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Tropical Andean Forests Are Highly Susceptible to Nutrient Inputs—Rapid Effects of Experimental N and P Addition to an Ecuadorian Montane Forest

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Abstract

Tropical regions are facing increasing atmospheric inputs of nutrients, which will have unknown consequences for the structure and functioning of these systems. Here, we show that Neotropical montane rainforests respond rapidly to moderate additions of N (50 kg ha^{-1} yr^{-1}) and P (10 kg ha^{-1} yr^{-1}). Monitoring of nutrient fluxes demonstrated that the majority of added nutrients remained in the system, in either soil or vegetation. N and P additions led to not only an increase in foliar N and P concentrations, but also altered soil microbial biomass, standing fine root biomass, stem growth, and litterfall. The different effects suggest that trees are primarily limited by P, whereas some processes—notably aboveground productivity—are limited by both N and P. Highly variable and partly contrasting responses of different tree species suggest marked changes in species composition and diversity of these forests by nutrient inputs in the long term. The unexpectedly fast response of the ecosystem to moderate nutrient additions suggests high vulnerability of tropical montane forests to the expected increase in nutrient inputs.

Introduction

Since the 1950/60s, anthropogenic changes to the cycling of the key nutrients nitrogen (N) and phosphorus (P) have dramatically altered the structure and functioning of many ecosystems in the world's industrialized regions [1–6]. Elevated N and P inputs affect virtually all components and processes of terrestrial and aquatic ecosystems, including plant growth, plant longevity and stress tolerance, plant community composition and diversity, biotic interactions (plant-plant, plant-fungus, plant-animal), the composition and activity of heterotrophic communities, and the storage and cycling of carbon, nutrients and water [7–9]. This is because primary production is limited by N or P, or both in the vast majority of ecosystems around the globe [8,10–14].

In the past 30 years, research has focused on the structural and functional responses of temperate and boreal forests to atmospheric N inputs [15–18] because the bulk of fertilizer use worldwide was in the industrialized nations of the northern hemisphere. Furthermore, these regions had particularly pronounced gaseous NO_x emissions originating from the combustion of fossil fuels and NH_3 emissions from animal production [1]. However, this situation is changing rapidly. With the expansion of

industrial agriculture into many tropical and southern hemispheric regions, the spread of N and P compounds to adjacent and more distant non-agricultural ecosystems in these regions has been greatly increased [1,19–20]. In the future, tropical forests will be increasingly exposed to airborne N and P inputs. Higher P inputs are mostly due to the deposition of dust [4,21–24], but sources of N can be varied, including oxidised and reduced N compounds emitted with farming, livestock breeding and the combustion of fossil fuels, and N released through biomass burning with the conversion of tropical forests [1,25–26].

Although tropical forests are likely to be sensitive to these changes, the size and direction of their responses are unclear [7,20,27–31]. A number of fertilization experiments in tropical forests have investigated responses to experimental high-dose treatments with N, or N and P (100–300 kg N ha^{-1} yr^{-1} and/or 50–100 kg P ha^{-1} yr^{-1}) [30,32–40]. These experiments typically focused on selected ecosystem properties, such as changes in tree growth, fine litter production or soil carbon pools, but did not provide comprehensive insight into ecosystem responses to elevated N and P loads. Here, we report data from a nutrient manipulation experiment (NUMEX) investigating the response of an old-growth montane forest ecosystem in the Andes of southern

Ecuador to moderate N (50 kg ha^{-1} yr^{-1}) and/or P (10 kg ha^{-1} yr^{-1}) additions considering a multitude of response variables. The study allows comprehensive insight into how highly diverse tropical montane forest ecosystems respond to moderate nutrient additions such as those predicted by climate change scenarios.

Results and Discussion

A large number of ecosystem properties and functions exhibited marked responses to the experimental nutrient additions after only one year.

Soil nutrient pools and soil biological activity

Nutrient addition did not result in a significant increase of the organic layer N pool while the P pool increased after combined addition of N and P (Fig. 1). The reason is likely the relatively high N stock in the thick organic layer. An adjacent micro-catchment between 1850 and 2150 m a.s.l. had 0.9–21 Mg ha^{-1} N in the organic layer while the P stock ranged from 30–700 kg ha^{-1} [41]. Soil microbial biomass decreased after adding N (Fig. 2a), whereas P addition had no effect. A decrease in microbial biomass after N addition suggests detrimental effects of nitrogen on soil microorganisms and has been seen in other studies [3,42], in particular with lignin decomposing fungi [43–44]. Conforming to the assumption of detrimental effects on microorganisms using complex organic compounds the metabolic activity of microorganisms significantly increased after N addition (Fig. 2b), indicating a shift towards microorganisms predominantly using more easily available carbon resources.

All treatments resulted in slightly higher net N mineralization rates, the increase after N addition was marginally significant (p = 0.08, Fig. 2c). N$_2$O emissions tended to increase after N+P addition (p = 0.05) but not after addition of N or P only (Fig. 2d). This suggests that N is needed as substrate for denitrification while P is limiting the respective organisms.

