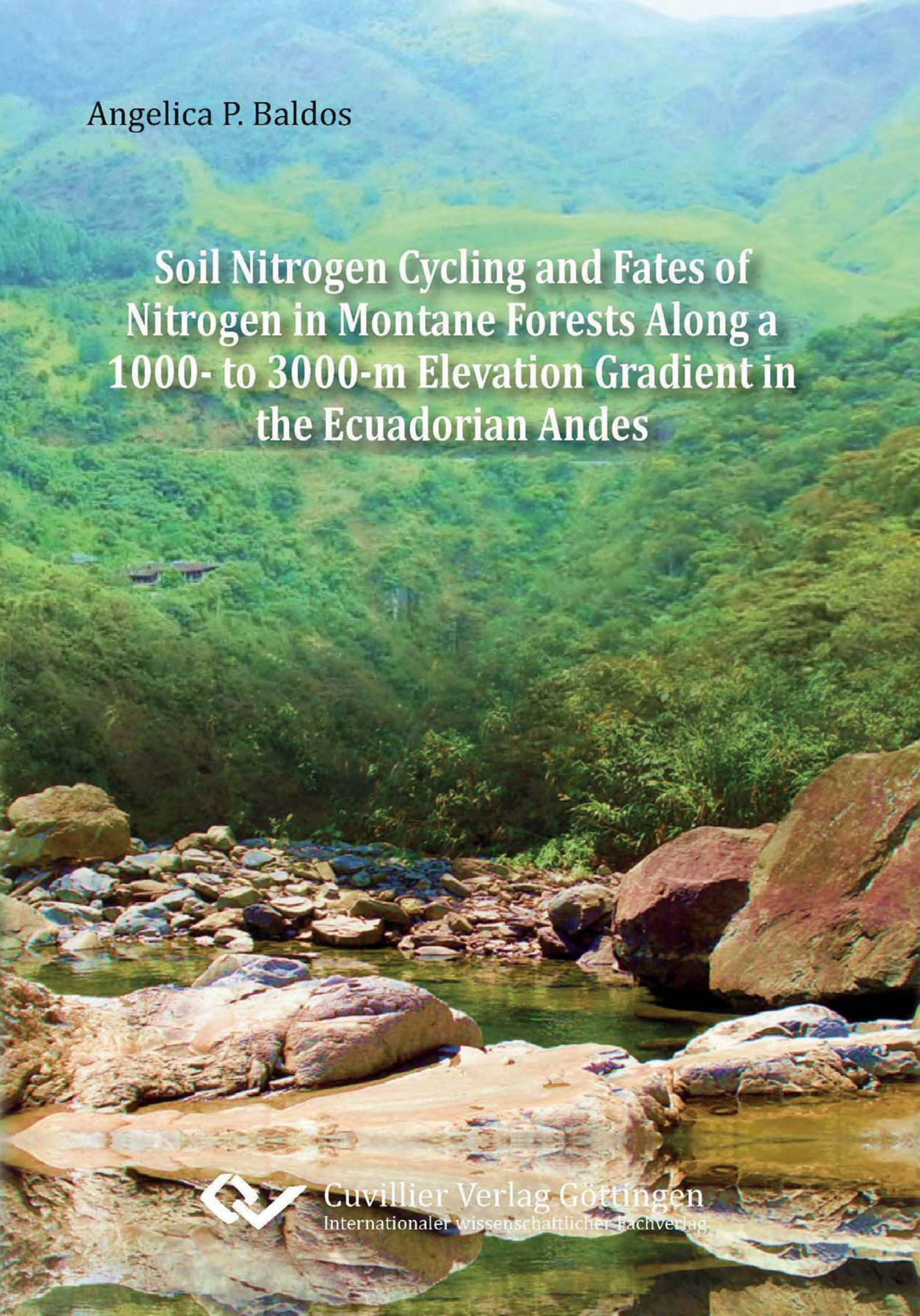
Angelica P. Baldos

Soil Nitrogen Cycling and Fates of Nitrogen in Montane Forests Along a 1000- to 3000-m Elevation Gradient in the Ecuadorian Andes



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Soil Nitrogen Cycling and Fates of Nitrogen in Montane Forests Along a 1000- to 3000-m Elevation Gradient in the Ecuadorian Andes

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born in Tacloban, Philippines

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For everyone who asks, receives; the one who seeks, finds; and to the one who knocks, the door will be opened. - *Matthew 7:8*

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SUMMARY

Tropical montane rainforests host an exceptional number of threatened species, are important water sources, and also contain large carbon pools. In addition, tropical forests are both sinks and sources of important greenhouses gases (GHGs) and is the largest natural source of a potent greenhouse gas, nitrous oxide (N_2O). Threats to tropical montane forests are mostly anthropogenic in nature; added to these is the increasing atmospheric nutrient deposition which the tropics is facing today mainly from fertilizer and fossil fuel use and biomass burning. This is significant in terms of the global nitrogen (N) cycle whose critical product is a potent greenhouse gas, N_2O. Also, the N cycle is always tied to the carbon (C) cycle and climate, making these major biogeochemical cycles more complex. The global N cycle is one of the most anthropogenically altered nutrient cycles on earth. Although the soil internal N cycle plays an important role in the regulation of N retention and loss, there is still a scarcity of information how the increasing nutrient deposition will impact the soil N cycle as well as the long-term fates of soil mineral N in tropical montane forests.

Against this background, we assessed changes in the soil N cycle and characterized the fluxes and fates of mineral N in neotropical montane forests under elevated nutrients inputs by means of a nutrient manipulation experiment (i.e. N, P, N+P, and control). Starting in 2008, a fertilization experiment was conducted in montane forests in Ecuador at 1000 m, 2000 m and 3000 m elevations. Each elevation had four replicate plots (20 m x 20 m each) of these treatments: control, N (50 kg N ha^{-1} $year^{-1}$), P (10 kg P ha^{-1} $year^{-1}$) and combined N+P. Two field experiments were conducted for this thesis. The first experiment aimed to assess changes in gross rates of N production (N mineralization and nitrification) and retention: microbial immobilization of ammonium (NH_4^+) and nitrate (NO_3^-) and dissimilatory NO_3^- reduction to NH_4^+ (DNRA), with low additions of N, P and their combination, and identify the factors controlling these soil N-cycling rates at each elevation and across the elevation gradient. The second experiment aimed to assess the fluxes of NH_4^+ and NO_3^- in different component pools of

soil N and their short-term and long-term fates in tropical montane forest soils and determine the effects of chronic N addition on the net fluxes and fates of NH_4^+ and NO_3^-.

In the first experiment, we measured gross rates of soil N cycling in 2010 and 2011 using ^{15}N pool dilution techniques with in-situ incubation of intact soil cores. In control plots, gross rates of soil N cycling decreased with elevation increase, and microbial N retention rates were tightly coupled with mineral N production rates. At 1000 m and 2000 m, N additions increased gross N production rates and decreased microbial immobilization of NH_4^+ and NO_3^- and DNRA while at 3000 m, N additions increased gross N mineralization rates and decreased DNRA; NH_4^+ and NO_3^- immobilization rates were lower than their production rates. At all elevations, decreased microbial retention of N was accompanied by decreased microbial biomass C and microbial C:N ratio. P addition did not affect any of the soil N-cycling processes. Our results signified that four years of N addition, at a rate expected to occur at these sites, decoupled microbial N production from N retention, indicating potential for N losses. The change from closely coupled to leaky soil N cycling in response to N addition suggests deleterious effects of N losses. The responses of soil N-cycling processes were consistent across elevations, suggesting vulnerability of tropical montane forests to increases in N deposition.

In the second experiment, we assessed the fluxes and fates of NH_4^+ and NO_3^- in different component pools of soil N and their short-term and long-term fates in tropical montane forest soils under elevated N inputs. At 1000 m and 3000 m elevations in the control and N-fertilized plots only, we traced the fates of NH_4^+ and NO_3^- for one year (started in 2010 and ended in 2011) using ^{15}N tracers ($^{15}NH_4^+$ and $^{15}NO_3^-$). In these montane forest soils, both forms of inorganic N were equally utilized relative to their internal supply, indicating a lack of preferential retention of either form of mineral N. Patterns of N uptake by microbes and plant roots might be influenced more by low N availability rather than the energetic advantages associated with using the more reduced forms of N for growth. The influx of mineral N into the soil organic N pool was largely contributed by turn-over of microbes at the 1000 m elevation and by both microbes and fine roots at the 3000 m elevation indicating that microbial turn-over and release of N from fine-roots are important controls in the retention of N in the soil organic N

pool of these tropical montane forests. In these N-poor montane forests the form of inorganic N is unimportant to the long-term retention of N. The long-term fate of N in the soil organic N pool was the result of the rapid influx of both forms of inorganic N, mainly through microbes and fine roots. Under N addition, N retention decreased in the microbial and fine root pools which consequently decreased N retention in the soil organic N pools. N addition seemed to have shifted the importance of microbial and plant controls on the influx of N in the soil organic N pool. The reduced N retention under elevated N inputs indicate potential for N losses and also highlight the central role of biotic controls on the storage of N in soils as well as the role of the stored N in C cycling.

The relatively rapid response (i.e. within 4 years) of the soil internal N cycle and the shift in importance of microbial and plant controls on the retention of N in soil, suggest that greater attention be paid to the biological implications of increased soil N availability and decreased microbial N retention in response to increase in atmospheric N deposition onto montane forests. Many of these responses may be only observed after several years. Our results support the importance of large scale long-term manipulation experiments. Further investigations should focus on 1) the long-term fates of N, 2) effects of elevated N-input on soil chemical characteristics and microbial community structure and function and 3) the stability or losses of soil organic matter and rock-derived nutrients. This will increase our understanding of how increases in N deposition affect the biogeochemistry of tropical montane forests.

ZUSAMMENFASSUNG

Tropische Bergregenwälder weisen eine außergewöhnliche Anzahl bedrohter Arten auf, sind wichtige Wasserquellen und beinhalten große Kohlenstoffvorräte. Zusätzlich sind tropische Wälder zugleich Senken und Quellen wichtiger Treibhausgase (GHG) und die größte natürliche Quelle eines starken Treibhausgases, dem Lachgas (N_2O). Bedrohungen von tropischen Bergwäldern sind meist anthropogener Natur; dazu kommt die steigende atmosphärische Nährstoff-Deposition, die den Tropen heute hauptsächlich durch Düngemittel, Nutzung fossiler Brennstoffe und Biomasse-Verbrennung zu Teil wird. Dies ist insofern bedeutsam, dass N2O ein entscheidendes Produkt im globalen Stickstoff (N)-Kreislaufs ist. Auch ist der N-Kreislauf immer mit dem Kohlenstoff (C)-Kreislauf und dem Klima gekoppelt und erhöht die Komplexität dieser wichtigen biogeochemischen Kreisläufe. Der globale N-Kreislauf ist einer der am stärksten anthropogen veränderten Nährstoffkreisläufe der Erde. Obwohl der bodeninterne N-Kreislauf eine wichtige Rolle bei der Regulation der N-Rückhaltung und des N-Verlustes spielt, gibt es immer noch wenige Informationen darüber, die die verstärkte Nährstoff-Deposition den N-Kreislauf beeinflussen wird, als auch über den langfristigen Verbleib von mineralischem Stickstoff im Boden tropischer Bergwälder.

Vor diesem Hintergrund erhoben wir die Änderungen im Boden-N-Kreislauf und charakterisierten die Flüsse und den Verbleib von mineralischem N in neotropischen Bergwäldern unter erhöhten Nährstoffeinträgen mit Hilfe eines Nährstoff-Manipulations-Experiments (d.h. N, P, N+P und Standard). Ein 2008 gestartetes Düngungs-Experiment wurde in Bergwäldern Ecuadors auf 1000 m, 2000 m und 3000 m Höhe durchgeführt. Jede Höhe wies vier Plot-Wiederholungen (je 20 m x 20 m) folgender Behandlung auf: Standard, N (50 kg N ha^{-1} $Jahr^{-1}$), P (10 kg P ha^{-1} $Jahr^{-1}$) und N+P kombiniert. Für diese Abschlussarbeit wurden zwei Feldexperimente durchgeführt. Das erste Experiment zielte darauf ab, Änderungen in den Bruttoraten von N-Produktion und N-Rückhaltung bei geringer Zufuhr von N, P und ihrer Kombination zu ermitteln. Zur N-Produktion gehören N-Mineralisation und Nitrifikation und zur N-Retention gehören mikrobielle Immobilisierung von Ammonium (NH_4^+) und Nitrat (NO_3^-)

sowie dissimilatorische NO_3^--Reduktion zu NH_4^+ (DNRA). Außerdem war es das Ziel, Faktoren zu identifizieren, die diese N-Kreislaufraten auf jeder Höhe einzeln und über den Gradienten der Höhe kontrollieren. Das zweite Experiment zielte darauf ab, Flüsse von NH_4^+ und NO_3^- in verschiedenen Teilen des Boden-Stickstoffs zu ermitteln und ihren kurzfristigen und langfristigen Verbleib in tropischen Bergwäldern zu ermitteln. Außerdem sollten die Effekte chronischer N-Zufuhr auf die Nettoflüsse und den Verbleib von NH_4^+ und NO_3^- bestimmt werden.

Im ersten Experiment maßen wir die Bruttoraten des Boden-N-Kreislaufs in 2010 und 2011 mit der ^{15}N pool dilution-Technik mit in-situ-Inkubation intakter Bodenzylinder. In den Standard-Plots verringerten sich die Bruttoraten des N-Kreislaufs mit steigender Höhe, und mikrobielle N-Rückhaltungsraten waren eng mit mineralischen N-Produktionsraten gekoppelt. Auf 1000 m und 2000 m erhöhte die N-Zufuhr die Brutto-N-Produktionsraten und verringerte mikrobielle Immobilisation von NH_4^+ und NO_3^- und DNRA - während N-Zufuhr auf 3000 m die N Mineralisationsraten erhöhte und DNRA verringerte; NH_4^+ und NO_3^--Immobilisationsraten waren geringer als ihre Produktionsraten. Auf allen Höhen wurde verringerte mikrobielle N-Rückhaltung von verringerter mikrobieller C-Biomasse und verringertem C:N-Verhältnis begleitet. P-Zufuhr beeinflusste keinen der N-Kreislaufprozesse. Unsere Ergebnisse zeigen, dass vier Jahre N-Zufuhr mit einer für diese Gebiete erwarteten Rate die mikrobielle N-Produktion von der N-Rückhaltung entkoppelt hat, was auf potenzielle N-Verluste hindeutet. Der Wechsel von einem eng gekoppelten zu einem durchlässigen N-Kreislauf als Reaktion auf N-Zufuhr legt einen schädlichen Effekt von N-Verlust nahe. Die Reaktionen der Boden-N-Kreislauf-Prozesse waren über die Höhen konsistent, was auf Störanfälligkeit der tropischen Bergwälder auf erhöhte N-Deposition hindeutet.

Im zweiten Experiment maßen wir die Flüsse und den Verbleib von NH_4^+ und NO_3^- in verschieden Teilen des Boden-Stickstoffs und ihren kurzfristigen und langfristigen Verbleib in tropischen Bergwald-Böden unter erhöhter N-Zufuhr. Nur auf 1000 m und 3000 m Höhe in den Standard-Plots und N-Dünungs-Plots, verfolgten wir den Verbleib von NH_4^+ und NO_3^- für ein Jahr (von 2010 bis 2011) mit ^{15}N-Tracern ($^{15}NH_4^+$ und $^{15}NO_3^-$). In diesen Bergwaldböden wurden

beide Formen anorganischen Stickstoffs gleichsam relativ zu ihren internen Vorräten genutzt, was ein Fehlen bevorzugter Rückhaltung einer der Formen von N andeutet. Muster der N-Aufnahme von Mikroorganismen und Pflanzenwurzeln könnten eher durch N-Verfügbarkeit als den energetischen Vorteilen beim Wachsen, die mit der Nutzung reduzierterer Formen von N verbunden sind, beeinflusst sein. Der Zufluss des mineralischen N in die organischen Boden-N-Vorräte wurde Großteils vom Umsatz der Mikroben auf 1000 m Höhe und von Mikroben und Feinwurzeln auf 3000 m beigesteuert. Dies deutet an, dass mikrobieller Umsatz und die Freigabe von N aus Feinwurzeln wichtige Kontrollfaktoren in der Rückhaltung von N im organischen Boden-N-Vorrat dieser tropischen Bergwälder sind. In diesen N-armen Bergwäldern ist die Form des anorganischen N unwichtig für die Langzeit-Rückhaltung von N. Der langfristige Verbleib von N im organischen Boden-N-Vorrat war das Resultat des rapiden Zuflusses beider anorganischer N-Formen, hauptsächlich durch Mikroben und Feinwurzeln. Unter N-Zufuhr verringerte sich die N-Rückhaltung in den mikrobiellen und den Feinwurzel-Vorräten. N-Zufuhr scheint die Wichtigkeit der mikrobiellen und pflanzlichen Kontrollfaktoren über den Zufluss von N in den organischen Boden-N-Vorrat verlagert zu haben. Die reduzierte N-Rückhaltung unter erhöhten N-Einträgen deutet Potenzial für N-Verluste an und verdeutlicht die zentrale Rolle der biotischen Kontrollfaktoren auf die Speicherung von N in Böden sowie die Rolle des gespeicherten N im C-Kreislauf.

