrate quality/quantity) did not change. Given that organic layers of tropical montane forests are reported to store large amounts of potentially mineralizable nutrients (Wilcke et al. 2002), the root proliferation in the organic layer in our montane site (accounting for 41% of the total fine-root mass within 1 m depth; Hölscher et al. 2009) is possibly a response to this large pool of nutrients. The added N may have favored C assimilation by microbes resulting in increased microbial biomass which, in turn, resulted in a positive feedback by increased mineralization of nutrients stored in the organic layer and consequently increased plant-derived inputs (i.e., increased leaf litterfall by 1–2 yr N addition to our montane site; Appendix A; Adamek et al. 2009).

The increased NO_3^- losses at 1.5 m depth suggest increases in losses of base cations (Matson et al. 1999). The Andisol in our montane site had initially low base saturation and the presence of an organic layer, which is a characteristic feature of this montane forest, may play an important role in buffering nutrient losses. This study is still ongoing, and it remains to be seen whether microbial biomass and N immobilization will keep pace with increasing N availability, and how long the initial increase in aboveground net primary production can be sustained with the changes in other nutrients after long-term N addition.

Implications of ^{15}N recoveries at T_0 on N retention

$^{15}NH_4^+$ recoveries in our lowland site were comparable with those reported from an old-growth lowland forest on an Oxisol in Brazil (Sotta et al. 2008), while $^{15}NH_4^+$ recoveries in our montane site were higher than the values reported from old-growth montane forests on Inceptisols in Hawaii (Hall and Matson 1999) and Indonesia (Corre et al. 2006). The complete recovery of injected $^{15}NH_4^+$ in the NH_4^+ pool suggests that rapid reactions of NH_4^+ to organic N pool (e.g., physical condensation reactions with phenolic compounds; Nömmik and Vahtras 1982) and fixation on clay minerals (Davidson et al. 1991) were not important for NH_4^+ retention in our study sites.

The low $^{15}NO_3^-$ recoveries in the lowland and montane control plots were comparable with those reported from Indonesian montane forests on Inceptisols (Corre et al. 2006) and from Ecuadorian forests on Andisols (Arnold et al. 2009), while the high $^{15}NO_3^-$ recoveries in the lowland 9-yr N-addition plots were comparable with the values reported from an Amazonian lowland forest on an Oxisol (Sotta et al. 2008). The recoveries of injected $^{15}NO_3^-$ in the NH_4^+ pool suggest either fast reduction of NO_3^- to NH_4^+, possibly by Fe^{2+} (Ottley et al. 1997), dissimilatory NO_3^- reduction to NH_4^+ (Silver et al. 2001), or both. Our measured ^{15}N recoveries in the extractable organic N pool were comparable with the values reported from an Amazonian lowland forest on an Oxisol (Sotta et al. 2008) and from Ecuadorian forests on Andisols (Arnold et al. 2009). Previous studies that reported fast reaction of NO_3^- to extractable organic N were disputed by Colman et al. (2007). These authors demonstrated that NO_3^- concentrations are underestimated by their standard continuous-flow injection colorimetry (CFIC) method with NH_4Cl + EDTA buffer, that the underestimation of NO_3^- is correlated with soluble iron concentrations, and that this interference does not appear when they substituted an imidazole buffer. The three tests we conducted to address this issue confirmed that this fast (presumably abiotic) reaction of NO_3^- to extractable organic N was not caused by an analytical artifact. First, our standard CFIC method with NH_4Cl buffer (no EDTA) showed 1:1 correlation with imidazole buffer for NO_3^- analysis in our soil extracts (Appendix B), and both buffers gave virtually the same NO_3^- values for the range of iron concentrations tested (Appendix C). Second, the iron concentrations in our soil extracts (ranging from 0.23 to 1.65 mg Fe/L) were very low compared to the level (>5 mg Fe/L; Appendix C) that may cause analytical interference. Third, we quantitatively recovered the amount of NO_3^- added to our soil extracts using our standard method (data not shown). In addition, most of the injected $^{15}NO_3^-$ was recovered in the insoluble N

pool (calculated as total ^{15}N recovery – ^{15}N recovery in extractable N), from which determination of extractable N by persulfate digestion is unaffected by iron (Colman et al. 2007). The only hypothesis currently under consideration for the fast reaction of NO_3^- to organic N is that of Davidson et al. (2003, 2008): NO_3^- is reduced to NO_2^- in soil microsites by reduced metals in the soil (e.g., Fe(II)), followed by reaction of NO_2^- with organic matter to produce organic N and by regeneration of reducing microsites by heterotrophic activity in a C-rich medium. Our results suggest that abiotic processes may be more important for retention of NO_3^- than of NH_4^+.

Conclusions

N-loss responses of the lowland and montane forest soils to elevated N input were indicated by the changes in soil-N cycling and not by vegetation response to N addition. The lowland forest on a fertile Inceptisol, with large soil-N-cycling rates and net primary production not limited by N, showed large N-oxide emissions. First-year N addition did not further increase the large N-cycling rates and N-oxide emissions. Only chronic (9 yr) N addition substantially increased gross N-mineralization rates, which were possibly due to improved substrate quality; microbial biomass and NH_4^+ immobilization decreased and consequently increased gross nitrification rates, NO_3^- leaching, and N-oxide emissions. On the other hand, the montane forest on an acidic, low base saturation Andisol, with small soil N-cycling rates and N-limited aboveground net primary production, showed small N-oxide emissions. First-year N addition showed immediate increases in mineral N production rates and N losses, which were paralleled by an increase in microbial biomass in the organic layer. The instantaneous increase in NO_3^- leaching was possibly due to the combination of increased NO_3^- availability and sandy loam soil texture, which may offer less hydraulic resistance to the high drainage flux in this wet montane forest. Also, in both lowland and montane forest soils, there was rapid reaction of NO_3^- to organic N, which may play an important role in regulating N losses. Our results show that soil type, presence of an organic layer, changes in soil N cycling, and hydrological properties (e.g., related to soil texture and rainfall) may be more important indicators than vegetation as N sink on how tropical forest soils respond to elevated N input. Further investigations on long-term fates of N, effects of N-induced changes in soil chemical characteristics on stability or losses of soil organic matter and rock-derived nutrients, and changes in microbial structure, function and enzyme activity will increase our understanding of how increases in N deposition affect the biogeochemistry of tropical forests.

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APPENDIX A

Tree responses to elevated N input in the lowland and montane forests (*Ecological Archives* E091-116-A1).

APPENDIX B

Comparison between imidazole buffer (Colman et al. 2007) and our standard method of NH_4Cl buffer (without ethylenediamine tetraacetic acid, EDTA) for NO_3^- determination from our soils extracted with 0.5 mol/L K_2SO_4 (*Ecological Archives* E091-116-A2).

APPENDIX C

Comparison between imidazole buffer (Colman et al. 2007) and our standard method of NH_4Cl buffer (without ethylenediamine tetraacetic acid, EDTA) for NO_3^- determination from standard (0.5 mol/L K_2SO_4) solutions containing 4.9 mg NO_3^--N/L with increasing Fe^{2+} levels (*Ecological Archives* E091-116-A3).