The assumption that P addition stimulated microorganisms which are responsible for N transformations is further supported by the finding that the combined addition of N and P had a significant effect on the NH$_4$-N/NO$_3$-N ratio in mineral soil solution (Fig. 2e–f). Although we added urea, which is primarily a source of NH$_4^+$, NO$_3^-$ concentrations increased after combined N and P addition while NH$_4^+$ concentrations did not. This was probably because of the combination of stimulated nitrification and microbial NH$_4^+$ retention. Our finding that only N and P addition together stimulated N mineralization (and possibly also nitrification) is different than reports from other tropical sites where N addition alone stimulated nitrification and triggered NO$_3^-$ losses to the subsoil. This difference may be the result of no - or less pronounced - P limitation of the involved microorganisms [27,45]. In contrast to many temperate forests where NO$_3^-$ is the most abundant N form [46], NO$_3^-$ only accounted for 3–5% of total N in the soil solutions from our study site. Instead, the soil N pool was predominantly made up of dissolved organic nitrogen (DON) and NH$_4^+$ (contributing 50–70% and 27–43% respectively) [47]. This implies that small changes in N mineralization and nitrification rates can have a large impact on NO$_3^-$ concentrations and fluxes. Our results indicate that the addition of different nutrients may stimulate or inhibit different processes in the ecosystem resulting in a complex system response to nutrient deposition.

Nutrient cycling

Increased N and P contents in litterfall and throughfall (Figs. 3a–f) indicate that a large proportion of the added nutrients was taken up by trees and subsequently accelerated nutrient cycling through higher N and P return with litterfall and leachate [48] and through stimulated litter decomposition by higher N and P concentrations [49]. The annual increase in N and P fluxes in litterfall and throughfall after fertilization was equivalent to 25.4% and 26.7% of the applied N, after N addition and after N and P addition, respectively, and 3.8% and 6.1% of the applied P, after P addition and after N and P addition, respectively. Neither organic layer N and P pools nor N and P losses to the atmosphere or to the subsoil were significantly increased by N or P addition (Figs. 2d and 3i, j), suggesting that the bulk of N and P added was retained in the ecosystem [48]. The N and P effects on nutrient cycling were interrelated. Phosphorus increased the retention of N in the system since aboveground N losses were slightly reduced (Fig. 2d) and losses by leaching were negligible (Fig. 3i–j). We attribute this mainly to the stimulation of the N-mineralizing microbial community by P addition as reflected by the significantly increased net N mineralization rates [50]. Furthermore, N application increased the P return with litterfall and throughfall when added in combination with P (N+P treatment: Figs. 3d,f). The positve effects
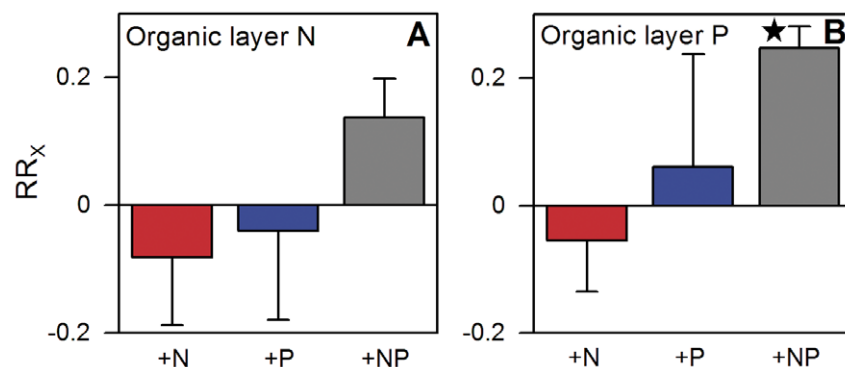

Figure 1. Effects of one year of experimental nutrient addition on various soil nutrient pools of a montane forest in Ecuador. Effects are presented as natural-log transformed response ratios (RR$_X$) in which the parameter in the enriched treatment is divided by its value in the control treatment and then ln-transformed. Hence, a value of 0.2 indicates a value in the manipulated treatment that is c. 23% higher than in the control, while a value of 0.5 indicates a 65% increase. Error bars indicate plus or minus one standard error. Data of the control treatment (mean ±1 SE) are given in parentheses below. Asterisks indicate significant differences to the control (P≤0.05). a. Organic layer nitrogen pool (3.79±0.31 Mg N ha^{-1}). b. Organic layer phosphorus pool (98.6±6.8 kg P ha^{-1}).
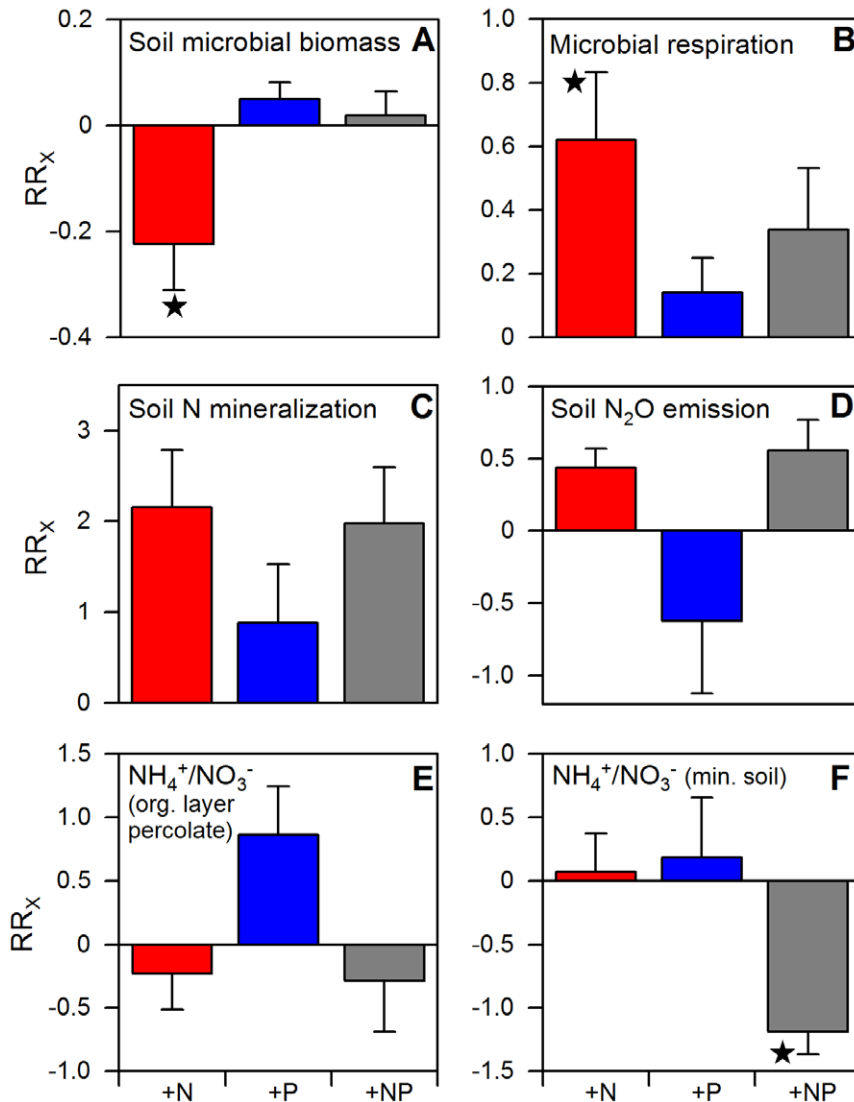doi:10.1371/journal.pone.0047128.g001