Die relativ schnelle Reaktion (d.h. innerhalb von 4 Jahren) des internen Boden-N-Kreislaufs und die Verlagerung der Wichtigkeit von mikrobiellen und pflanzlichen Kontrollfaktoren auf die Rückhaltung von N im Boden deutet darauf hin, dass größere Aufmerksamkeit auf die biologischen Implikationen erhöhter N-Verfügbarkeit und verringerter mikrobieller N-Rückhaltung als Reaktion auf erhöhte atmosphärische N-Deposition in Bergwäldern verwendet werden sollte. Viele dieser Reaktionen könnten erst nach mehreren Jahren beobachtbar sein. Unsere Ergebnisse unterstützen die Wichtigkeit von großskalierten, langfristigen Manipulations-Experimenten. Weitere Untersuchungen sollten den Fokus auf 1) den langfristigen Verbleib von N, 2) die Effekte erhöhten N-Inputs auf bodenchemische Eigenschaften und mikrobielle Gemeinschaftsstruktur und -funktion und 3) die Stabilität oder

Verluste organischen Bodenmaterials und gesteinsbürtiger Nährstoffe legen. Dies wird unser Verständnis darüber erweitern, wie die Erhöhung der N-Deposition die Biogeochemie von tropischen Bergwäldern beeinflusst.

CHAPTER I General Introduction

Tropical montane forests

Tropical montane forests are mostly found at elevations ranging from 1500 – 3500 meters above sea level (masl) in large inland mountains (e.g. Andes), but could be found also at lower elevations in areas close to the coast and in insular mountains (Bubb et al. 2004; Hamilton 1995). With increasing altitude in montane areas, temperature (Walker and Flenley 1979) and amount of precipitation (Bendix et al. 2008) change. Unlike the forests found in lower elevations in the humid tropics, vegetation in the montane forests is usually characterized by the reduced stature of trees, its trunks and branches often gnarled and twisted, crowns that are dense and compact, and with sclerophyllous leaves. Stem density also increases with elevation, with lichens, mosses, bryophytes, and filmy ferns becoming common and comprising a high proportion of the biomass (Nadkarni 1984). Soil is often wet or waterlogged with thick organic layers and consequently a slow release of mineral nutrients (Edwards and Grubb 1977; Tanner et al. 1998), that points to a possible nutrient limitation in these ecosystems. In the eastern Andean tropical montane forests, organic layer thickness increase with elevation (Wilcke et al. 2002), and this has been tied to the soil N availability of this ecosystem (Wolf et al. 2012).

There are several threats to tropical montane forests, mostly anthropogenic in nature, including the increasing atmospheric nutrient deposition (which include key nutrients such as nitrogen (N) and, phosphorus (P)), that the tropics are facing today (Hietz et al. 2011; Homeier et al. 2012) mainly from fertilizer and fossil fuel use (Galloway et al. 1994, 2008) and biomass burning (Crutzen and Andreae 1990; Cochrane 2003). Biomass burning is a major contributor to the increased deposition of nutrients, including N, especially in Ecuador (Fabian et al. 2005).

Anthropogenic alterations to the global N and P cycles

All ecosystems receive N inputs from atmospheric deposition. These inputs are often small, ranging from 1 to 5 kg ha^{-1} $year^{-1}$, in the ecosystems downwind from pollution-free open-ocean waters (Hedin et al. 1995). Human activities are now the major source of N deposited in many

areas of the world (Chapin et al. 2011). Inputs from anthropogenic sources of N to ecosystems can be quite large, for example 10 to 20 kg ha^{-1} $year^{-1}$ in northeastern US or 50 to 100 kg ha^{-1} yr^{-1} in northern China (Chapin et al. 2011). In Ecuadorian montane forests, the annual N deposition rate for the period 1998 to 2010 was 14 to 45 kg N ha^{-1} $year^{-1}$ (Homeier et al. 2012). Anthropogenic activities such as land-use changes and biomass burning have accelerated the movement of N not only within, but also between ecosystems (Vitousek et al. 1997). The distribution of fixed N can range from regional (mineral N deposition on land) to global (production of GHGs) and fixed N can occur in various forms. The Haber process fixes more N than any other anthropogenic process, and projections are that it will reach 165 Tg $year^{-1}$ by 2050 (Galloway et al. 2004). Elevated N input to terrestrial ecosystems can change the plant species composition (Vitousek et al. 1997), decrease plant diversity (Bobbink et al. 1998; Phoenix 2006), can lead to soil acidification (van Breemen et al. 1982) and declining soil fertility, pollute ground and surface waters (Aber et al. 1998; Schulze 1989), contribute to the formation of acid rain and smog through increased emissions of N_2O and NO (Vitousek et al. 1997).

Unlike carbon (C)and N, phosphorus (P) has only a tiny gaseous component and no biotic pathway that brings the new P into ecosystems. Until recently, available P in ecosystems were derived from organic forms and recycled tightly within terrestrial ecosystems. The physical transfers of P globally are constrained by a lack of gaseous component. P moves around the atmosphere primarily through wind erosion and runoff of particulates in rivers and streams to oceans. Global atmospheric P deposition is estimated to be around 3 Tg $year^{-1}$ (Smil 2000; Ruttenberg 2004) of which 4.8% of this is anthropogenic in source (Mahowald et al. 2008). In Ecuadorian montane forests, the annual P deposition rate was 0.4 to 4.9 kg P ha^{-1} $year^{-1}$ in 1998 to 2010 (Homeier et al. 2012). The major alteration to the global P cycle caused by anthropogenic activities is the acceleration of the entry of P into the biosphere from the mining of P-bearing rock such as phosphate apatite, and loss of P from soil into aquatic ecosystems (Bennett & Carpenter 2002). Because the P commonly limits production in lakes, P fertilization

of freshwater ecosystems can lead to eutrophication and associated negative consequences for aquatic organisms and humans.

Impacts of elevated nutrient deposition in tropical forests

Tropical forests are both sinks and sources of important greenhouse gases (GHGs) because they generate a third of the global net primary production (Field et al. 1998; Malhi and Phillips 2004) and is the largest natural source of a potent GHG, nitrous oxide (N_2O) (Bouwman et al. 1995). Although biogeochemical cycles in tropical forest ecosystems are highly vulnerable to climate change (Malhi & Phillips 2004), the soil N cycle that regulates the production of these trace gas fluxes as well as the availability of N to plants have rarely been studied in tropical montane forests.

Increased N inputs to tropical forest soils have been tied to increases in soil N-oxide ($NO + N_2O$) emissions, nitrate (NO_3^-) leaching, decrease in microbial N immobilization, and differential changes in plant productivity, soil carbon dioxide (CO_2) fluxes and soil carbon (C) dynamics (e.g. Hall and Matson 2003; Lohse and Matson 2005; Koehler et al. 2009a, 2009b; Corre et al. 2010, 2013; Cusack et al. 2011a; Wright et al. 2011). On the other hand, studies on how P addition affects soil N-cycling in tropical montane forests are rare, and to our knowledge only one study so far reported on P-addition effects on net rates of soil N cycling and N_2O emissions where net rates of soil N-cycling and soil N_2O emissions were not affected by P addition alone but only by combined N + P addition (Martinson et al. 2013). These results suggest deleterious consequences on the environment. These processes are strongly controlled by internal transformations of N in the soil, illustrating the importance of quantifying gross rates of soil N-cycling, which separate mineral N production processes from N retention processes, in order to understand the mechanisms of how changes in soil N-cycling rates with elevated N input result in changes in N losses.

Studies on retention and fates of N in tropical forests are rare because the studies conducted so far have only traced ^{15}N within days (e.g. Templer et al. 2008) but are relatively well-studied in some temperate forest ecosystems. Temperate forests generally demonstrate

that the soil is an important and large sink of N over a long-term period (e.g. Emmett and Quarmby 1992; Buchmann et al. 1996; Magill et al. 1997; Nadelhoffer et al. 1999). On the other hand, in a subtropical lower montane forest in Puerto Rico, NO_3^- transformation processes (i.e. nitrification with subsequent dissimilatory NO_3^- reduction to NH_4^+ and uptake of NH_4^+ by plants) play a major role of N retention during a 7-day ^{15}N-tracing study (Templer et al. 2008). A meta-analysis of ^{15}N tracing studies conducted across ecosystem types show that N retention was influenced by ecosystem type, vegetation type, mycorrhyzal type, soil C:N ratio, disturbance history and even the method of ^{15}N application (Templer et al. 2012). They also showed that above a certain threshold of added N (i.e. 46 kg N ha^{-1} $year^{-1}$) total ecosystem N retention decreased. Since studies assessing short-term N cycles mostly exclude competition from plant uptake it is also of interest to study the long-term N cycling, which involves several short-term N-cycling events and the recycling of N within the plant-soil-microbe system. This links the short-term fates of N into long-term patterns of retention in an ecosystem. Because the N cycle is closely linked with the C cycle, the fates of N in the soil influence the ability of the soil to sequester C (Cusack et al. 2011b; Templer et al. 2012). How increase in nutrient deposition affects the long-term fates of soil mineral N in tropical montane forest soils remain poorly understood.

The N cycle

The N cycle (Fig. 1) describes the movement and the transformation of N_2 into various organic or inorganic forms in various oxidation states in the atmosphere, biosphere, and geosphere interface, each of which has consequences for the ecosystem (Brady and Weil 2002). The major N transformations, which are N fixation, N assimilation, N mineralization, nitrification, and denitrification are mainly facilitated by microbes (Fig. 1).

N-fixation is the conversion of N_2 to biologically available forms (Eq. 1). N fixation can be done either by lightning or by nitrogen-fixing organisms. The energy from lightning causes nitrogen (N_2) and water (H_2O) to combine to form ammonia (NH_3) and nitrates (NO_3^-). Biological N fixation occurs when atmospheric N_2 is converted to NH_3 by an enzyme called

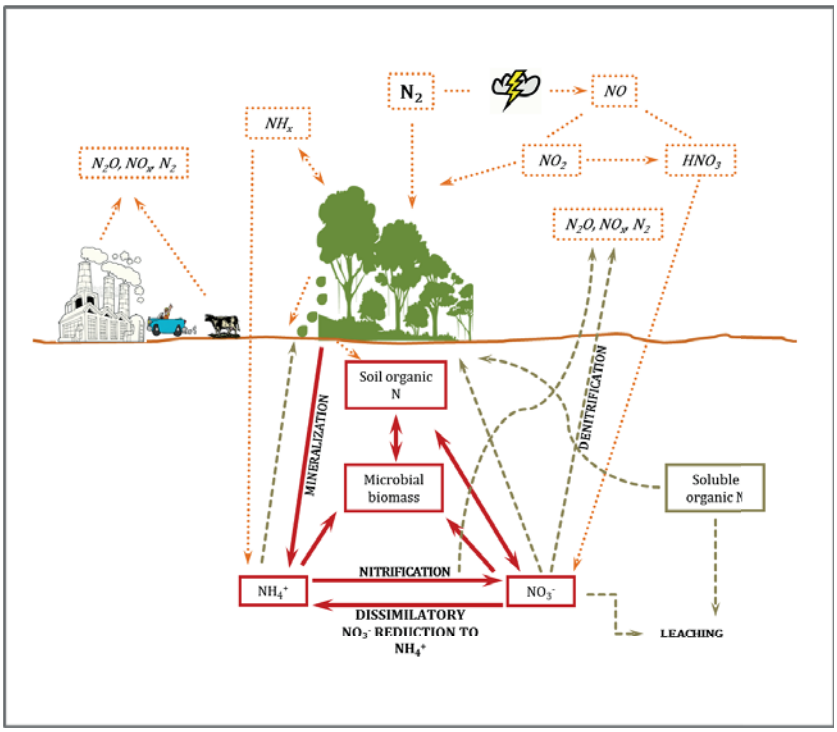

Fig. 1 A simplified model of the nitrogen cycle showing the N transformations in the atmosphere and N inputs to the N cycle (orange arrows), the soil N cycle (red arrows), N assimilation and losses from the soil N cycle (green arrows). (Adapted from Arnold 2008, PhD dissertation)

nitrogenase. The process is coupled to the hydrolysis of 16 equivalents of ATP and is accompanied by the co-formation of one molecule of H_2.

Eq. 1 $N_2 + 8H + 8e^- \rightarrow 2\ NH_3 + H_2$

Fixed N is then converted to NH_4^+ (Eq. 2). NH_4^+ and NH_3 are closely related. NH_4^+ is a positively charged ion (cation) and is therefore attracted to negatively charged soil particles hence it is

Eq. 2 $NH_3 + H_2O \rightarrow NH_4^+ + OH^-$

held as an exchangeable cation in soil and does not easily leach. NH_3 on the other hand is a gas that once formed can quickly escape from soil to the air. ***Nitrification*** is the conversion of NH_4^+ to NO_3^-, where NH_4^+ is first oxidized to NO_2^- (Eq. 3) performed by oxidizing bacteria and *Archaea*, then NO_2^- is further oxidized to NO_3^- (Eq. 4) done by the bacteria *Nitrobacter*. Oxidation of NO_2^- usually proceeds at a more rapid rate so NO_2^- is a relatively rare form of inorganic soil N. NO_3^- is a negatively charged anion and is highly soluble so it moves with water and can easily be leached. NH_4^+ and NO_3^- make up the bulk of soil inorganic N and are the principal forms available for plant growth (e.g. Walworth 2013).

Eq. 3 $2\ NH_4^+ + 3O_2 \rightarrow 2\ NO_2^- + 2\ H_2O + 4H^+$

Eq. 4 $2\ NO_2^- + O_2 \rightarrow 2\ NO_3^-$

Plants ***assimilate N*** in the form of NH_4^+ or NO_3^- or they may also assimilate dissolved organic N (DON). The transformation of organic N into NH_4^+, when the decomposers including bacteria, fungi and protozoa use up the amino groups of dead biomass is N ***mineralization***.

NO_3^- can be chemically reduced to through the process of denitrification. ***Denitrification*** is the transformation of NO_3^- and NO_2^- to N_2 (Eq. 5). This process (Eq. 6) is anaerobic and carried out by denitrifying bacteria such as *Pseudomonas*. This process is important in that it removes fixed N (i.e. NO_3^-) from the ecosystem and returns it to the atmosphere in a biologically inert

Eq. 6 $NO_3^- \rightarrow NO_2^- \rightarrow NO + N_2O \rightarrow N_2$

Eq. 7 $2\ NO_3^- + 10e^- + 12\ H^+ \rightarrow N_2 + 6\ H_2O$

form (N_2) and also plays an important role in the removal of unwanted NO_3^-in bodies of water where NO_3^- accumulation might lead to undesirable consequences such as algal blooms (e.g. Bernhard 2012). However during denitrification, gaseous N losses occur in the form of N-oxides that may contribute to environmental pollution and global warming.

One major part of the N cycle is the soil internal N cycle (Fig. 1, red arrows) because it regulates nutrient availability to plants as well as the loss of potentially harmful N through emissions of GHGs and the leaching of NO_3^-. Beside N mineralization and nitrification, there are three other transformation processes that are important in the N cycling within the soil. First, is microbial immobilization of mineral and organic N through the incorporation into microbial biomass that is released again after the organisms die. Second, abiotic NH_4^+ and NO_3^- retention by NH_4^+ fixation of clay minerals (Davidson et al. 1991) or physical condensation reactions with phenolic compounds (Nömmik 1970; Nömmik and Vahtras 1982; Johnson et al. 2000), and NO_3^- reduction to NO_2^-, which readily reacts with soil organic matter (Smith and Chalk 1980; Azhar et al. 1986; Thorn and Mikita 2000). Third, the conversion of NO_3^- to NO_2^-, and then to NH_4^+ which is known as the dissimilatory NO_3^- reduction to NH_4^+ (DNRA) (Silver et al. 2001, 2005; Sotta et al. 2008).

The NH_4^+ produced by N mineralization has several potential fates. In addition to being absorbed by plants or microbes, NH_4^+ readily absorbs to the negatively charged surfaces of soil minerals and organic matter, reducing the concentration of NH_4^+ in the soil solution. NH_4^+ can also be oxidized mainly by bacteria to NO_2^- and NO_3^- or converted to NH_3, and lost to the atmosphere. The potential fates of NO_3^- are being absorbed by plants and microbes, exchanged on anion exchange sites, or loss from the ecosystems via denitrification or leaching. Some microbes also absorb NO_3^- and reduce it to NH_4^+ through DNRA to NH_4^+. Gaseous losses of N from ecosystems course through major processes like NH_3 volatilization, nitrification, and denitrification. These processes release N as NH_3 gas, N_2O, NO, and N_2 and the gas fluxes are controlled by the rates of soil processes and by soil and environmental characteristics that regulate diffusion rates through soils. N is lost by leaching of DON from all ecosystems and as NO_3^- from NO_3^- rich ecosystems. NO_3^- leached from terrestrial ecosystems moves into groundwater to lakes and rivers, and is subsequently lost to the atmosphere through denitrification or transported to the ocean (Chapin et al. 2011).