Figure 2. Effects of one year of experimental nutrient addition on biological soil activity of a montane forest in Ecuador. a. Soil microbial biomass in May 2009 (5881±25 µg C_{mic} g^{-1} soil dry mass). b. Respiration of soil microorganisms in May 2009 (5.066±0.36 µl O_2 mg C_{mic}^{-1} h^{-1}). c. Net N mineralization in September 2008 (23.4±10.5 ng N cm^2 h^{-1}). d. Annual emission of N_2O (0.25±0.03 kg N ha^{-1} yr^{-1}). e. Mean NH_4^+/NO_3^- ratio of organic layer percolate from February 2008 to January 2009 (15.7±6.7) and f. mean NH_4^+/NO_3^- ratio of mineral soil solution from February 2008 to January 2009 (8.9±2.0). Error bars indicate plus or minus one standard error. Data of the control treatment (mean ±1 SE) are given in parentheses in the legend. Asterisks indicate significant differences to the control (P≤0.05). For interpretation of graph see legend of Fig. 1.
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of N addition on litter P concentration and P return with litterfall and throughfall might be a result of better soil P availability to plants by increased extracellular phosphatase activity after N addition. This has been observed in several temperate and tropical forest studies [51–53].

Tree biomass and forest productivity

Stand leaf area index (LAI) tended to increase after addition of N and P alone (although not significantly), while leaf litter production increased only after N+P addition (Figs. 4a,c). Since N fertilization tended to increase the specific leaf areas and foliar N concentrations of the four most common tree species (Table 1; difference significant only for *Myrcia* sp.), two foliage attributes generally associated with a shorter leaf lifespan [54], we infer that N addition has stimulated leaf production. The LAI increase after P addition probably resulted from an extension of mean leaf

lifespan as indicated by the observed slight reduction in litter production. This is supported by results from Hawaiian montane forests, where leaf production and leaf longevity were increased after P addition [55].

Stand basal area increment as a proxy of aboveground productivity tended to increase in all fertilization treatments (Fig. 4b), as reported from other Neotropical montane forests after addition of N [34,39,55], P [34,39] or N and P [35].

All fertilizer treatments resulted in a marked reduction in standing tree fine root biomass (by 15–28%) with the effect being strongest after P addition (Fig. 4d). This treatment also led to a strong increase in standing fine root necromass, while the N and N+P treatments had no effect on necromass (Fig. 4e). Presumably, the accumulation of fine root necromass in the P treatment resulted from reduced root litter decay due to an unfavorable litter N:P ratio for decomposers [56]. A decline of fine root biomass and
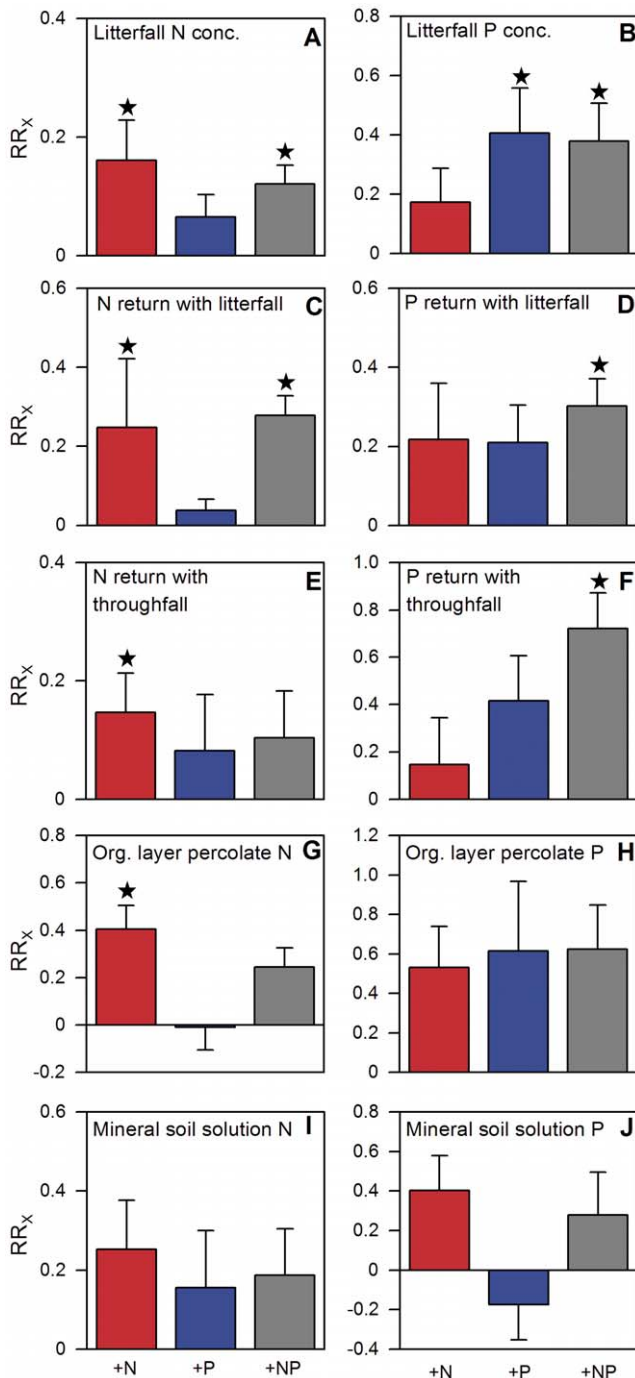
Figure 3. Effects of one year of experimental nutrient addition on nutrient cycling of a montane forest in Ecuador. a. Nitrogen and b. phosphorus concentrations of the litterfall in January 2009 after one year of nutrient addition ($0.87\pm0.03\%$ N and $0.025\pm0.006\%$ P). Total annual return of c. nitrogen and d. phosphorus with litterfall (41.7 ± 5.0 kg N ha^{-1} yr^{-1} and 1.49 ± 0.24 kg P ha^{-1} yr^{-1}). Total annual return of e. nitrogen and f. phosphorus with throughfall (10.1 ± 1.0 kg N ha^{-1} yr^{-1} and 0.15 ± 0.03 kg P ha^{-1} yr^{-1}). g. Annual flux of nitrogen and h. phosphorus found in the organic layer percolate (14.65 ± 1.21 kg N ha^{-1} yr^{-1} and 0.13 ± 0.02 kg P ha^{-1} yr^{-1}). i. Annual flux of nitrogen and j. phosphorus found in the soil solution at 0.3 m soil depth (3.26 ± 0.59 kg N ha^{-1} yr^{-1} and 0.03 ± 0.004 kg P ha^{-1} yr^{-1}). Error bars indicate plus or minus one standard error. Data of the control treatment (mean ±1 SE) are given in parentheses in the legend. Asterisks indicate significant differences to the control (P≤0.05). For interpretation of graph see legend of Fig. 1.
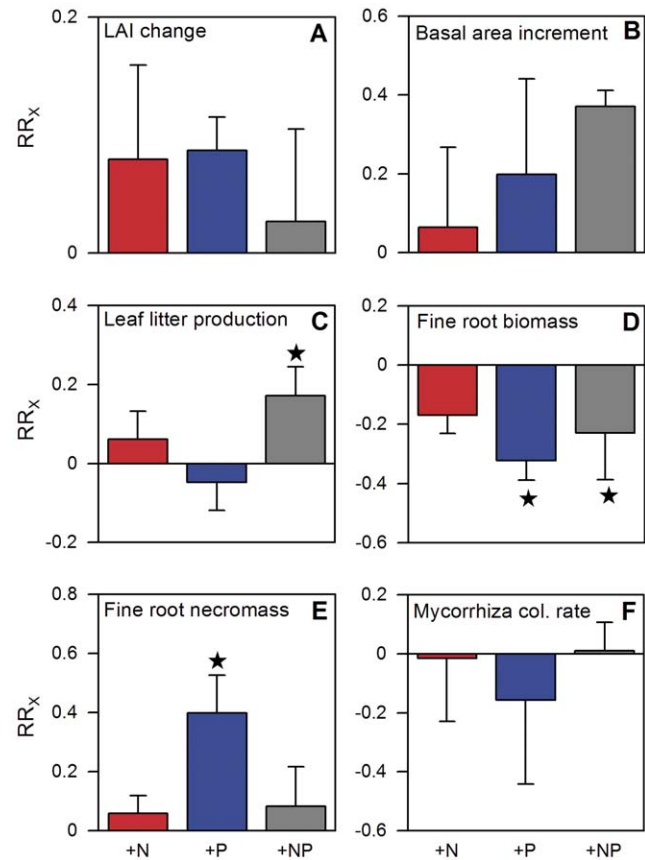doi:10.1371/journal.pone.0047128.g003

Figure 4. Effects of one year of experimental nutrient addition on vegetation related parameters of a montane forest in Ecuador. a. Relative change of leaf area index (LAI) after one year of nutrient addition (measurements from January 2009 were compared to measurements prior to nutrient addition in January 2008; control mean changed from 4.6 ± 0.2 in 2008 to 4.7 ± 0.4 in 2009). b. Plot basal area increment from February 2008 to January 2009 (0.111 ± 0.018 m^2 ha^{-1}). c. Annual leaf litter production from February 2008 to January 2009 (3.46 ± 0.46 Mg ha^{-1}). d. Fine root biomass in January 2009 (443 ± 28 g m^{-2}), e. fine root necromass in January 2009 (426 ± 29 g m^{-2}), and f. rate of fine root colonization by arbuscular mycorrhizal fungi in January 2009 ($53.3\pm6.2\%$). Error bars indicate plus or minus one standard error. Data of the control treatment (mean ±1 SE) are given in parentheses in the legend. Asterisks indicate significant differences to the control (P≤0.05). For interpretation of graph see legend of Fig. 1.
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a concurrent increase of dead roots after nutrient addition have also been shown in other montane forests after N addition [57: Puerto Rico] or after N, P and N+P addition [58: Hawaii].