Hypotheses

This thesis has two major experiments. The first study investigates the impact of four years of low additions of N, P, and N + P on gross rates of mineral N production (i.e. N mineralization and nitrification) and retention (microbial immobilization of NH_4^+ and NO_3^-, and DNRA) in three montane forest soils along a 3000-m elevation gradient in the Ecuadorian Andes (Fig. 2). The hypotheses for this experiment are:

(1) in control plots, gross rates of mineral N production are closely coupled with or equal to rates of microbial N retention, and soil N-cycling rates will decrease along the 3000-m elevation gradient

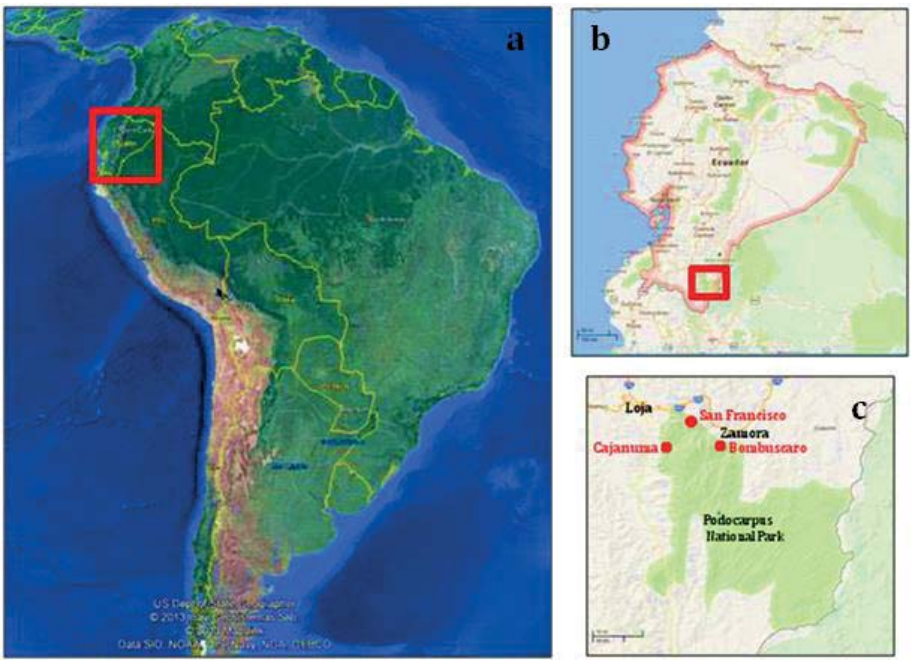

Fig. 2 The study sites. (a) The study was conducted in the eastern slopes of the Ecuadorian Andes. (b) In the south of Ecuador covering the provinces of Loja and Zamora, three sites along an elevation gradient were chosen for the NUMEX. (c) The three sites were inside (Bombuscaro at 1000 masl and Cajanuma at 3000 masl) and adjacent to (San Francisco at 2000 masl) the Podocarpus National Park. Maps are from Google Earth (a) and Google Maps (b, c)

(2) N, P and combined N + P additions may alleviate nutrient constraints on microbial activity and may increase gross rates of mineral N production across the elevation gradient, and consequently

(3) soil N cycling will become uncoupled (i.e. N production rates surpass N retention rates) if the microbial N retention rates does not keep pace with the increase in gross N production rates.

The second study investigates the effects of four years of low N addition on the fates and fluxes of N in a premontane forest at 1000 m elevation on a Cambisol soil that has no organic layer, but only a thin layer of decomposing leaves (Ol layer) covering the mineral soil, and in an upper montane forest at 3000 m elevation on a Histosol soil with a thick organic layer covering the mineral soil, both situated on a 2000-m elevation gradient in the Ecuadorian Andes. The soil N availability (i.e. based on gross rates of N cycling) of these sites were low (Chapter 2) in comparison to the gross N-cycling rates of montane forests in Andosol soils (Arnold et al. 2009; Corre et al. 2013). The hypotheses for this experiment are:

(1) in these montane forest soils with low N availability, NH_4^+ and NO_3^- are retained largely through root and microbial uptake, and

(2) chronic N addition will reduce N retention in these soils

Project objectives

Projected increases in atmospheric N deposition in tropical regions are thought to alter rates of soil N cycling (Galloway et al. 2008) and to my knowledge very few studies have been conducted so far on the impacts of chronic nutrient input in neotropical montane forests. Also, nutrient controls on soil N cycling and the characterization of the fluxes and the fates of N have rarely been studied in neotropical montane forests. Therefore, the main objectives of this study were to identify nutrient controls on soil N cycling and to characterize fluxes and fates of N along a 3000-m elevation gradient in the Ecuadorian Andes by means of a nutrient manipulation experiment (NUMEX; i.e. N, P, N+P, and control) (Fig. 3).

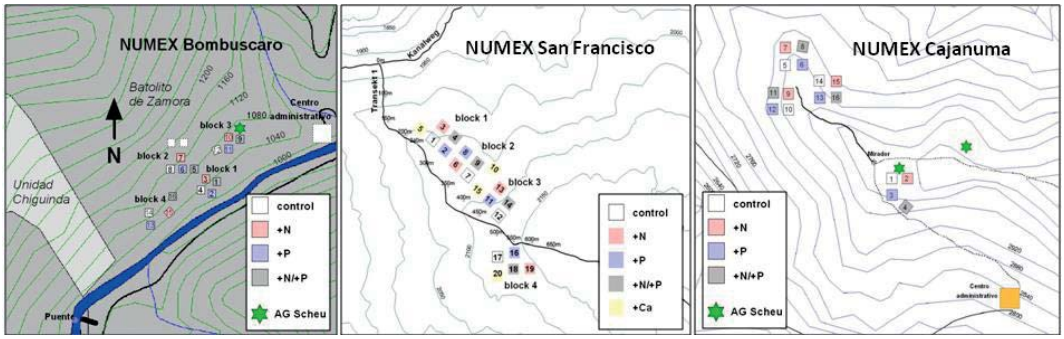

Fig. 3 Experimental design of each study site. Maps from J. Homeier

The objectives for the first study were to:

(1) assess the changes in gross rates of N production (N mineralization and nitrification) and retention (microbial immobilization of NH_4^+ and NO_3^- and dissimilatory NO_3^- reduction to NH_4^+, DNRA) with N, P and combined N + P additions, and

(2) identify the factors controlling these soil N-cycling rates at each elevation and across the elevation gradient.

For the second study, the objectives were to:

(1) assess the net fluxes of NH_4^+ and NO_3^- in the different components of soil N and their short-term and long-term fates in tropical montane forest soils with low N availability, and

(2) determine the effects of chronic N addition on the net fluxes of NH_4^+ and NO_3^- in the different soil N pools and on their fates in these montane forest soils

CHAPTER II Responses of soil nitrogen cycling to nutrient inputs in montane forests along a 3000-m elevation gradient in the Ecuadorian Andes

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An improved version is submitted to Ecology.

Abstract

Large areas in the tropics receive elevated atmospheric nutrient inputs. Presently, little is known on how soil nitrogen (N) cycling in tropical montane forests will respond to such increased nutrient inputs. We assessed how gross rates of N production (N mineralization and nitrification) and retention (microbial immobilization of NH_4^+ and NO_3^- and dissimilatory NO_3^- reduction to NH_4^+ (DNRA)) change with elevated N and phosphorus (P) inputs in montane forest soils at 1000, 2000 and 3000 m elevations in south Ecuador. At each elevation, four replicate plots (20 x 20 m each) of control, N (50 kg N ha^{-1} $year^{-1}$), P (10 kg P ha^{-1} $year^{-1}$) and combined N+P additions were established in 2008. We measured gross N-cycling rates in 2010 and 2011, using ^{15}N pool dilution techniques with in-situ incubation of intact soil cores. In control plots, gross N-cycling rates decreased with increasing elevation, and microbial N retention were tightly coupled with mineral N production. At 1000 m and 2000 m, 4-year N and N+P additions increased gross N production rates and decreased NH_4^+ and NO_3^- immobilization and DNRA relative to the control. At 3000 m, 4-year N and N+P additions increased gross N mineralization rates, decreased DNRA and although NH_4^+ and NO_3^- immobilization were not different from the control plots these rates were lower than their respective production rates. At all elevations, decreased microbial N retention was accompanied by decreased microbial biomass C and C:N ratio. P addition did not affect any of the soil N-cycling processes. Our results signified that four years of N addition, at a rate expected to occur at these sites, uncoupled microbial N production and retention, indicating a potential for N losses. This consistent response of soil N-cycling processes across elevations suggests vulnerability of tropical montane forests to increases in N deposition.

Introduction

Large areas in the tropics receive elevated inputs of nutrients through atmospheric deposition (Hietz et al. 2011; Homeier et al. 2012) that mainly originate from anthropogenic sources such as fertilizer use, fossil fuel (Galloway et al. 1994; 2008) and biomass burning (Crutzen and Andreae, 1990; Cochrane, 2003). Elevated nutrient deposition not only occurs in areas with high population densities but also in areas far from the source of nutrients. For example, southern Ecuadorian montane forests receive elevated atmospheric nitrogen (N) and phosphorus (P) deposition that are largely attributed to biomass burning in the Amazon basin (Fabian et al. 2005). How tropical montane forests react to elevated N and P inputs has been evaluated in field experiments, where N and P were added and the response of ecosystem processes were measured. In most of these studies, relatively high amounts of either N (with rates from 125 to 300 kg N ha^{-1} $year^{-1}$), P (with rates from 50 to 100 kg P ha^{-1} $year^{-1}$) or a combination of N + P were added to the soil. The major focus of these studies was how vegetation reacted to elevated N and P inputs. These studies showed that addition of N, P or combined N + P increased either stem diameter growth, litter production or both in montane forests of Jamaica (at 1600 m elevation on a Histosol soil; Tanner et al. 1990), Venezuela (at 2500 m on a Histosol soil; Tanner et al. 1992), Colombia (at 865 m on a Leptosol soil; Cavelier et al. 2000), Panama (at 1200 m on Andosol soil; Adamek et al. 2009), Puerto Rico (at 640 m on Acrisol soil; Cusack et al. 2011a), and Peru (at 1000 m to 3000 m; Fisher et al. 2013). Fine-root biomass either increased, did not change or decreased with N and/or P addition in montane forests in Panama (at 1200 m on Cambisol and Andosol soils; Cavelier 1992; Adamek et al. 2011), Hawaii (at 1176 m and 1134 m on Inceptisol and Oxisol soils; Ostertag, 2001) and Puerto Rico (Cusack et al. 2011a). For a montane forest at 2000 m elevation on a Cambisol soil in southern Ecuador, one of the sites where our present study was conducted, leaf litter production and stand basal area increased while fine-root biomass decreased in the first year of N, P and combined N + P additions despite the low application rates (50 kg N ha^{-1} $year^{-1}$ and 10 kg P ha^{-1} $year^{-1}$) (Homeier et al. 2012). The fast response of this Ecuadorian montane forest was attributed to accelerated nutrient (e.g. N

and P) cycling between vegetation and soil, suggested by higher N and P return with litterfall, throughfall and percolate below the organic layer (Wullaert et al. 2010; Homeier et al. 2012). Thus, soil N cycling was suggested as the regulator of these above-ground responses.

On the other hand, some of the deleterious consequences of increased N input in tropical forests are increases in soil NO (a precursor to tropospheric ozone formation and acid rain) and N_2O emissions (a potent greenhouse gas) and NO_3^- leaching, which contributes to soil acidification and leaching of base cations (e.g. Matson et al. 1999; Koehler et al. 2009; Corre et al. 2010). These pathways of N losses have in common that they are related to the rates of microbial N cycling in soils. In a montane forest at 1200 m elevation on an Andosol soil in Panama, 1-4 years of N addition (125 kg N ha^{-1} $year^{-1}$) increased gross rates of soil N mineralization and nitrification, leading to increases in soil N-oxide ($NO + N_2O$) emissions and NO_3^- leaching (Koehler et al. 2009; Corre et al. 2010, 2013). Also in Hawaiian montane forests at ~1200 m elevation, 11-13 years of N addition to an N-limited forest on an Andosol soil and 5-7 years of N addition to a P-limited forest on a Ferralsol soil increased gross rates of soil N mineralization and nitrification and decrease microbial N retention, leading to higher soil N-oxide emissions and NO_3^- leaching (Hall and Matson 2003; Lohse and Matson 2005). These studies illustrate the importance of quantifying gross rates of soil N cycling, which separate mineral N production processes from microbial N retention processes, in order to understand the mechanisms of how changes in soil N-cycling rates with elevated N input result in changes in N losses.

Studies on how P addition affects soil N cycling in tropical montane forests are rare, and to our knowledge only one study so far reported on P-addition effects on net rates of soil N cycling and N_2O emissions. Although net rates of soil N cycling only indicate the amounts of mineral N available for plant uptake, these indices are commonly used because its measurement is less laborious and inexpensive. In montane forests along 1000 – 3000 m elevation gradient in southern Ecuador, where our present study was also conducted, net soil N-cycling rates and soil N_2O emissions were not affected by P addition alone but only by combined N + P addition (at 50 kg N ha^{-1} $year^{-1}$ and 10 kg P ha^{-1} $year^{-1}$; Martinson et al. 2013). Furthermore, two years of N and

combined N + P additions in forest at 1000 m elevation on a Cambisol soil did not change its already high net nitrification rate but rapidly increased soil N_2O emissions. At 2000 m elevation on a Cambisol soil and at 3000 m elevation on a Histosol soil, N and combined N + P additions resulted in small increases of the previously undetectable net nitrification activity and soil N_2O emissions but only in the second year of treatment. Together, these results suggest that changes in soil N cycling and the accompanying changes in N_2O emissions, despite with low levels of nutrient additions, depended on the initial soil N status (i.e. fast and large response at 1000 m elevation with large initial net nitrification and small and delayed response at 2000 m and 3000 m elevations with undetectable initial net nitrification). In summary, quantifying the changes in gross rates of soil N cycling in response to changes in nutrient inputs in these montane forest soils in southern Ecuador will provide direct evidence to explain the vegetation response (Homeier et al. 2012), soil N_2O emissions (Martinson et al. 2013) and N leaching (Wullaert et al. 2010).

In our present study, our objectives were to (1) assess the changes in gross rates of N production (N mineralization and nitrification) and retention (microbial immobilization of NH_4^+ and NO_3^- and dissimilatory NO_3^- reduction to NH_4^+, DNRA) with N, P and combined N + P additions, and (2) identify the factors controlling these soil N-cycling rates at each elevation and across the elevation gradient. We report on the impact of 4 years of N, P, and combined N + P additions at low rates (i.e. 50 kg N ha^{-1} $year^{-1}$ and 10 kg P ha^{-1} $year^{-1}$), which are in the range of expected nutrient depositions in these montane forests in southern Ecuador (Homeier et al. 2012). We hypothesized that (1) in control plots gross rates of mineral N production are closely coupled with or equal to rates of microbial N retention, and soil N-cycling rates decrease along 1000-3000 m elevation gradient, (2) N, P and combined N + P additions may alleviate nutrient constraints on microbial activity and may increase gross rates of mineral N production across the elevation gradient, and consequently (3) soil N cycling will become uncoupled (i.e. N production rates surpass N retention rates) if the microbial N retention does not keep pace with the increase in gross N production rates.

Materials and Methods

Site description and soil characteristics

The study sites are within and adjacent to the Podocarpus National Park in the Cordillera de Consuelo, a mountain range forming part of the eastern chain of the Andes. We conducted our study in an on-going nutrient manipulation experiment (NUMEX) that was established in 2008 in southern Ecuador. The NUMEX consists of three sites along an elevation gradient: 990 to 1100 m above sea level (Bombuscaro, 4.115^{O} S, 78.968^{O} W), 1950 to 2100 m (San Francisco, 3.982^{O} S, 79.083^{O} W) and 2900 to 3050 m (Cajanuma, 4.110^{O} S, 79.178^{O} W) (Fig. 2). The site at 1000 m has an old-growth premontane forest growing on a Dystric Cambisol soil from parent material of deeply-weathered granitic rock. The soil has no organic layer, but only a thin layer of decomposing leaves (Ol layer) covers the mineral soil. The site at 2000 m consists of an old-growth lower montane forest growing on a Stagnic Cambisol soil whereas the site at 3000 m has an old-growth upper montane forest growing on a Stagnic Histosol soil; both soils have parent material of metamorphosed schist and are covered by an organic layer ranging in thickness from 10 to 40 cm (Homeier et al. 2008, 2010; Martinson et al. 2013). The annual mean air temperature and precipitation were respectively 19.4 °C and 2.2 m year^{-1} at 1000 m, 15.7 °C and 1.9 m year^{-1} at 2000 m, and 9.4 °C and 4.5 m year^{-1} at 3000 m (Moser et al. 2007). No clear seasonal patterns were observed for precipitation (Emck 2007), soil temperature and soil moisture content at all elevations (Martinson et al. 2013). Ambient nutrient deposition in the area near the site at 2000 m was 14 to 45 kg N ha^{-1} year-1 and 0.4 to 4.9 kg P ha^{-1} year^{-1} from 1998 to 2010 (Homeier et al. 2012). The forest stand characteristics of the three study sites were reported earlier by Martinson et al. (2013).

The initial soil characteristics (measured in 2007 before to the start of treatment) were reported by Martinson et al. (2013) and soil characteristics were determined again in April 2012 after four years of treatment. Organic layer (present only in the sites at 2000 m and 3000 m) and mineral soil (at depths of 0 to 5, 5 to 10, 10 to 25, and 25 to 50 cm) were sampled from one soil profile per replicate plot. Samples were air-dried, ground and analyzed for total C and N using a

CN analyzer (Elementar Vario EL; Elementar Analysis Systems GmbH, Hanau, Germany) and for ^{15}N natural abundance using isotope ratio mass spectrometry (IRMS; Delta Plus, Finnigan MAT, Bremen, Germany). Soil pH was measured from a mixture of soil and distilled water with a ratio of 1:4. Effective cation exchange capacity (ECEC) of the mineral soil was determined from air-dried and sieved (2 mm) soils; these were percolated with unbuffered 1 mol/L NH_4Cl and percolates were analyzed for cation concentrations using an inductively coupled plasma-atomic emission spectrometer (Spectroflame, Spectro Analytical Instruments, Kleve, Germany). Base saturation was calculated as percentage exchangeable base cations. All soil samples were analyzed at the laboratory of Soil Science of Tropical and Subtropical Ecosystems (SSTSE), University of Goettingen, Germany. We did not find any significant differences in these soil characteristics among treatments at each elevation after 4 years of treatment, and we reported the values for the top 5-cm depth in Table 1.

Experimental design

The NUMEX is a full-factorial fertilization experiment, set up in a stratified random design with four replicate blocks where treatments (N-, P-, combined N + P-additions and control) were assigned randomly per block (Fig. 3). The size of each treatment plot was 20 m x 20 m, and plots were separated by at least 10 m. Replicate blocks covered short topographic gradients; in these forests, short topographic gradients may result in differences in nutrient availability and soil conditions (Wolf et al. 2011), and hence they were considered as statistical blocks. We applied N and P at rates which were low compared to other nutrient manipulation experiments in tropical montane forests (e.g. Hall and Matson 2003; Corre et al. 2010). Treatments started in 2008; fertilizers were applied by hand to the plots as evenly as possible at a rate of 50 kg urea-N ha^{-1} $year^{-1}$ and 10 kg P $ha^{-1}year^{-1}$ (analytical grade $NaH_2PO_4.2H_2O$), divided in two equal applications per year.

Gross rates of N production and retention

We used the ^{15}N pool dilution techniques (Davidson et al. 1991) for measurements of gross rates of soil mineral N production: mineralization and nitrification. We conducted these measurements in October 2010 (third year of treatment) for the control and N-addition plots only and in October 2011 (fourth year of treatment) for all treatment plots. In each plot, four intact soil cores (5-cm height and 250-cm^3 volume) were taken at the top 5-cm depth; two were injected with $(^{15}NH_4)_2SO_4$ solution and the other two with $K^{15}NO_3$ solution. Each soil core received five 1-ml injections of the solutions that contained 30 μg N ml^{-1} with 99% ^{15}N enrichment, equivalent to an average rate of 2.6 $\pm$ 0.5 μg N g^{-1}. One soil core from each labeled pair was broken up, mixed well in a plastic bag and subsampled for 0.5 M K_2SO_4 extraction 10 minutes after ^{15}N injection. The other intact soil core from each labeled pair was placed in a plastic bag, inserted back into the soil to incubate in-situ for 1 day and extracted with K_2SO_4. From the 1-day incubated soil cores, microbial immobilization of NH_4^+ and NO_3^- (as measures of microbial N retention) were determined from the $^{15}NH_4^+$- and $^{15}NO_3^-$-labelled cores, respectively, by $CHCl_3$ fumigation immediately after harvesting the cores. A subsample of about 25 g fresh weight were fumigated with $CHCl_3$ for 5 days and afterwards extracted with 0.5 M K_2SO_4. All extracts were immediately frozen and kept frozen during transport by air to Germany, where further analysis was conducted at the SSTSE, University of Goettingen. Concentrations of NH_4^+ (Berthelot reaction method; Skalar Method 155-000), NO_3^- (copper-cadmium reduction method with NH_4Cl buffer but without ethylenediamine tetraacetic acid; Skalar Method 461-000) and organic N in the extracts (persulfate digestion to convert all dissolved N into NO_3^- followed by the same method for NO_3^- analysis) were analyzed using continuous flow injection colorimetry (Cenco/Skalar Instruments, Breda, Netherlands). ^{15}N analysis from NH_4^+, NO_3^- and organic N pools followed the same ^{15}N diffusion procedures described in our earlier studies (e.g. Corre et al. 2007, 2010), and ^{15}N was determined using IRMS (Delta C, Finnigan MAT, Bremen, Germany).

Calculation of gross rates of N mineralization, nitrification, NH_4^+ and NO_3^- immobilization followed the equations given by Davidson et al. (1991). Rates of DNRA (also a

measure of microbial retention for NO_3^-) were calculated from the $^{15}NO_3^-$-injected soil cores following the same calculations used by Silver et al. (2001, 2005). Gravimetric moisture content was determined from each soil core to calculate the dry mass of K_2SO_4-extracted soil. All rates were converted from mass basis to area basis using the average soil bulk densities for each elevation, which were measured in all plots at the top 5-cm depth: 0.84, 0.18 and 0.11 g cm^{-3} for the 1000 m, 2000 m and 3000 m elevations, respectively. We also calculated the relative change in soil N-cycling rates in the N-, P- and N + P-addition plots from the respective control plots at each elevation as: (treatment - control) ÷ control. A similar index was used by Homeier et al. (2012) and Cleveland and Townsend (2006) to indicate the direction and magnitude of change in the processes due to the treatment in relation to the control.

Other supporting variables

Soil microbial biomass C and N in the top 5-cm depth were determined in October 2010 (only in control and N-addition plots) and 2011 (all treatment plots), using the $CHCl_3$ fumigation-extraction method (Brookes et al. 1985; Davidson et al. 1989). From an intact soil core taken from each plot, part was extracted immediately with 0.5 M K_2SO_4 and part was fumigated with $CHCl_3$ for 5 days and then extracted. 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, CA, USA). Organic N in the extracts was determined using persulfate digestion and the digests analyzed using continuous flow injection colorimetry (copper–cadmium reduction method; Skalar Method 461-000; Cenco/Skalar Instruments, Breda, Netherlands). Microbial biomass C and N were calculated as the difference in extractable organic C and N between the fumigated and unfumigated soils divided by $k_C = 0.45$ and $k_N = 0.68$ (Brookes et al. 1985).

From the soil samples taken at the depths of 0-5, 5-10, 10-25 and 25-50 cm in April 2012 (after four years of treatment), the ^{15}N natural abundance signatures were determined and the ^{15}N enrichment factor (ε) for the entire 50-cm depth was calculated using the Rayleigh equation (Mariotti et al. 1981): $\varepsilon = d_s - d_{so} / \ln f$, where d_s is the $\delta^{15}N$ value at various depths in

the soil profile, d_{so} is the $\delta^{15}N$ value of the input substrate (or the reference depth, which we took as the top 5-cm depth) and f is the remaining fraction of total N (i.e. total N at certain a depth divided by the total N at the top 5-cm depth). The ^{15}N natural abundance signatures and ^{15}N enrichment factors of tropical forest soils have been used as an indirect indicator of soil N-cycling status (e.g. Sotta et al. 2008; Arnold et al. 2009; Corre et al. 2010) and soil N_2O emissions (e.g. Purbopuspito et al. 2006; Wolf et al. 2011).

Statistical analysis

Tests for normality using Kolmogorov-Smirnov D statistic and for equality of variance using Levene statistic (Sokal and Rohlf 1981) were first conducted for each parameter. Parameters that showed heterogeneous variance were log transformed. Differences among treatments for each elevation or among elevations for each treatment were assessed using one-way analysis of variance with Tukey-HSD test for multiple comparisons. Differences between the two sampling periods (i.e. October 2010 and 2011) for the control and N-addition plots at each elevation were assessed using Paired T test. Correlations among soil-N cycling rates and soil factors were conducted separately for the control plots across elevations and for all treatments (including the controls) at each elevation using Pearson correlation test. The first was to assess which soil factors affect soil N cycling under unaltered conditions of nutrient availability, and the second was to determine which soil factors affect soil N cycling with four years of altered N and P levels at each elevation. Means and standard errors of the four replicate blocks for each treatment were reported as measures of central tendency and dispersion, respectively. Levels of significance were defined at $P \leq 0.05$. Analyses were conducted using XLStat Pro 2012.6 (Addinsoft SARL 2012).

Table 1 Soil characteristics (mean ± SE, n = 4) of top 5-cm depth (corresponding to mineral soil for the sites at 1000 m and to organic layer for the sites at 2000 m and 3000 m) of montane forest soils across 1000-3000 m elevation gradient after 4 years of treatment

Elevation and treatment[a]	Total C[b] (kg C m^{-2})	Total N[b] (g N m^{-2})	C:N ratio[b]	pH-H_2O[b]	ECEC[c] ($mmol_c$ kg^{-1})	Base saturation (%)	Exchangeable cations for the site at 1000 m (mmolc kg^{-1}) and total cation concentrations (mg g^{-1}) for the sites at 2000 m and 3000 m			
							Ca	Mg	K	Al
1000 m above sea level (masl)										
Control	2.5 ± 0.7	167.5 ± 34.6A	13.7 ± 1.2[B]	4.3 ± 0.2[A]	74.8 ± 18.1	45.3 ± 8.0	23.6 ± 8.3	6.8 ± 1.7	3.5 ± 1.0	32.9 ± 9.7
Nitrogen	2.6 ± 0.6	171.6 ± 26.6	14.1 ± 1.4	3.9 ± 0.2	68.5 ± 7.8	37.8 ± 10.2	11.6 ± 0.5	6.6 ± 3.9	7.7 ± 4.3	35.2 ± 12
Phosphorus	2.1 ± 0.4	139.3 ± 13.3	14.4 ± 1.3	4.3 ± 0.2	74.15 ± 6.0	47.5 ± 17.5	21.8 ± 8.5	9.6 ± 4.9	2.8 ± 0.5	34.5 ± 14.1
Nitrogen + phosphorus	2.5 ± 0.8	181.3 ± 34.8	12.9 ± 1.6	3.8 ± 0.1	72.2 ± 7.3	24.5 ± 4.0	12.9 ± 4.3	4.3 ± 0.6	2.6 ± 0.3	48.3 ± 2.4
2000 masl										
Control	4.4 ± 0.1	166.7 ± 12.1A	26.2 ± 2.2[B]	4 ± 0.1[AB]	-	-	0.3 ± .06	0.4 ± 0.04	0.9 ± 0.05	2.5 ± 0.3
Nitrogen	4.3 ± 0.0	169.3 ± 5.8	25.5 ± 0.9	4 ± 0.2	-	-	0.5 ± 0.2	0.5 ± 0.1	1.0 ± 0.1	4.1 ± 0.6
Phosphorus	4.2 ± 0.1	160.7 ± 4.1	26.5 ± 1.0	3.9 ± 0.1	-	-	0.5 ± .07	0.5 ± 0.03	1.2 ± 0.08	3.4 ± 0.8
Nitrogen + phosphorus	4.3 ± 0.0	167.0 ± 9.9	25.9 ± 1.2	3.7 ± 0.2	-	-	0.5 ± 0.1	0.5 ± 0.05	1.1 ± 0.08	4.3 ± 0.7
3000 masl										
Control	2.6 ± 0.1	75.3 ± 3.7B	34.7 ± 1.4[A]	3.7 ± 0.0[B]	-	-	0.3 ± 0.1	0.1 ± 0.07	2.3 ± 1.4	5.8 ± 2.7
Nitrogen	2.5 ± 0.1	86.1 ± 6.0	28.3 ± 3.0	3.9 ± 0.2	-	-	0.4 ± 0.1	0.3 ± 0.2	2.8 ± 1.3	5.1 ± 2.6
Phosphorus	2.6 ± 0.1	84.5 ± 5.8	31.7 ± 2.5	3.7 ± 0.1	-	-	0.3 ± 0.1	0.4 ± 0.2	1.8 ± 0.9	3.9 ± 1.6
Nitrogen + phosphorus	2.6 ± 0.1	94.7 ± 3.4	27.6 ± 1.7	3.8 ± 0.1	-	-	0.3 ± .03	0.3 ± 0.2	2.5 ± 1	4.8 ± 1.3

[a] Control plots had no fertilizer addition; nitrogen plots were fertilized with 50 kg urea-N ha^{-1} $year^{-1}$; phosphorus plots were fertilized with 10 kg P ha^{-1} $year^{-1}$ of analytical grade $NaH_2PO_4.2H_2O$; nitrogen + phosphorus plots received combined application of the same fertilizers. All fertilizers were split in two equal applications per year and broadcasted by hand. There was no significant treatment effect detected at all elevations.

[b] Means with different capital letters indicate significant differences across the elevation gradient for the control plots (one-way ANOVA with Tukey HSD at $P \leq 0.05$).

[c] ECEC – Effective cation exchange capacity

Results

Gross rates in N production and retention in control forest soils

The two measurement periods of soil N cycling (October 2010 and 2011) did not show significant differences in any of the parameters measured (all $P = 0.30$ to 0.98), and hence values presented in Table 2 were averages of these two sampling periods. With increasing elevation, gross N mineralization ($P = 0.02$), gross nitrification ($P < 0.00$), NH_4^+ immobilization ($P < 0.00$), NO_3^- immobilization ($P < 0.00$), DNRA rates ($P < 0.00$) and microbial C ($P = 0.03$) decreased while microbial C:N ratio increased ($P = 0.05$; Table 2). At each elevation, soil mineral N production rates were not different from microbial N immobilization rates (gross N mineralization = NH_4^+ immobilization, $P = 0.30$ to 0.50; gross nitrification = NO_3^- immobilization, $P = 0.50$ to 0.67; Table 2). DNRA rates were on average 28 %, 46 % and 36 % of gross nitrification rates at 1000 m, 2000 m and 3000 m elevations, respectively, and in comparable percentages in proportion to NO_3^- immobilization rates (28 %, 50 % and 47 % in the same elevation sequence) (Table 2). The ^{15}N natural abundance signatures of soils and ^{15}N enrichment factors over a depth of 50 cm decreased with increasing elevation ($P = 0.05$; Fig. 4). Soil C stock in the top 5 cm did not differ across elevations ($P = 0.10$) whereas the C:N ratio ($P < 0.00$) increased and soil N stocks ($P = 0.02$) and pH ($P = 0.01$) decreased with increasing elevation (Table 1).

Considering all control plots across elevations, gross N mineralization was positively correlated with NH_4^+ immobilization, DNRA and pH but was negatively correlated with microbial C:N ratio. Gross nitrification was positively correlated with NO_3^- immobilization but was negatively correlated with both microbial and soil C:N ratios. Microbial C:N ratio was positively correlated with soil C:N ratio (Table 3). Gravimetric moisture content did not differ across elevations ($P = 0.10$) and was not correlated with any parameters of soil N cycling (data not shown).

Nutrient addition effects on gross rates of N production and retention

The two measurement periods of soil N cycling in the N-addition plots (October 2010 and October 2011) also did not differ in all parameters measured ($P = 0.35$ to 0.80) and these two periods were averaged in assessing the treatment effects at each elevation. At the 1000 m elevation, four years of N and combined N + P additions increased gross N mineralization ($P = 0.03$) and gross nitrification rates ($P = 0.05$), and decreased NH_4^+ immobilization ($P = 0.05$), NO_3^- immobilization ($P = 0.05$), DNRA rates ($P = 0.05$), microbial C ($P = 0.05$), microbial C:N ratio ($P = 0.05$) (Table 2) and K_2SO_4-extractable C ($P < 0.00$; data not reported). P addition did not affect any of the measured parameters (i.e. gross rates of N production, N retention and microbial biomass). To get an index of N mineralization that only includes the influence of substrate quantity and quality and accounts for the differences in the amount of microbial biomass, we expressed N mineralization activity per unit microbial biomass (i.e. specific gross N mineralization = gross N mineralization ÷ microbial N). Specific gross N mineralization rates increased in the N- and N + P-addition plots ($P = 0.05$; Fig. 5). Considering all treatment plots at 1000 m elevation, negative correlations were found for both gross N mineralization and gross nitrification with microbial C:N ratio and for NH_4^+ immobilization with gross nitrification (Table 4). NO_3^- immobilization and DNRA were both positively correlated with K_2SO_4-extractable C (Table 4). Gravimetric moisture content did not differ among treatments at each elevation ($P = 0.44$ to 0.52) and was not correlated with any parameters of soil N cycling in all elevations (data not shown).