The observed decrease of fine root biomass does not necessarily indicate a reduced fine root production. More likely is a stimulation of fine root production by nutrient addition and a concurrent increase of fine root turnover [59,60], the combination of both effects could result in a reduced standing fine root biomass.

The root colonization by arbuscular mycorrhizal fungi (AMF) was not significantly affected by nutrient addition, with values remaining more or less constant at about 50% (Fig. 4f). This result contrasts with the rapid response of fine root biomass and does not fit to the predictions of the functional equilibrium model of AMF [61]. Empirical data show root colonization by AMF to be reduced when N and particularly P availability is increased [62]. These results indicate that in our study area the addition of P (and to a lesser extent of N) relaxed the growth limitation imposed by P

Table 1. Effects of nutrient addition on foliar nutrient concentrations and leaf morphology of the four most common tree species of a montane forest in Ecuador.

			Deviation from control (%)		
	Tree species	control	+N	+P	+NP
Foliar N (mg g^{-1})	Graffenrieda	12.2	+5	±0	+4
	Myrcia sp.	11.3	**+9**[*]	−6	**+11**[*]
	Hieronyma	13.8	+1	−4	**+11**[*]
	Alchornea	13.8	+7	**+15**[*]	**+14**[*]
Foliar P (mg g−1)	Graffenrieda	0.43	−2	**+37**[*]	+21
	Myrcia sp.	0.43	**+28**[*]	−5	**+33**[*]
	Hieronyma	0.54	+2	+11	**+28**[*]
	Alchornea	0.71	−11	**+34**[*]	**+42**[*]
Foliar N/P ratio	Graffenrieda	31	+7	**−31**[*]	−17
	Myrcia sp.	27	−17	−4	−18
	Hieronyma	26	−3	**−14**[*]	**−14**[*]
	Alchornea	20	**+16**[*]	**−15**[*]	**−21**[*]
Leaf area (cm^2)	Graffenrieda	178.2	+28	−1	+15
	Myrcia sp.	17.9	+3	−15	+12
	Hieronyma	26.8	−13	−29	−23
	Alchornea	30.5	−8	+1	+2
Specific leaf area (cm^2 g^{-1})	Graffenrieda	38.4	+9	+7	+10
	Myrcia sp.	40.2	+2	−5	+2
	Hieronyma	69.3	+6	+1	−7
	Alchornea	40.9	+13	+5	+12

Given are the absolute values for the control treatment and the percental effects of the treatments. Asterisks indicate significant differences to control (P<0.05). The number of sampled trees was for Graffenrieda emarginata: 5 (control), 6 (+N), 6 (+P) and 6 (+NP), for Myrcia sp.: 6, 5, 5 and 6, for Hieronyma fendleri 2, 5, 5 and 3 and for Alchornea lojaensis: 5, 4, 5 and 4.
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(and N) scarcity and prompted the trees to allocate more carbon into aboveground structures and productivity. Our finding that fine root biomass was significantly reduced upon N and P addition, while the mycorrhizal infection of the trees remained unchanged, points at the importance of AMF functions other than nutrient acquisition in controlling the plant-fungus interaction [63], or resource uptake rates that are independent of infection rate.

The stand-level N and P use efficiencies in the control plots were in the upper range of values reported from other tropical forests [64] (Fig. 5). Addition of nitrogen, phosphorus, or both, led to a significant decrease (10–25%) in the N or P use efficiencies, respectively, within one year, suggesting rapid relaxation from growth limitation by P and N and most likely decreased nutrient resorption efficiencies of the vegetation. Lower nutrient resorption efficiencies with increasing green leaf nutrient status were also reported by Kobe et al. [65] for a global data set of perennial plant species.

Divergent tree species growth responses

We found a highly variable response of stem diameter growth upon N and/or P addition among the tree species in the fertilized plots. Depending on species, growth rates were either higher or lower relative to the control plots (Table 2). However, two of the most common species (Hieronyma fendleri and Alchornea lojaensis) showed increases in stem diameter growth rates after addition of N, P or N+P, while two other species (Graffenrieda emarginata and Myrcia sp.) tended to reduce growth upon N or P fertilization. The divergent growth response of different tree species is in agreement

with fertilization studies from other tropical montane forests [33,34]. It appears that the responsiveness to N or P addition of stem diameter growth is highly species-specific and that while some species will increase, others will reduce their competitive strength with continued nutrient addition, likely resulting in species composition changes and diversity reductions in this species-rich forest over time [7,66–67]. Since the forest canopy will become denser in response to fertilization (increasing the LAI) and the trees will be relieved of growth constraints due to limited N and/or P availability, light competition will become more important with increasing input of nutrients. These changes may reduce the competitive ability of the seedlings and saplings of the currently abundant tree species, and will probably result in their eventual replacement by species adapted to more fertile soils. Changes in tree species composition (from slow-growing species adapted to nutrient-poor soils to faster growing species adapted to more fertile soils) will most likely accelerate the projected shifts in the C cycle by increasing the biomass turnover rate. The N:P ratio in leaf biomass of the unfertilized trees (means of 20–31 in the four most common species) provides support for the conclusion that tree growth in the studied forest is mainly limited by P [68]. Consequently, P was accumulated to a larger extent in the foliage than N after addition of P or N, and the N:P ratio responded more to P addition (decrease) than to N addition (no uniform effect; Table 1). The increased foliar N concentrations in three of the four studied common tree species after addition of N or N+P, respectively, should result in higher photosynthetic carbon gain, since photosynthetic capacity is closely related to foliar N [69].
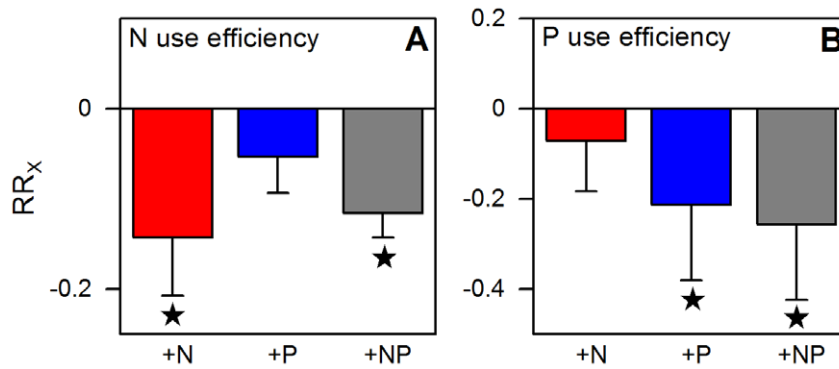
Figure 5. Nutrient use efficiencies and monthly nutrient return with litterfall. Nutrient use efficiencies (i.e. the ratio of total litterfall dry mass to nutrient content [64]) of the different treatments in the studied montane forest in Ecuador after one year of nutrient addition (samples from January 2009, means of N = 24 litter traps per treatment). a. N use efficiency (116.5 ± 3.8 g g^{-1}), b. P use efficiency (4751 ± 782 g g^{-1}). Error bars indicate plus or minus one standard error. Data of the control treatment (mean ± 1 SE) are given in parentheses in the legend. Asterisks indicate significant differences to the control ($P \leq 0.05$). For interpretation of graph see legend of Fig. 1.
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Conclusions

Overall, the strong and complex short-term response of the tropical montane forest ecosystem to moderate nutrient inputs suggests major consequences of expected future nutrient inputs into these ecosystems. This is particularly evident at our study site. The effects that we observed were larger than those reported from tropical lowland forests on more fertile soils, where only long-term nutrient addition resulted in significant effects [40]. Several of the responses to nutrient addition are similar to those known from other tropical montane forests, where they occurred either after chronic nutrient addition or after fertilization with higher amounts of N.

Provided that these initial trends persist, continued addition of substantial amounts of N and P will probably result in taller forests with a higher above-ground biomass but smaller below-ground biomass [60]. However, the below-ground response of the system to nutrient addition is still poorly understood. Given the large stocks of carbon in the organic layer, stimulated mineralization and soil respiration rates and less belowground C sequestration may turn these ecosystems into a significant future source of CO_2 to the atmosphere.

Table 2. Effects of nutrient addition on annual stem diameter growth of the four most common tree species of a montane forest in Ecuador.

Tree species	control	Deviation from control (%)		
		+N	+P	+NP
	(mm)			
Graffenrieda emarginata	0.84 (49)	−12 (49)	−21 (47)	+16 (54)
Myrcia sp.	0.80 (17)	−39 (15)	−50* (16)	−5 (11)
Hieronyma fendleri	0.07 (6)	+157 (22)	+71 (13)	+414* (13)
Alchornea lojaensis	0.12 (20)	+33* (18)	+17 (16)	+42* (9)
all other species pooled	0.28 (78)	−11 (94)	+82 (77)	+25 (89)

Given are the absolute values for the control treatment (February 2008–January 2009) and the percental effects of the treatments. Asterisks indicate significant differences to the control (P<0.05).
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Further studies have to show how nutrient cycles and key ecosystem services such as carbon storage will adjust to continuing input of moderate amounts of N and P and how community composition will change in the long run.

Cross-study comparisons of nutrient manipulation experiments could contribute to a better understanding of ecosystem responses to increasing nutrient deposition, but the currently published studies are hard to compare due to different levels of fertilizer addition and methodological differences among the various studies. A network of coordinated experiments adding low amounts of nutrients to tropical forests, that covers a wide range of environmental conditions (climate, soil), would be the method choice of obtaining general patterns of tropical forest ecosystem responses to increasing nutrient availability.

Materials and Methods

Study area

The study was conducted at about 2000 m elevation, in a tropical montane moist forest of the San Francisco Reserve in the Andes of southern Ecuador (3°58′S, 79°04′W) (Fig. S1). This forest is in nearly pristine condition, and is one of the best-studied tropical montane forests worldwide, known for its extraordinary richness in tree species as well as other plant and animal groups [70–71]. The forest harbors more than 300 tree species with Lauraceae, Melastomataceae and Rubiaceae being the plant families with the highest species numbers. The study site has a mean annual precipitation of ~2200 mm and an annual mean temperature of ~15°C. The most abundant soil types at the study site are Cambisols that developed on paleozoic metamorphosed schists and sandstones. Soils are heterogeneous but usually nutrient-poor (thick organic layers can harbor locally high nutrient stocks, but often these are only slowly bioavailable) [41]. Estimates of total annual nutrient depositions (based on the monitoring of bulk and dry deposition between 1998 and 2010) range from 14–45 kg N ha^{-1} and 0.4–4.9 kg P ha^{-1} for the study area.

All necessary permits were required for the described field studies.

Experimental design

A full-factorial nutrient manipulation experiment (NUMEX) was conducted in 16 plots of 400 m^2 (20 m×20 m) consisting of four treatments (N, P, N+P, control) with four replicates in a

stratified random design in four blocks at 2020–2120m a.s.l. (Fig. S1). Minimum distance between two plots was 10 m.