At the 2000 m elevation, also N and combined N + P additions increased gross N mineralization ($P = 0.05$) and gross nitrification rates ($P = 0.05$) and decreased NH_4^+ immobilization ($P = 0.05$), NO_3^- immobilization ($P = 0.05$), DNRA rates ($P < 0.00$), microbial C ($P = 0.05$), microbial C:N ratio ($P = 0.05$) (Table 2) and K_2SO_4-extractable C ($P < 0.00$; data not reported). No significant effect on any of the parameters was observed for P addition. Specific gross N mineralization rates increased in N- and N + P-addition plots ($P = 0.05$; Fig. 5). Positive

correlations were found for NH_4^+ immobilization with microbial C, gross nitrification with DNRA, and DNRA with K_2SO_4-extractable C (Table 4).

At the 3000 m elevation, N and combined N + P additions increased gross N mineralization rates ($P = 0.01$) and decreased DNRA rates ($P < 0.00$), microbial C ($P = 0.05$), microbial C:N ratio ($P < 0.00$) (Table 2) and K_2SO_4-extractable C ($P < 0.00$; data not reported). NH_4^+ and NO_3^- immobilization rates in N- and N + P-addition plots were not different from the control plots ($P = 0.65$ to 0.74), but compared to their production rates NH_4^+ immobilization rates were lower than gross N mineralization rates ($P = 0.04$) and NO_3^- immobilization rates were lower than gross nitrification rates ($P = 0.05$) in the N- and N + P-addition plots. P addition also did not affect gross rates of N transformations and microbial biomass. Specific N mineralization rates increased in N- and N + P-addition plots ($P = 0.05$; Fig. 5). Gross N mineralization and gross nitrification were positively correlated and both were negatively correlated with microbial C:N ratio (Table 4). DNRA was also positively correlated with K_2SO_4-extractable C (Table 4).

We compared across elevations the effects of either N, P or combined N + P additions. The magnitude and direction of relative changes (i.e. [treatment – control] ÷ control) in gross N mineralization, NH_4^+ immobilization (Fig. 6a), gross nitrification, NO_3^- immobilization and DNRA rates (Fig. 6b) were comparable among elevations for each treatment ($P = 0.29$ to 0.80).

Discussion

Patterns and controlling factors of gross rates of microbial N production and retention in control forest soils along the elevation gradient

We looked if there were trends in N-cycling rates of montane forest soils measured so far by comparing our measurements with values from studies that employed in-situ incubation of intact soil cores, considering that storage and subsequent laboratory incubation considerably alter soil N-cycling rates (Arnold et al. 2008). Our measured gross N transformation rates were larger than the values reported for an old-growth montane forest on Acrisol soil at 650-750 m

elevation in Puerto Rico (Silver et al. 2001; Templer et al. 2008). Our values were however lower than those reported for old-growth forests on Andosol soils along 630-1500 m elevation gradient in northwestern Ecuador (Arnold et al. 2009) and for an old-growth montane forest on Andosol soil at 1200 m elevation in Panama (Corre et al. 2010, 2013). The annual air temperature and rainfall were respectively 19 °C and 4.5 m for the Puerto Rican site, 18-22 °C and 2.4-4.9 m for the northwestern Ecuadorian sites, and 20 °C and 5.5 m for the Panamanian site. The temperatures in two of our sites (16 °C at 2000 m and 9 °C at 3000 m) may have contributed to their low N-cycling rates. However, our site at the 1000 m elevation had comparable temperature (19 °C) as these other Andosol soils and yet its soil N-cycling rates were lower. Furthermore, precipitation influences soil N availability through frequency and intensity of anaerobic conditions that affect decomposition. This was shown by the decreasing soil mineral N concentrations across a precipitation gradient of 2-5 m $year^{-1}$ in Hawaiian montane forests at 1300 m elevation (Schuur and Matson, 2001). The annual precipitation at our sites (~2 m $year^{-1}$ at 1000 m and 2000 m elevations, and 4.5 m $year^{-1}$ at 3000 m elevation) were comparable to the sites in northwestern Ecuador and even lower than the Panamanian site. Thus, rainfall could not explain the low soil N-cycling rates measured at our sites. Differences in soil N-cycling rates among montane forest soils have been attributed to differences in forest types and soil types, presence or absence of organic layer, thickness of organic layer and temperature (e.g. Hall and Matson 2003; Arnold et al. 2009; Corre et al. 2010, 2013; Martinson et al. 2013). In general, the Andosol soils with thin organic layer (<10 cm thick) and relatively high temperatures in northwestern Ecuador (Arnold et al. 2009) and Panama (Corre et al. 2010, 2013) have larger rates of soil N cycling than other montane forest soils measured so far, i.e. Acrisol soil in Puerto Rico (Silver et al. 2001) and our present sites (Cambisol without an organic layer at 1000 m elevation and Cambisol and Histosol soils with thick organic layer (> 10 cm) and low temperatures at 2000 m to 3000 m elevations).

We examined the factors that influence the soil N-cycling rates in our montane forests. The pattern of decreasing gross rates of soil N-cycling with increasing elevation strongly signified a decreasing soil N availability along our elevation gradient. A similar pattern of

decreasing gross N mineralization rates along a 350-1500 m elevation gradient was observed for old-growth forests in northwestern Ecuador (Arnold et al. 2009). In our study, the decreasing soil N availability was mirrored by the increasing thickness of organic layers with increasing elevation, which also reflects the climatic limitation (e.g. decreasing temperature) on decomposition and the feedbacks between vegetation and soil nutrient availability (Wolf et al. 2011; Martinson et al. 2013). The latter was indicated by the 1) increasing C:N ratios of litterfall and organic layer with increasing elevation (Wolf et al. 2011) and 2) decreasing specific gross N mineralization (i.e. decreasing substrate quality and quantity) with increasing elevation (Fig. 5, control plots). The influence of substrate quality was signified by the positive correlation between soil C:N ratio and microbial C:N ratio which, in turn, was negatively correlated with gross N mineralization and gross nitrification across elevations (Table 3). The influence of substrate quantity was supported by the decreasing amount of litterfall-N along the same elevation gradient (Wolf et al. 2011). Lastly, soil chemical characteristics (i.e. decreasing pH with increasing elevation; Table 1) may also have limited the microbial activity at the higher elevations, as shown by the positive correlation between soil pH and gross N mineralization across elevations (Table 3). In summary, unfavorable soil biochemical conditions (pH and soil C:N ratio), decreasing substrate quantity and quality (litterfall-N, and C:N ratios of soil, litterfall and microbial biomass), increasing organic layer thickness and decreasing temperatures contributed to decreasing soil N availability along our elevation gradient.

Two important implications of the pattern of soil N availability across our elevation gradient are its significance on forest productivity and its use for prediction of soil N-oxide emissions. In our elevation gradient, total litter production and increment of stand basal area decrease with increasing elevation (Wolf et al. 2011), reflecting the pattern of soil N availability. Also, in tropical forest soils the rates of soil N-cycling are directly related to the rates of soil N-oxide emissions (Davidson et al. 2000).

Table 2 Mean (± SE, n = 4)a gross rates of soil N cycling and microbial biomass in the top 5-cm depth of montane forest soils across 1000-3000 m elevation gradient

Elevation and treatment[b]	Gross N mineralization (mg N m^{-2} d^{-1})	NH_4^+ immobilization (mg N m^{-2} d^{-1})	Gross nitrification (mg N m^{-2} d^{-1})	NO_3^- immobilization (mg N m^{-2} d^{-1})	Dissimilatory NO_3^- reduction to NH_4^+ (mg N m^{-2} d^{-1})	Microbial C (g C m^{-2})	Microbial N (g N m^{-2})	Microbial (C:N ratio)
1000 m above sea level (masl)								
Control[c]	235 ± 30 $^{A, b}$	210 ± 14 $^{A, a}$	88 ± 12 $^{A, b}$	90 ± 12 $^{A, a}$	25 ± 2 $^{A, a}$	68 ± 8 $^{A, a}$	5 ± 1 $^{A, a}$	13 ± 0.2 $^{A, a}$
Nitrogen[c]	505 ± 9 ^{a}	112 ± 24 ^{b}	115 ± 10 ^{a}	42 ± 11 ^{b}	14 ± 4 ^{b}	51 ± 1 ^{b}	5 ± 0.6 ^{a}	10 ± 0.2 ^{b}
Phosphorus	316 ± 109 ^{ab}	171 ±105 ^{ab}	81 ± 19 ^{b}	77 ± 30 ^{ab}	18 ± 4 ^{ab}	64 ± 7 ^{ab}	5 ± 1 ^{a}	13 ± 1 ^{a}
Nitrogen + phosphorus	567 ± 98 ^{a}	84 ± 51 ^{b}	119 ± 12 ^{a}	28 ± 13 ^{b}	17 ± 7 ^{b}	60 ± 2 ^{b}	6 ± 0.5 ^{a}	10 ± 1 ^{b}
2000 masl								
Control[c]	191 ± 53 $^{AB, b}$	223 ± 29 $^{A, a}$	35 ± 2 $^{A, b}$	32 ± 4 $^{B, a}$	16 ± 3 $^{B, a}$	65 ± 8 $^{AB, a}$	4 ± 0.1 $^{A, a}$	16 ± 0.2 $^{B, a}$
Nitrogen[c]	444 ± 20 ^{a}	111 ± 12 ^{b}	61 ± 5 ^{a}	20 ± 6 ^{b}	10 ± 1 ^{b}	43 ± 5 ^{b}	3 ± 0.1 ^{a}	14 ± 0.5 ^{b}
Phosphorus	266 ± 65 ^{ab}	185 ± 28 ^{ab}	41 ± 12 ^{b}	33 ± 15 ^{ab}	12 ± 2 ^{a}	47 ± 12 ^{ab}	3 ± 1 ^{a}	15 ± 2 ^{ab}
Nitrogen + phosphorus	343 ± 22 ^{a}	144 ± 16 ^{b}	77 ± 7 ^{a}	16 ± 8 ^{b}	4 ± 3 ^{b}	43 ± 9 ^{b}	3 ± 1 ^{a}	14 ± 0.3 ^{b}
3000 masl								
Control[c]	60 ± 10 $^{B, b}$	87 ± 20 $^{B, a}$	25 ± 6 $^{B, a}$	19 ± 6 $^{B, a}$	9 ± 2 $^{C, a}$	39 ± 2 $^{B, a}$	2 ± 1 $^{A, a}$	19 ± 0.2 $^{B, a}$
Nitrogen[c]	144 ± 31 ^{a}	71 ± 13 ^{a}	31 ± 6 ^{a}	17 ± 7 ^{a}	4 ± 2 ^{b}	29 ± 3 ^{b}	2 ± 0.4 ^{a}	14 ± 1 ^{b}
Phosphorus	104 ± 43 ^{ab}	107 ± 25 ^{a}	28 ± 6 ^{a}	20 ± 7 ^{a}	6 ± 1 ^{ab}	49 ± 11 ^{ab}	3 ± 1 ^{a}	16 ± 1 ^{ab}
Nitrogen + phosphorus	214 ± 18 ^{a}	70 ± 10 ^{a}	40 ± 8 ^{a}	17 ± 6 ^{a}	3 ± 2 ^{b}	29 ± 3 ^{b}	3 ± 1 ^{a}	10 ± 1 ^{b}

[a] Means with different capital letters indicate significant differences across the elevation gradient for the control plots, and means with small letters indicate significant differences among treatments at each elevation (one-way ANOVA with Tukey HSD at $P \leq 0.05$).

[b] Control plots had no fertilizer addition; nitrogen plots were fertilized with 50 kg urea-N ha^{-1} $year^{-1}$; phosphorus plots were fertilized with 10 kg P ha^{-1} $year^{-1}$ of analytical grade $NaH_2PO_4.2H_2O$; nitrogen + phosphorus plots received combined application of the same fertilizers. All fertilizers were split in two equal applications per year and broadcasted by hand.

[c] Values for control and nitrogen-addition plots are means of two measurements made in October 2010 and in October 2011, after three and four years of treatment, respectively. The two measurement periods of each treatment did not differ (Paired T test at $P = 0.30$ to 0.98), and hence were averaged for each treatment

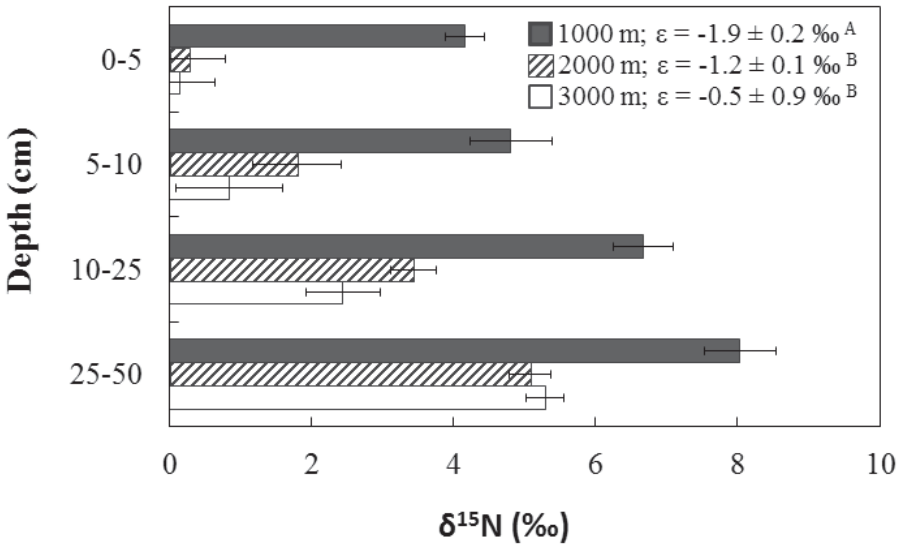

Fig. 4 $\delta^{15}N$ profiles (‰; means ± SE, n = 4) and ^{15}N enrichment factors (ε; means ± SE, n = 4) of the control forests across the elevation gradient (m above sea level). Means of ^{15}N enrichment factors with different letters indicate significant differences across the elevation gradient (one-way ANOVA with Tukey HSD at $P \leq 0.05$).

This has been supported by the direct relationships between gross rates of soil N-cycling, ^{15}N natural abundance signatures of soil or litterfall (e.g. Arnold et al. 2009; Corre et al. 2010) and the overall ^{15}N enrichment factor of the soil (Sotta et al. 2008) as well as by the direct relationships between ^{15}N natural abundance signatures of soil or litterfall and soil N-oxide losses (Purbopuspito et al. 2006; Wolf et al. 2011). Large ^{15}N natural abundance signatures and ^{15}N enrichment factors of the soil indicate high soil N-cycling rates and N losses because fractionation during nitrification and denitrification leaves the isotopically heavy N behind and allows the loss of isotopically light N from the ecosystem (Amundson et al. 2003). Along our elevation gradient, the decrease in ^{15}N natural abundance signatures and ^{15}N enrichment factors of the soils corroborated the decrease in soil N availability (Fig. 4 and Table 2) and the decrease in soil N-oxide emissions with increase in elevation (Wolf et al. 2011; Martinson et al. 2013). With this link between soil N availability, forest productivity, ^{15}N natural abundance signatures and N-oxide emissions, our results supported Wolf et al.'s (2011) recommended method of estimating large-scale N-oxide fluxes from these montane forest soils in south Ecuador. They suggest using predictive relationships of N-oxide fluxes with ^{15}N natural abundance signatures

(soils or litterfall) and/or forest productivity as a fast and less labor-intensive method of estimating N-oxide fluxes on a large scale.

The dominant fate of the produced NH_4^+ and NO_3^- in the control forest soils was microbial immobilization, indicated by comparable rates of N production and retention (Table 2) and by positive correlations of gross N mineralization with NH_4^+ immobilization and gross nitrification with NO_3^- immobilization (Table 3). This signified that the soil N-cycling in these forests was tightly coupled. Similar findings have been observed in montane forest soils in Puerto Rico (Silver et al. 2001; Templer et al. 2008), northwestern Ecuador (Arnold et al. 2009) and Panama (Corre et al. 2010, 2013). The closely-coupled soil N-cycling in our control forest soils was also supported by the low soil N_2O and NO emissions, especially at 3000 m elevation that showed net consumption (negative fluxes) of atmospheric N_2O and NO (Wolf et al. 2011; Martinson et al. 2013) and had the lowest fluxes reported so far compared to other montane forests (e.g. Cambisol soils at 1800 m and 2500 m elevations in Indonesia, Purbopuspito et al. 2006; Cambisol soil at 1300 m elevation in Hawaii, Holtgrieve et al. 2006; Andosol soil at 1200 m elevation in Panama, Koehler et al. 2009). DNRA, which is a N retention mechanism through microbial conversion of NO_3^- to NH_4^+ (Silver et al. 2001), was increasingly important with decreasing soil N availability along the elevation gradient (i.e. DNRA rates were half of the NO_3^- immobilization rates at 2000 m and 3000 m elevations; Table 2). Since NH_4^+ immobilization rates were 2 to 6 times greater than gross nitrification rates, DNRA would further retain N in our montane forest soils.

Nutrient-addition effects on gross rates of microbial N production and retention

Across all three montane forest sites, we observed a consistent pattern of response of soil N-cycling to low rates of N, P, and combined N + P additions. Gross rates of microbial N production increased whereas microbial N retention decreased after four years of N and combined N + P additions (Table 2 and Fig. 6), suggesting uncoupled soil N-cycling rates at all elevations. Since P addition did not have an effect on any of the soil N-cycling parameters measured across the

elevation gradient, the effects of combined N + P addition is probably in response to the addition of N. The absence of P-addition effects could either be that the amount of P added (10 kg P ha^{-1} $year^{-1}$) was too low to elicit any responses in soil N-cycling rates or that soil N-cycling was not sensitive to P at least up to four years of low P addition.

Generally, mineral N production rates are influenced by the amount of microbial biomass and microbial community composition as well as substrate quality and quantity. The increases in gross rates of N mineralization and nitrification with N or combined N + P additions were influenced more by the increases in substrate quality and quantity, as supported by the increases in specific gross N mineralization rates in these plots at all elevations (Fig. 5), and possibly less likely by the amount of microbial biomass since microbial C contents had decreased in these treatment plots. Increase in substrate quality was also suggested by the negative correlations of gross rates of N mineralization and nitrification with microbial C:N ratios (i.e. at 1000 m and 3000 m; Table 4), assuming that microbial C:N ratio is stoichiometrically related to the substrate C:N ratio (Hart et al. 1994). A support for the increase in substrate quantity was the larger litterfall-N after one year of N or combined N + P additions compared to the control at the 2000 m elevation (Homeier et al. 2012). Our findings corroborated those reported for the montane forests in Hawaii (on Cambisol soil at 1200 m elevation; Hall & Matson, 2003) and in Panama (on Andosol soil at 1200 m elevation; Corre et al. 2010, 2013), where gross rates of mineral N production increased after 10 years (Hawaii) and 1-4 years (Panama) of N additions (with rates of 100 to 125 kg N ha^{-1} $year^{-1}$).

On the other hand, the decrease in microbial immobilization of NH_4^+ and NO_3^- with N or combined N + P additions could be attributed to the decrease in microbial C in these plots at all elevations (Table 2). This was supported by the positive correlation between NH_4^+ immobilization and microbial C (i.e. at 2000 m elevation; Table 3). Studies in tropical lowland and temperate forests consistently reported a decrease in microbial biomass and a reduced N immobilization relative to N production with chronic N addition (e.g. Compton et al. 2004; DeForest et al. 2004; Venterea et al. 2004; Corre et al. 2003, 2007, 2010).

Table 3 Pearson correlation coefficients ($n = 12$) among rates of soil N cycling, microbial C, microbial C:N ratio, pH-H_2O, total N, and soil C:N ratio of soil in the top 5-cm depth, conducted across sites for all control plots

	NH_4^+ immobilization	Gross nitrification	NO_3^- immobilization	DNRA	Microbial C	Microbial C:N ratio	pH-H_2O	Total N	C:N ratio
Gross N mineralization	**0.87***	0.45	0.43	**0.83***	0.31	**-0.80***	**0.81***	0.55	-0.64
NH_4^+ immobilization		0.44	0.48	0.55	0.50	-0.50	0.42	0.46	-0.66
Gross nitrification			**0.73***	0.63	0.16	**-0.81***	0.61	0.47	**-0.83***
NO_3^- immobilization				0.65	0.27	-0.79	0.65	0.48	-0.72
DNRA					0.36	-0.64	0.61	0.51	-0.64
Microbial C						-0.08	0.29	0.65	-0.34
Microbial C:N ratio							-0.61	-0.68	**0.89***
pH-H2O								0.57	-0.66
Total N									-0.32

* $P \leq 0.05$

[a] DNRA – dissimilatory NO_3^- reduction to NH_4^+

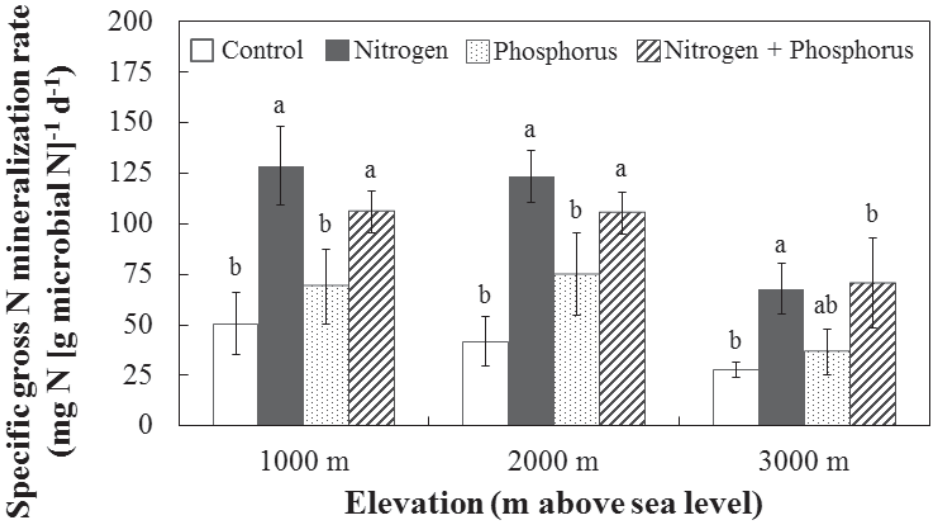

Fig. 5 Specific gross N mineralization rates (specific gross N mineralization rates = gross N mineralization rate/ microbial N; mg N g microbial N^{-1} d^{-1}) of the montane forests across the elevation gradient. Means with different letters indicate differences between treatments for each site (one-way ANOVA with Tukey HSD at $P \leq 0.05$).

There is a growing interest on how increases in N depositions in tropical forests change microbial community structure, enzyme activity or structural fractions of soil organic matter, which may link to the changes in the retention of N and C in these forests. In one of the few studies on this topic, 5 years of N addition (50 kg N ha^{-1} $year^{-1}$) in two forests in Puerto Rico (on Acrisol soils at 260 m and 640 m elevations) showed differential effects: in the lower elevation forest, bacterial decomposers and hydrolytic enzyme activities increased and were associated with a decrease in labile C compounds; in the upper elevation forest, fungal abundance and oxidative enzyme activities increased and were associated with a decrease in poor quality C compounds (Cusack et al. 2011b). From our study sites, changes in microbial community composition, enzyme activities or structural fractions of soil organic matter have not yet been investigated. We only had indirect indicators of changes in microbial community and soil C fraction: the decreases in microbial C:N ratios with N or N + P additions at all elevations (Table 2) suggest a shift toward a bacterial-dominated microbial community, and the decrease in K_2SO_4-extractable C with N or N + P additions may indicate a reduction in labile C fraction. Soil chemical characteristics also showed, although not statistically significant, a decrease in base saturation, an increase in exchangeable Al in the mineral soil (i.e. at 1000 m elevation) and an

increase in total Al concentration in the organic layer (i.e. at 2000 m elevation) in N- and N + P-addition plots relative to the control plots (Table 1). We speculate that N addition had possibly shifted microbial community to a more bacterial-dominated and reduced labile C; these, together with the changes in soil chemical characteristics, resulted in reduced microbial biomass C and consequently reduced microbial N immobilization. We compared the rates of microbial N-production and N-retention processes to assess how uncoupled or leaky the soil N-cycling had become due to N addition. Gross nitrification increased when microbial NH_4^+ immobilization decreased in N- and N + P-addition plots (Table 2), suggesting that increased availability of substrate may have enabled the nitrifiers to compete more for NH_4^+ and organic N. This was supported by the positive correlation of gross nitrification with gross N mineralization at 3000 m elevation, which had initially the lowest soil N availability, and by the negative correlation of gross nitrification with NH_4^+ immobilization at the 1000 m elevation, which had initially the highest soil N availability (Table 4). Moreover, the importance of DNRA as a NO_3^- retention process had declined in N- and N + P- addition plots, particularly at 3000 m elevation where DNRA was only one-third of NO_3^- immobilization as opposed to one-half in the control plots (Table 2). Several factors could influence DNRA (e.g. soil redox status, soil C and NO_3^- pools; Silver et al. 2001, 2005), but the only parameter that correlated with DNRA rates in our sites was the K_2SO_4-extractable C (Table 4), suggesting that the labile soil C pool may be more important than the NO_3^- pool in N-enriched conditions. In summary, as opposed to the control and P-addition plots, the N- and N + P-addition plots at all elevations changed from closely coupled to leaky soil N-cycling (i.e. NH_4^+ immobilization $<$ gross N mineralization; NO_3^- immobilization + DNRA $<$ gross nitrification), which signals a potential for deleterious effects of N losses from the soil (e.g. NO_3^- leaching, N-oxide emissions).

Conclusions

For the control forests, gross rates of mineral N production were closely coupled with microbial N retention and N-cycling rates decreased with increasing elevation, supporting our first hypothesis. The link between soil N-cycling rates, ^{15}N natural abundance signatures of soil or

litterfall, forest productivity and soil N-oxide emissions opens the possibility of using easily-measurable proxy variables (e.g. stand basal area increment, litter production or ^{15}N signatures of soil or litterfall) for estimating large-scale N-oxide fluxes. These montane forests showed similar responses to 4 years of N and combined N + P additions. Increased gross rates of mineral N production with decreased rates of microbial N retention were largely an effect of N addition since P addition alone did not affect any of these processes. This partly supported our second hypothesis. At all elevations, the change from closely coupled to leaky soil N-cycling in response to N addition supported our third hypothesis. Since soil N availability of these montane forests was generally low, the increases in soil N-cycling rates may also bring beneficial effects on forest productivity (as was observed after the first year of treatment at the 2000 m site), if such increase in N availability will not bring limitation of other nutrients in the long run. The relatively rapid response (i.e. within 4 years) to a low rate of N addition, at a level expected to occur in our study area, suggests that greater attention should be paid to the biological implications on montane forests of such uncoupled soil N cycling in response to increases in N deposition. Many of these responses may be only observed after several years with large spatial variability, and hence the need of a long-term manipulation experiment at a large scale. Our results also point out the need of complementary studies to link the changes in the soil N cycling with changes in structural fractions of soil organic matter, microbial community function, enzyme activity and ultimately in the long-term fates of N and C in these montane forests.

Table 4 Pearson correlation coefficients ($n = 16$) among rates of soil N cycling, microbial C, microbial N, microbial C:N ratio, extractable C, in the top 5-cm depth, conducted across treatments for each site

	NH_4^+ immobilization	Gross nitrification	NO_3^- immobilization	DNRA	Microbial C	Microbial N	Microbial C:N ratio	Extractable C
1000 m above sea level (masl)								
Gross N mineralization	-0.44	0.44	-0.37	0.08	0.28	0.19	**-0.66***	-0.37
NH_4^+ immobilization		**-0.67***	0.39	0.37	-0.34	-0.37	0.24	0.41
Gross nitrification			-0.22	-0.04	0.26	0.12	**-0.70***	-0.38
NO_3^- immobilization				0.62	0.05	-0.21	0.32	**0.72***
DNRA					0.40	0.20	-0.11	**0.73***
Microbial C						0.72	-0.06	0.15
Microbial N							0.08	0.07
Microbial C:N ratio								0.68
2000 masl								
Gross N mineralization	-0.37	-0.22	-0.12	-0.00	0.34	0.14	-0.03	-0.35
NH_4^+ immobilization		-0.31	0.12	0.43	**0.68***	-0.12	0.16	0.26
Gross nitrification			-0.32	**0.68***	0.10	0.28	-0.12	-0.23
NO_3^- immobilization				0.25	0.36	-0.38	0.34	0.26
DNRA					0.55	0.15	0.06	**0.78***
Microbial C						0.54	-0.32	0.40
Microbial N							0.22	-0.06
Microbial C:N ratio								0.16
3000 masl								
Gross N mineralization	-0.40	**0.81***	0.07	-0.27	0.00	0.05	**-0.73***	0.41
NH_4^+ immobilization		-0.05	0.37	-0.09	0.34	-0.10	-0.14	-0.05
Gross nitrification			-0.14	-0.38	0.02	0.27	**-0.69***	0.36
NO_3^- immobilization				0.24	0.27	-0.07	0.16	0.57
DNRA					0.04	0.29	0.54	**0.69***
Microbial C						-0.09	-0.07	-0.01
Microbial N							0.01	-0.44
Microbial C:N ratio								0.64

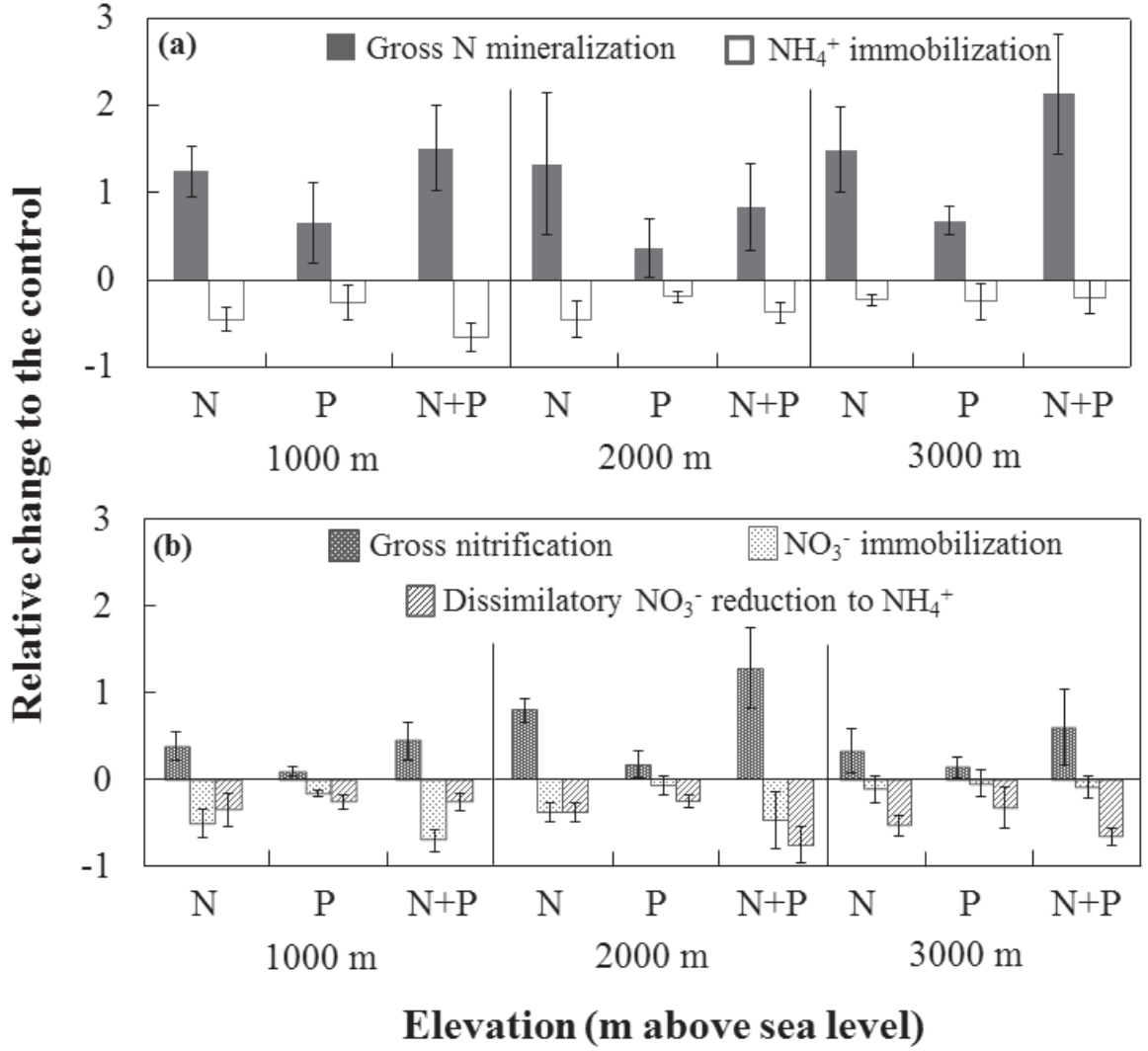

Fig. 6 Changes (mean ± SE, n = 4) in (a) gross N mineralization and NH_4^+ immobilization rates, and (b) gross nitrification, NO_3^- immobilization, and dissimilatory NO_3^- reduction to NH_4^+ (DNRA) rates in the nitrogen- (N), phosphorus- (P), combined nitrogen and phosphorus- (N+P) addition plots relative to the control plots at each forest site (relative change to the control plots = (control – treatment)/control). Relative change (to the control plots) in gross N mineralization, NH_4^+ immobilization, gross nitrification, NO_3^- immobilization, and DNRA rates were comparable among sites for all treatments.