Nitrogen and phosphorus were added at an annual rate of 50 kg N ha^{-1} as urea and 10 kg P ha^{-1} as monosodium phosphate. The fertilizer was dispersed homogeneously over the plots with two application dates per year (January 26 and July 26) starting in 2008.

The dominant tree species in the NUMEX plots, making up about one quarter of all stems (dbh≥10 cm), was *Graffenrieda emarginata* Ruiz & Pav. (Melastomataceae); other frequently found species were *Myrcia* sp. nov. (Myrtaceae), *Alchornea lojaensis* Secco and *Hieronyma fendleri* Briq. (both Euphorbiaceae). The mean number of trees, mean tree diameter and stem basal area per plot (pre-fertilization survey of trees ≥10 cm dbh) were 45.7, 15.0 cm and 0.91 m^2, respectively. Average stand height was 12 to 14 m.

Organic layer nutrient pools

In April 2009, the soil organic layer (including the Oi, Oe, and Oa horizons) was sampled with a 0.2×0.2 m frame to the depth of the organic layer/mineral soil boundary at five randomly selected points within each permanent plot. Samples were dried to constant mass at 40°C.

The organic horizons were separated from the underlying mineral soil at the point where bulk density abruptly increases from <0.2 g cm^3 in the organic layers to >1 g cm^3 in the mineral soil [41].

The N concentrations in the ground samples were determined with a CHNS-analyzer (Vario EL Cube, Elementar Analysensysteme GmbH). After microwave digestion with HNO$_3$ (Mars 5 Xpress, CEM Corporation, Matthews, NC), the total phosphate concentration was detected photometrically (Continuous Flow Analyser; Bran+Luebbe GmbH, Norderstedt, Germany). Soil bulk density was determined with two additional samples per plot which were dried at 105°C for 24 h. Detailed results are shown in Table S1.

Microbial biomass

In May 2009, three samples per plot were collected in the upper organic layer to a depth of 5 cm using a metal corer (5 cm diameter). The upper litter layer (1–2 cm) was removed and the three samples were pooled and stored at 5°C. Before measurements, roots >2 mm were removed from the soil and the remaining material was chopped to pieces of <25 mm^2, homogenized and pre-incubated at 20°C for five days.

Respiration of soil microorganisms was measured as O$_2$ consumption using an automated electrolytic O$_2$ microcompensation apparatus [72]. Respiration was measured at hourly intervals at 22°C for 24 h. Basal respiration (BR) of microorganisms was calculated as the mean oxygen consumption rates of hours 14–24 after the start of the measurements without addition of substrate. Microbial biomass carbon (C$_{mic}$) was calculated from substrate induced respiration measuring the respiratory response to D-glucose which activates the metabolism of living microorganisms in the soil [73]. After adding Glucose (80 mg g^{-1} dry mass in 300 µl deionized water) the mean of the lowest three readings within the first 5–10 h was taken as maximum initial respiratory response (MIRR; µg O$_2$ h^{-1} g^{-1} soil dry mass). Microbial biomass (µg C g^{-1} soil dry mass) was calculated as 38×MIRR [74].

Net rates of nitrogen cycling in the soil

Net rates of N cycling in the soil were measured in October 2008 using the buried bag method [75]. In each plot a soil sample was taken from 0 to 5 cm depth. One subsample was extracted immediately in the field with 0.5 mol L^{-1} K$_2$SO$_4$ to determine initial NH$_4^+$ and NO$_3^-$ levels (T$_0$). The other sample was put into a plastic bag, reburied in the soil, incubated for ten days and afterwards extracted with 0.5 mol L^{-1} K$_2$SO$_4$ (T$_1$). The plastic bag was closed with a rubber band to prevent rain coming in but not too tight to permit air exchange. Net N mineralization and nitrification rates were calculated as the difference between T$_1$- and T$_0$- NH$_4^+$ and NO$_3^-$ concentration.

Trace gas measurements

Nitrous oxide was measured monthly using static vented chambers. Four permanent chamber bases made of polyvinyl chloride (area 0.04 m^2, height 0.25 m, ~0.02 m inserted into the soil) were randomly placed in four of six subplots per plot at least four weeks before the first measurement, resulting in 16 chamber bases per block and 48 in total. Four gas samples (100 ml each) were removed at 2, 14, 26 and 38 min after chamber closure with an acrylonitrile butadiene styrene (ABS) lid and stored in pre-evacuated glass containers (60 mL) with stopcocks [76]. Gas samples were transported to the laboratory in Loja (Ecuador) within two days and analyzed using a gas chromatograph (Shimadzu GC-14B, Duisburg, Germany) equipped with an electron capture detector (ECD) and an autosampler [77]. Gas concentrations were determined by comparison of integrated peak areas of samples to standard gases (320, 501, 1001 and 3003 ppb N$_2$O; Deuste Steininger GmbH, Mühlhausen, Germany). 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 [76]. Zero fluxes were included.

Litterfall

Six litter traps (each 0.36 m^2 in surface area positioned 1 m above ground) were randomly placed in each plot. The litterfall was collected every four weeks starting on November 6th, 2007. The collected samples were oven-dried at 60°C before determining the dry weight.

Leaf morphology

Leaf samples from sun-exposed branches of each of 4–5 trees per treatment from four common species (*Alchornea lojaensis*, *Graffenrieda emarginata*, *Hieronyma fendleri* and *Myrcia* sp. nov.) were collected in January 2009 to quantify changes in leaf morphology and foliar nutrient concentrations one year after the onset of the experiment. For each sample 10–25 fresh leaves were scanned using a flat bed scanner (CanonScan LIDE 30, Canon). The images were analyzed subsequently with the WinFolia 2001a software (Regent Instruments Inc., Quebec, Canada) for calculation of leaf area. The leaves were then dried at 60°C to constant mass. Specific leaf area (SLA) was calculated as the ratio of leaf area and leaf dry weight.

Foliar and litter nutrient contents

The concentrations of total C and N in leaf and litter mass were determined with a C/N elemental analyzer (Vario EL III, elementar, Hanau, Germany). The concentrations of total P were analyzed using an Inductively Coupled Plasma Analyzer (Optima 5300DV ICP-OES, Perkin Elmer) after digesting the samples with concentrated HNO$_3$.

Throughfall and soil solutions

Throughfall was collected with 20 randomly distributed, fixed-positioned funnel gauges in each plot. The volume of throughfall water was measured in the field with a graduated cylinder, and the

samples were then bulked according to their relative volume to result in a single sample per plot per collecting date.

Litter leachate was collected using three zero-tension lysimeters per plot, which consisted of plastic boxes with a collecting surface area (polyethylene net) of 0.15 m×0.15 m, installed below the organic layer. All collected litter leachate samples of a plot were bulked to yield a single sample per plot per collecting date.

In each plot, mineral soil solution was collected using three suction lysimeters (ceramic suction cups with 1 μm pore size) at 0.15 and 0.30 m depth, installed so that bulking of the soil solution per soil depth occurred *in situ*.

Throughfall, litter leachate and mineral soil solutions were sampled fortnightly. After collecting the mineral soil solution, a vacuum was applied to the suction lysimeters in order to collect sufficient sample for the next sampling period.

After field collection, throughfall, litter leachate, and soil solution samples were transported to our field laboratory where an aliquot was filtered (ashless filters with pore size 4–7 μm, folded filter type 389; Munktell & Filtrak GmbH, Bärenstein, Germany) and frozen until transport to Germany for further analysis. Samples were analyzed for concentrations of NH_4^+, NO_3^-, total dissolved N, and total dissolved P using continuous flow analysis (CFA, Bran+Luebbe GmbH, Norderstedt, Germany). Dissolved organic nitrogen concentrations were calculated as the difference between total dissolved N and the sum of total inorganic nitrogen (NH_4^++NO_3^-), assuming that NO_2^- concentrations were negligible.

Leaf area index

The LAI was quantified in the plots with two LAI-2000 plant canopy analyzers (LI-COR Inc., Lincoln, NE, USA). The LAI measurements were conducted in the remote mode, i.e. by synchronous readings below the canopy at 2 m height above the forest floor and in a nearby open area ("above-canopy" reading) using two devices. One measurement was done above each litter trap and a second at the same time outside the forest. Measurements were done in January 2008 (before 1st fertilization) and in January 2009 (one year after the 1st fertilization).

Stem diameter growth and basal area growth per plot

The stem diameter growth of all trees present with a dbh≥10 cm was monitored in the 16 plots every six weeks with permanent girth-increment tapes (D1 dendrometer, UMS, Munich; 713 stems in total). The cumulative increase in plot basal area per year was calculated as the sum of all tree basal area increments in a plot between February 15th, 2008 (after the first fertilization) and January 19th, 2009.

Fine root biomass

For measuring fine root biomass, we took six root samples per plot to a depth of 20 cm using a soil corer of 3.5 cm in diameter in January 2009. The soil samples were transferred to plastic bags and transported to the laboratory, where processing of the stored samples (4°C) took place within six weeks. In the lab, the samples were soaked in water and cleaned from soil residues using a sieve

with a mesh size of 0.25 mm. Only fine roots (roots <2 mm in diameter) of trees were considered for analysis. Live fine roots (biomass) were separated from dead rootlets (necromass) under the stereomicroscope based on color, root elasticity, and the degree of cohesion of cortex, periderm and stele [78–79]. The fine root biomass of each sample was dried at 70°C for 48 h and weighed.

Mycorrhiza

Root colonization by arbuscular mycorrhizal fungi was measured at 200× magnification following clearing and staining with 0.05% Trypan Blue according to [80], additionally including fungal structures as defined in [81].

Statistical analyses

The effects of N and/or P addition on the various investigated parameters were expressed by a response ratio metric ($RR_X = ln$ (measured value in nutrient addition treatment/measured value in the control)) [8] in order to compare the response of plant- and soil-related state variables or flux parameters in relative terms. Non-transformed data are shown in Table S2.

Effects of the addition of N and/or P on individual parameters were analyzed using linear mixed models (package lme4, R version 2.13.0) [82]. We included the fertilization treatments as fixed effects and the factor "block" as a random factor in the models, since in most parameters, samples were nested within plots. P-values for the fixed effects were calculated with the "cftest" function of the package "multcomp" (R version 2.13.0) [82].

Supporting Information

Figure S1 Location of the study area in southern Ecuador and outline of the Ecuadorian Nutrient Manipulation EXperiment (NUMEX).
(DOC)

Table S1 Soil nutrient status of the experimental plots in July 2007 prior to the first fertilization.
(DOC)

Table S2 Ranges and means of all parameters shown in Figures 1–5.
(DOC)

Author Contributions

Conceived and designed the experiments: JH DH CL MM MCR SS EV WW. Performed the experiments: JH DH TC NLC GOM NP MCR DS HW. Analyzed the data: JH DH TC GOM DS HW. Wrote the paper: JH DH CL SS WW.

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