CHAPTER III Fluxes and fates of nitrogen in soils of old-growth montane forests in the Ecuadorian Andes with four years of elevated nitrogen input

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Abstract

Nitrogen (N) retention in tropical forest soils and how its mechanisms change with increase in N input are of current interest because of their link to soil carbon dynamics and the presently increasing N deposition in tropical regions. We conducted an in-situ ^{15}N pulse chase study to: (1) assess the net fluxes and fates of mineral N (NH_4^+ and NO_3^-) in tropical montane forest soils with low N availability, and (2) determine the effects of four years of low N addition on the fluxes and fates of mineral N in these forest soils. Our study sites were located in the Ecuadorian Andes at 1000 m and 3000 m elevations, where control (no manipulation) and N-addition (50 kg urea-N ha^{-1} $year^{-1}$) treatments, with four replicate plots (20 m x 20 m each), have been established since 2008. From November 2010 to October 2011, we traced the fates of NH_4^+ and NO_3^- in different soil N pools (NH_4^+, NO_3^-, extractable organic N, microbes, fine roots and soil organic N), using one-time application of either $^{15}NH_4^+$ or $^{15}NO_3^-$ at very low amounts but 99 % ^{15}N enriched.

Both ^{15}N tracers showed similar dynamics: a redistribution phase (i.e. short-term fate) characterized by large fluxes and fast transfers of ^{15}N among N pools, and an equilibrium phase (i.e. long-term fate) depicted by small fluxes and stable transfers of ^{15}N among N pools. The short- and long-term fates of either ^{15}N tracer also showed similar mechanisms of paths and fluxes among of different N pools in both forest sites, signifying the lack of preferential retention for either NH_4^+ or NO_3^-. In control plots of both forest sites, the short-term fate signified a complete recovery of both ^{15}N tracers with the largest sink in the soil organic N pool (52-62 % of the added ^{15}N), which was mainly contributed by microbial N turnover and fine-root related N release. The long-term fate showed low ^{15}N flux rates among N pools but a consistent flux into the slowly cycling soil organic N pool, suggesting that internal soil N cycling might not be largely dependent on the recycling of recently incorporated N in the soil organic matter. The low N availability in these control forest soils might have induced the efficient and equal retention of both NH_4^+ and NO_3^- relative to their availability.

Four years of low N addition resulted in reduced N retention with long-term fate in soil organic N pool accounting only 30-38 % of the added ^{15}N tracers. This was because the

movement of N out of microbial and fine-root N pools did not lead to its accumulation in soil organic N. These results robustly show that these Andean montane forests are highly sensitive to changes in N deposition at a rate expected to occur at these sites. Therefore, greater attention should be paid to the biological implications of increased N deposition with the consequent decrease in N retention, and thus possibly also carbon, in these forests.

Introduction

The tropics are experiencing high rates of nitrogen (N) deposition (Hietz et al. 2011; Homeier et al. 2012) even in areas far from anthropogenic sources of N. In Ecuador, the major source of deposited nutrients, including N, is biomass burning in the Amazon (Fabian et al. 2005). Additional N inputs that exceed the N demand of an ecosystem may lead to nutrient imbalances in vegetation, changes in biodiversity, reduced productivity and increased N losses (e.g. Bernhard 2012). N additions to tropical forest soils to simulate increase in atmospheric N deposition have shown increases in soil N-oxide ($NO + N_2O$) emissions, nitrate (NO_3^-) leaching, decrease in microbial N immobilization, and differential changes in plant productivity, soil carbon dioxide (CO_2) fluxes and soil carbon (C) dynamics (e.g. Hall and Matson 2003; Lohse and Matson 2005; Koehler et al. 2009a, 2009b; Corre et al. 2010, 2013; Cusack et al. 2011a; Wright et al. 2011). These results suggest deleterious consequences on the environment and changes in soil C storage. These processes are strongly controlled by internal transformations of N in the soil, and because N cycle is closely linked with C cycle the fates of N in the soil influence the ability of the soil to sequester C (Cusack et al. 2011b; Templer et al. 2012).

Retention of N in an ecosystem occurs through plant and microbial uptake and assimilation into biomass, storage as soil organic N, or adsorption of inorganic N onto soil exchange sites and soil organic matter. The fates of inorganic N in an ecosystem can be characterized in terms of the dynamics of N fluxes in the plant-soil-microbe system over time. Kaye and Hart (1997) characterized N fluxes among plant, microbe and soil that occur within days to weeks without recycling as short-term and fluxes that happen over months to years with recycling as long-term cycling. Short-term ^{15}N-tracing studies in forest soils show microbial biomass and soil organic matter as greater sinks than plant roots of added ^{15}N-ammonium (NH_4^+) or $^{15}NO_3^-$ during 0.1-16 days in an unpolluted temperate forest in Chile (Perakis and Hedin 2001). In another study, microbial biomass is not a significant sink but rather plant roots for added $^{15}NH_4^+$ or $^{15}NO_3^-$ during 1 day and only an important sink for $^{15}NH_4^+$ after 7 days in a subtropical lower montane forest in Puerto Rico (Templer et al. 2008). Rapid, direct

incorporation of N into the soil without going through the microbial pathway has been observed as well by other studies in temperate forest soils (e.g. Dail et al. 2001; Perakis and Hedin 2001; Davidson et al. 2003) although the mechanism remains disputed. Studies assessing short-term N cycles mostly exclude competition from plant uptake. Long-term N cycling involves several short-term N-cycling events and the recycling of N within the plant-soil-microbe system links the short-term fates of N into long-term patterns of retention in an ecosystem. The long-term fates of inorganic N in tropical forest soils are not well known because the studies conducted so far have only traced ^{15}N within days (e.g. Templer et al. 2008).

Studies on retention and fates of N in some temperate forest ecosystems generally demonstrate that the soil is an important and large sink of N over a long-term period (e.g. Emmett and Quarmby 1992; Buchmann et al. 1996; Magill et al. 1997; Nadelhoffer et al. 1999). In an unpolluted temperate forest in Chile, rapid and possibly direct incorporation into the slow turn-over pool of soil organic matter appeared to be the dominant mechanism of NH_4^+ and NO_3^- retention during a 2-year ^{15}N-tracing study (Perakis and Hedin 2001). At different levels of N inputs, some components of the forest floor differ in importance as N sinks with soil organic matter dominating as N sink under low levels of N input and shifting to coarse roots and particulate organic matter under high levels of N input (Perakis et al. 2005). On the other hand, in a subtropical lower montane forest in Puerto Rico, NO_3^- transformation processes (i.e. nitrification with subsequent dissimilatory NO_3^- reduction to NH_4^+ and uptake of NH_4^+ by plants) play a major role of N retention during a 7-day ^{15}N-tracing study (Templer et al. 2008). A meta-analysis of ^{15}N tracing studies conducted across ecosystem types show that N retention was influenced by ecosystem type, vegetation type, mycorrhyzal type, soil C:N ratio, disturbance history and even the method of ^{15}N application (Templer et al. 2012). They also showed that above a certain threshold of added N (i.e. 46 kg N ha^{-1} $year^{-1}$) total ecosystem N retention decreased. How increase in N deposition affects the long-term fates of soil mineral N in tropical montane forest soils remain poorly understood.

In this study, we investigate on the effects of four years of low N addition on the long-term fates of N in a premontane forest at 1000 m elevation on a Cambisol soil with no organic

layer and in an upper montane forest at 3000 m elevation on a Histosol soil with a thick organic layer covering the mineral soil. The soil N availability (i.e. based on gross rates of soil N cycling) of these sites were low (see Chapter 2) in comparison to the gross N-cycling rates of tropical montane forests on Andosol soils (Arnold et al. 2009; Corre et al. 2013). We hypothesized that: (1) in montane forest soils with low N availability, NH_4^+ and NO_3^- are retained largely through root and microbial uptake, and (2) chronic N addition to these soils will reduce N retention. Our objectives were to: (1) assess the net fluxes of NH_4^+ and NO_3^- in the different components of soil N and their short-term and long-term fates in tropical montane forest soils with low N availability, and (2) determine the effects of chronic N addition on the net fluxes of NH_4^+ and NO_3^- in the different soil N pools and on their fates in these montane forest soils.

Materials and Methods

Site description and experimental design

Our study area was located on two montane forests in south Ecuador, where a nutrient manipulation experiment (NUMEX) is on-going. These forest sites are within and adjacent to the Podocarpus National Park in the Cordillera de Consuelo, a mountain range forming part of the eastern chain of the Andes. We chose two sites: a premontane forest at 990–1100 m above sea level (Bombuscaro, 4.115^{O} S, 78.968^{O} W; hereafter referred as 1000 m elevation) and an upper montane forest at 2900–3050 m (Cajanuma, 4.110^{O} S, 79.178^{O} W; hereafter referred as 3000 m elevation). The site at 1000 m has an old-growth premontane forest growing on a Dystric Cambisol soil from parent material of deeply-weathered granitic rock. The soil has no organic layer but is covered only by a thin layer of decomposing leaves (Ol layer). The site at 3000 m has an old-growth upper montane forest growing on a Stagnic Histosol soil from a parent material of metamorphosed schist and is covered by an organic layer of 10 to 40 cm thick (Martinson et al. 2013). The annual mean air temperature and precipitation were respectively 19.4 °C and 2.2 m year^{-1} for the 1000 m and 9.4 °C and 4.5 m year^{-1} for the 3000 m (Moser et al. 2007). No clear seasonal patterns were observed for precipitation (Emck 2007) as well as for soil temperature and soil moisture contents at both sites (Martinson et al. 2013). Ambient N deposition in an area

at 2000 m elevation was 14 to 45 kg N ha^{-1} $year^{-1}$ during the period of 1998 – 2010 (Homeier et al. 2012). The forest stand characteristics of these sites were given by Martinson et al. (2013).

In each site, a full factorial nutrient manipulation experiment was set up in a stratified random design with treatments (N-, P-, combined N + P-additions and control) randomly assigned in each of the four replicate blocks. The size of each treatment plot was 20 m x 20 m, and was at least 10 m apart from each other. For our present study, we only included three replicate plots of the control and N-addition treatment. Compared to other nutrient manipulation experiments in tropical montane forests (e.g. Hall and Matson 2003; Corre et al. 2010), the rate of N applied was low and within the projected N deposition rate in these montane forests (Homeier et al. 2012). N addition started in 2008 at a rate of 50 kg urea-N ha^{-1} $year^{-1}$, divided in two equal applications per year. Granular urea was applied by hand as evenly as possible in the N-addition plots.

Soil characteristics

The initial soil characteristics were determined in 2007 prior to the start of nutrient manipulation and were reported by Martinson et al. (2013). Soil biochemical characteristics were determined again in April 2012 after four years of treatment. Organic layer (present only in the site at 3000 m) and mineral soil (at depth intervals of 0-5, 5-10, 10-25 and 25-50 cm) were sampled from one soil profile per replicate plot. Samples were air-dried, ground and analyzed for total C and N using a CN analyzer (Elementar Vario EL; Elementar Analysis Systems GmbH, Hanau, Germany). Soil pH was measured from a mixture of soil and distilled water with a ratio of 1:4. Effective cation exchange capacity (ECEC) of the mineral soil was determined from air-dried and sieved (2 mm) soils; these were percolated with unbuffered 1 M NH_4Cl and percolates were analyzed for cation concentrations using an inductively coupled plasma-atomic emission spectrometer (Spectroflame, Spectro Analytical Instruments, Kleve, Germany). Base saturation was calculated as percentage exchangeable base cations of the ECEC. All soil samples were analyzed at the Soil Science of Tropical and Subtropical Ecosystems (SSTSE), University of Goettingen, Germany. We did not find any significant differences in these soil characteristics

between control and N-addition plots at each elevation after four years of treatment (Appendix 1).

^{15}N pulse-chase tracing

We followed the fates of added $^{15}NH_4^+$ or $^{15}NO_3^-$ in the soil into different soil N pools at various times within one year from November 2010 (third year of treatment) to October 2011 (fourth year of treatment). The ^{15}N source was only applied once and was traced in NH_4^+, NO_3^-, extractable organic N, microbial biomass N, fine-root N and soil organic N (excluding root and microbial N) pools, which were all measured at 1, 7, 16, 20, 27, 91, 142 and 355 days after ^{15}N application, similar to the method employed by Perakis and Hedin (2001). In each replicate plot, we delineated two subplots of 1.5 m x 1 m each and positioned each subplot within 1 m of a canopy dominant tree. Prior to ^{15}N application, the few fresh or undecomposed fine litter were taken out by hand from the subplots. One subplot was applied with dissolved $(^{15}NH_4)_2SO_4$ and the other subplot with $K^{15}NO_3$. The rate of $(^{15}NH_4)_2SO_4$ (99% ^{15}N enriched) applied was 104 mg ^{15}N m^{-2} and for $K^{15}NO^3$ (99% ^{15}N enriched) was 3 mg ^{15}N m^{-2}. Each ^{15}N source was dissolved in 1.5 l distilled H_2O (equaling to 1 mm rain in application volume) and applied as evenly as possible onto the 1.5-m^2 area using a hand sprayer. The distance between these two subplots within each replicate plot was on average ~4 m. It was necessary to apply different rates of $^{15}NH_4^+$ and $^{15}NO_3^-$ so as not to change artificially the proportions of mineral N in these soils (i.e. gross rates of NH_4^+ transformations and concentrations predominated over gross rates of NO_3^- transformations and concentrations; Chapter 2) while being able to detect the applied ^{15}N in different N pools.

Prior to ^{15}N tracer application, soil mineral N was determined by in-situ extraction (as described by Corre et al. 2010); the control plots had extant NH_4^+ levels in the top 5 cm soil of 494 $\pm$ 7 mg N m^{-2} at 1000 m elevation and 238 $\pm$ 67 mg N m^{-2} at 3000 m elevation; the N-addition plots had 532 $\pm$ 108 mg N m^{-2} and 264 $\pm$ 53 mg N m^{-2} at 1000 m and 3000 m, respectively. The NO_3^- levels in the top 5 cm soil of the control plots were 37 $\pm$ 13 mg N m^{-2} at 1000 m elevation and 0.3 $\pm$ 0.3 mg N m^{-2} at 3000 m elevation; the N-addition plots had 151 $\pm$ 8

mg N m^{-2} and 11 ± 10 mg N m^{-2} at 1000 m and 3000 m, respectively. Thus, our applied $^{15}NH_4^+$ was 20-21 % and 39-44 % of the native NH_4^+ in the top 5 cm at 1000 m and 3000 m, respectively. The applied $^{15}NO_3^-$ was 2-8% of the extant NO_3^- in the top 5 cm at 1000 m; at 3000 m, the applied $^{15}NO_3^-$ was 27 % of the inherent NO_3^- in the N-addition plots and 10 times the native NO_3^- in the control plots, where NO_3^- levels measured monthly from April 2008 to September 2009 were all close to zero (Martinson et al. 2013).

On each sampling day and replicate plot, we took intact soil cores (8 cm diameter and 5 cm length) separately from each subplot labeled with $^{15}NH_4^+$ and $^{15}NO_3^-$. We limited the tracing to the top 5-cm depth in order for the work to be attainable. The cores included from the top partially decomposed fine litter down to 5 cm depth, but fresh leaf litter was excluded in order to include the same material throughout the one-year tracing period. Each core was broken up and mixed well in a plastic bag for determination of different N pools. First, soluble N pools (i.e. NH_4^+, NO_3^- and organic N) were extracted with 150 ml 0.5 M K_2SO_4 by shaking for 1 hour and filtering through K_2SO_4-prewashed filter papers (4 µm nominal pore size), and the extracts were immediately frozen. Frozen extracts were transported by air to Germany and kept frozen until analysis at SSTSE, University of Goettingen. Concentrations of NH_4^+ (analysed by Berthelot reaction method; Skalar Method 155-000), NO_3^- (analysed by copper-cadmium reduction method with NH_4Cl buffer but without ethylenediamine tetraacetic acid; Skalar Method 461-000) and total N in the extracts (analysed by persulfate digestion to convert all extracted N into NO_3^- followed by the same method for NO_3^- analysis; Stark and Hart 1996) were determined using continuous flow injection colorimetry (Cenco/Skalar Instruments, Breda, Netherlands). Extractable organic N was calculated as the difference between total extractable N and NH_4^+ + NO_3^- concentrations. For determination of ^{15}N enrichment, the same methods of serial ^{15}N diffusion for NH_4^+ and NO_3^- and a separate ^{15}N diffusion for persulfate digests (total extractable N converted to NO_3^-) described in our previous works (e.g. Corre and Lamersdorf 2004; Corre et al. 2007) were followed, and ^{15}N was analyzed using isotope ratio mass spectrometry (IRMS; Delta C, Finnigan MAT, Bremen, Germany). ^{15}N enrichment of the extractable organic N was calculated based on the isotope mixing equation using the difference in ^{15}N enrichments and N

concentrations between the total extractable N, NH_4^+ and NO_3^- pools. Blank correction was carried out as recommended by Stark and Hart (1996).

Second, microbial biomass C and N were determined from the remaining soil sample by $CHCl_3$ fumigation-extraction method; about 20 g of fresh soil was fumigated with $CHCl_3$ for 5 days and then extracted with 100 ml 0.5 M K_2SO_4 as above. Frozen extracts were also transported to SSTSE, University of Goettingen for analysis. 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, CA, USA). Organic N in the extracts was determined using persulfate digestion followed by NO_3^- analysis as mentioned above. Microbial biomass C and N were calculated as the difference in extractable organic C and N between the fumigated and unfumigated soils divided by $k_C = 0.45$ and $k_N = 0.68$ for a 5-day fumigation period (Brookes et al. 1985). ^{15}N enrichment of microbial N pool was determined by ^{15}N diffusion of the persulfate digest of fumigated sample and was calculated based on isotope mixing equation using the difference in ^{15}N enrichments and N concentrations between the fumigated and the corresponding unfumigated samples above. Gravimetric moisture content of each soil core was measured to adjust the field-moist weight to dry weight.

Third, live fine roots (≤2 mm diameter) were handpicked from the same soil core, washed with distilled H_2O to get rid completely of soil, frozen and transported to SSTSE, University of Goettingen. Finally, the remaining soil was frozen and transported to the same laboratory in Germany. Separately, the roots and soils were freeze-dried for ~5 days, ground and analyzed for total N and ^{15}N using the same CN analyzer and IRMS above. ^{15}N enrichment of the soil organic N was calculated based on isotope mixing equation using the difference in ^{15}N enrichments and N concentrations between total N, extractable N and microbial N. All N concentrations were converted from mass basis to area basis by multiplying with the sampling depth (top 5 cm) and the average of the measured soil bulk densities for each elevation (0.84 g cm^{-3} at 1000 m and 0.11 g cm^{-3} at 3000 m). In the case of fine-root N pool, we used the average of the measured fine root biomass (0.009 g cm^{-2} at 1000 m and 0.037 g cm^{-2} at 3000 m in the top 5 cm soil; Leuschner et al. 2007).

Calculations and statistical analysis

We calculated atom percent excess ^{15}N for each N pool by subtracting the measured ^{15}N natural abundance values (conducted prior to ^{15}N pulse-chase tracing) from the ^{15}N-enriched values. Recoveries of ^{15}N in each N pool were calculated as: atom percent excess $^{15}N/100$ x N pool ÷ ^{15}N applied x 100, where N pool and added ^{15}N (see above) are expressed on an area basis (see above). The ^{15}N recoveries in each pool serve as an index of the fate of N or its retention in that particular pool. We determined the net rates of ^{15}N flux for each N pool and the whole plot (i.e. sum of the component pools) from the least square linear regressions of ^{15}N recoveries vs. time (similar to that of Perakis and Hedin 2001). This yields net rates of ^{15}N flux that can be expressed equivalently as either the percentage of the originally added ^{15}N that is cycled per day or as the mass of ^{15}N tracer that is cycled per day. This serves as an index to characterize the movement of the added ^{15}N among different N pools over time. Also, comparison of net ^{15}N fluxes of the different N pools to net ^{15}N fluxes of the whole plot provides information on what controls the loss of ^{15}N tracers from the whole plot over time.

Parameters that showed non-normal distribution and heterogeneous variance were log transformed. We evaluated whether $^{15}NH_4^+$ or $^{15}NO_3^-$ addition changed the ambient levels of $^{15}NH_4^+$ or $^{15}NO_3^-$ by comparing measurements conducted before and 1 day after ^{15}N addition using Paired T test for each treatment and site. We used linear mixed effects (LME) model on our time-series data (i.e. N pools, ^{15}N atom percent excess, ^{15}N recoveries and net rates of ^{15}N fluxes). The ^{15}N tracer ($^{15}NH_4^+$ vs. $^{15}NO_3^-$) or treatment (control vs. N addition) at each site as well as site (1000 m vs. 3000 m) for the control forests only were considered fixed effects and time (sampling days) and replicate plots were random effects. In the LME model, we included either a variance function to allow for different variances of the response variable per level of the fixed effect, a first-order temporal autoregressive process that assumes that correlation between measurements decreased with increasing time difference or both if these improved the relative goodness-of-model fit based on Akaike Information Criterion. Mean values with ± 1 standard error, $n =$ number of replicate plots, P and F values with degrees of freedom are given

in tables, figures and appendices. Analyses were performed using R 2.11.1 (R Development Core Team 2010).

Results

Soil N pools and the dynamics of ^{15}N tracers in the control forests

In both forests at 1000 m and 3000 m elevations, the NH_4^+ and NO_3^- concentrations before and 1-d after application of the ^{15}N tracers did not differ (Paired T test; $P = 0.18$ to 0.35). Likewise, the extractable N pools (NH_4^+, NO_3^- and organic N; Table 1) did not differ between ^{15}N sources at each forest site across all sampling days (LME model; $F_{1, 4} = 1.8$ to 2.8 ; $P = 0.17$ to 0.25). All of these indicated that the applied ^{15}N did not affect the size of these extractable N pools. All N pools (LME model; $F_{1, 4} = 12.0$ to 18.7; $P = 0.01$ to 0.02) and microbial C (LME model; $F_{1, 4} = 9.0$; $P = 0.04$) were larger whereas microbial C:N ratio were smaller (LME model; $F_{1, 4} = 11.0$; $P = 0.03$) at 1000 m than at 3000 m (Table 1).

We observed two phases of ^{15}N tracer dynamics: (1) a redistribution phase that occurred generally during 1-20 days after ^{15}N addition, characterized by large fluxes and fast transfers of ^{15}N among N pools, and (2) an equilibrium phase that occurred during 20-355 days, depicted by small fluxes and stable transfers of ^{15}N among N pools (Fig. 7; Appendix 2). The addition $^{15}NH_4^+$ tracer at 1000 m showed that atom percent excess ^{15}N was largest in microbial biomass followed by fine roots, NH_4^+ and extractable organic N pools during 1 to 20 days and these ^{15}N enrichments decreased steadily thereafter (Fig. 7a; Table 2 for the averages of atom percent excess ^{15}N in the redistribution and equilibrium phases). Similar trends across time were observed at 3000 m (Appendix 2a; Table 2). The addition of $^{15}NO_3^-$ tracer showed that atom percent excess ^{15}N was largest in microbial biomass and fine roots both at 1000 m (Fig. 7c; Table 2) and 3000 m (Appendix 2c; Table 2). Obviously, the atom percent excess ^{15}N of N pools with $^{15}NH_4^+$ addition was larger than those with $^{15}NO_3^-$ addition (Figs. 7a, 7b; Appendix 2a, 2b) because the amount of added $^{15}NH_4^+$ was also larger than that of added $^{15}NO_3^-$, which was done in order not to change the inherent proportion NH_4^+ and NO_3^- in these sites (Table 1). These and the fact that different N pools have different amounts (i.e. extractable N pools were smaller than

microbial N, fine-root N and soil organic N; Table 1) necessitate characterization of the paths and kinetics of the ^{15}N tracers in the different pools by looking at the ^{15}N recoveries in each pool. The calculation of ^{15}N recoveries (see *Calculations*) accounts for the differences in N pool sizes. We used the ^{15}N recoveries to document the paths, fluxes and fates of $^{15}NH_4^+$ and $^{15}NO_3^-$ tracers based on the two clear phases of their kinetics: redistribution phase that corresponds to short-term fates and equilibrium phase that suggests the long-term fates. ^{15}N recovery in a N pool signify the percentage of initially added ^{15}N cycled per day or as the amount of added ^{15}N cycled per square meter per day.

Redistribution phase (1 – 20 days) of the ^{15}N tracers in control forests

At 1000 m, the addition of $^{15}NH_4^+$ tracer showed the highest ^{15}N recoveries in the soil organic N followed by microbial N and fine roots (Fig. 8a; Table 3). The addition of $^{15}NO_3^-$ tracer showed the same patterns (Fig. 8c; Table 3). The extractable N pools had only 0.1-1.5 % of the applied $^{15}NH_4^+$ or $^{15}NO_3^-$ (Table 3). The form of ^{15}N tracer did not have an effect on the ^{15}N recoveries in the different N pools and in the whole plot (all F and P values are reported in Appendix 3). At 3000 m, the ^{15}N tracer forms also did not affect the ^{15}N recoveries in the different N pools (Appendix 3) and ^{15}N recoveries were similarly the highest in soil organic N followed by microbial N and fine roots (Appendix 4a, 4c; Table 3). In both ^{15}N tracers, ^{15}N recoveries in microbial N were larger at 1000 m whereas the ^{15}N recoveries in the fine roots were smaller than those at 3000 m (Table 3; LME model; $F_{1,4} = 8.6$ to 14.6; $P = 0.02$ to 0.04); ^{15}N recoveries in the soil organic N did not differ between elevations (Table 3; LME model; $F_{1,4} = 3.1$ to 4.2; $P = 0.10$ to 0.15). Total ^{15}N recoveries in the whole plot were on average 85-91 % of the added ^{15}N (Fig. 8a, 8c, Appendix 4a, 4c; Table 3) and these values are not significantly different from 100 % recovery of added ^{15}N (LME model; $F_{1,4} = 0.98$ to 1.08; $P = 0.36$ to 0.38).

Since the ^{15}N tracer forms did not affect the ^{15}N recoveries in all N pools, we combined the ^{15}N recoveries from both $^{15}NH_4^+$ and $^{15}NO_3^-$ tracers in calculating the net tracer flux in each N

Table 5 Nitrogen pools (mean ± SE, $n = 3$)[a], microbial C and microbial C:N ratio across 1 to 355 days after ^{15}N ($^{15}NH_4^+$ and $^{15}NO_3^-$) application in the control and N addition plots. Values are for the top 5-cm depth, corresponding to the mineral soil for the forest at 1000 m elevation and organic layer for the forest at 3000 m elevation

Soil parameters	Control		4 years of N addition	
	1000 m elevation			
	$^{15}NH_4^+$ enriched	$^{15}NO_3^-$ enriched	$^{15}NH_4^+$ enriched	$^{15}NO_3^-$ enriched
NH_4^+ (mg N m^{-2})	217.1 ± 29.3b	266.8 ± 22.1	334.9 ± 14.38a	291.8 ± 41.7
NO_3^- (mg N m^{-2})	65.2 ± 1.8	60.6 ± 2.9b	70.6 ± 2.7	79.6 ± 6.2a
Extractable organic N (mg N m^{-2})	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1
Microbial N (g N m^{-2})	5.2 ± 0.7	7.0 ± 0.7	5. 9 ± 0.2	6.3 ± 0.3
Fine-root N (g N m^{-2})	1.7 ± 0.1	1.4 ± 0.1	1.9 ± 0.0	1.5 ± 0.1
Soil organic N (g N m^{-2})	176.6 ± 2.0	177.5 ± 3.0	179.6 ± 2.6	180.3 ± 3.2
Total N (g N m^{-2})	184.8 ± 2.3	187.3 ± 2.9	188.8 ± 2.7	189.6 ± 2.9
Microbial C (g C m^{-2})	70.8 ± 7.6a	89.8 ± 8.9a	55.9 ± 2.0b	54.4 ± 2.9b
Microbial C:N ratio	13.8 ± 0.4a	12.9 ± 0.2a	9.3 ± 0.3b	8.8 ± 0.2b
	3000 m elevation			
NH_4^+ (mg N m^{-2})	185.6 ± 28.5b	170.4 ± 7.1	252.35 ± 20.76a	216.53 ± 5.37
NO_3^- (mg N m^{-2})	22.7 ± 2.0	21.7 ± 1.1	29.68 ± 1.71	30.70 ± 3.19
Extractable organic N (mg N m^{-2})	0.9 ± 0.1	1.1 ± 0.1	1.15 ± 0.06	1.35 ± 0.08
Microbial N (g N m^{-2})	1.9 ± 0.1	2.0 ± 0.1	1.9 ± 0.1	2.1 ± 0.0
Fine-root N (g N m^{-2})	3.5 ± 0.2	3.4 ± 0.2	3.4 ± 0.2	3.4 ± 0.2
Soil organic N (g N m^{-2})	92.1 ± 0.3	91.9 ± 0.3	91.9 ± 2.3	91.5 ± 2.4
Total N (g N m^{-2})	98.6 ± 0.2	98.5 ± 0.3	98.6 ± 2.5	98.6 ± 2.5
Microbial C (g C m^{-2})	39.6 ± 2.3a	40.3 ± 1.6a	27.2 ± 1.8b	33.1 ± 0.5b
Microbial C:N ratio	20.9 ± 0.3a	20.4 ± 0.3a	14.7 ± 0.3b	15.6 ± 0.2b

[a] Means with different letters indicate significant differences between treatments (control vs N addition) for each ^{15}N source (either $^{15}NH_4^+$ or $^{15}NO_3^-$) and forest site (linear mixed effects model; $P \leq 0.05$). All N pools did not differ significantly between ^{15}N sources ($^{15}NH_4^+$ vs $^{15}NO_3^-$) at each treatment and forest site (linear mixed effects model at $F_{1,4} = 1.8$ to 2.8 ; $P = 0.17$ to 0.25).

[b] Control plots had no fertilizer addition; nitrogen plots were fertilized with 50 kg urea-N ha^{-1} $year^{-1}$ starting in 2008, split in two equal applications per year and broadcasted by hand. Sampling started in October 2010 and ended in November 2011, corresponding to 4 years of treatment.

Table 6 ^{15}N enrichments[a] (mean ± SE; $n = 3$) of N pools in the redistribution phase (1 to 20 days) and equilibrium phase (20 to 355 days) following ^{15}N ($^{15}NH_4^+$ or $^{15}NO_3^-$) application. Values are for the top 5-cm depth, corresponding to the mineral soil for the forest at 1000 m elevation and organic layer for the forest at 3000 m elevation

N pools	Redistribution phase ^{15}N atom % excess				Equilibrium phase ^{15}N atom % excess			
	Control		N addition		Control		N addition	
	$^{15}NH_4^+$	$^{15}NO_3^-$	$^{15}NH_4^+$	$^{15}NO_3$-	$^{15}NH_4^+$	$^{15}NO_3^-$	$^{15}NH_4^+$	$^{15}NO_3$-
1000 m elevation								
NH_4^+	0.24 ± 0.02	0.02 ± 0.01	0.22 ± 0.02	0.03 ± 0.00	0.11 ± 0.02	0.02 ± 0.00	0.10 ± 0.01	0.03 ± 0.00
NO_3^-	0.07 ± 0.01	0.01 ± 0.00	0.09 ± 0.01	0.01 ± 0.00	0.05 ± 0.02	0.01 ± 0.00	0.06 ± 0.02	0.02 ± 0.00
Extractable organic N	0.15 ± 0.02	0.03 ± 0.00	0.16 ± 0.01	0.03 ± 0.00	0.08 ± 0.01	0.02 ± 0.00	0.08 ± 0.01	0.02 ± 0.00
Microbial N	0.56 ± 0.06	0.19 ± 0.03	0.34 ± 0.04	0.16 ± 0.04	0.20 ± 0.03	0.06 ± 0.01	0.10 ± 0.01	0.05 ± 0.00
Fine-root N	0.37 ± 0.05	0.12 ± 0.02	0.20 ± 0.02	0.08 ± 0.01	0.17 ± 0.02	0.13 ± 0.00	0.09 ± 0.00	0.06 ± 0.00
Soil organic N	0.05 ± 0.00	0.01 ± 0.00	0.04 ± 0.00	0.00 ± 0.00	0.04 ± 0.01	0.01 ± 0.00	0.03 ± 0.00	0.00 ± 0.00
3000 m elevation								
NH_4^+	0.20 ± 0.03	0.01 ± 0.00	0.21 ± 0.03	0.01 ± 0.00	0.09 ± 0.03	0.00 ± 0.00	0.10 ± 0.02	0.00 ± 0.00
NO_3^-	0.09 ± 0.01	0.01 ± 0.00	0.10 ± 0.02	0.01 ± 0.00	0.07 ± 0.01	0.05 ± 0.00	0.08 ± 0.02	0.05 ± 0.00
Extractable organic N	0.16 ± 0.03	0.00 ± 0.00	0.19 ± 0.03	0.00 ± 0.00	0.10 ± 0.03	0.00 ± 0.00	0.09 ± 0.01	0.00 ± 0.00
Microbial N	0.59 ± 0.05	0.18 ± 0.02	0.35 ± 0.02	0.17 ± 0.03	0.21 ± 0.04	0.16 ± 0.00	0.15 ± 0.01	0.09 ± 0.00
Fine roots	0.35 ± 0.03	0.13 ± 0.00	0.22 ± 0.03	0.12 ± 0.01	0.20 ± 0.02	0.12 ± 0.00	0.09 ± 0.02	0.07 ± 0.00
Soil organic N	0.08 ± 0.01	0.07 ± 0.00	0.06 ± 0.00	0.00 ± 0.00	0.08 ± 0.01	0.06 ± 0.00	0.05 ± 0.01	0.03 ± 0.00

[a] Statistical tests are given in Appendix 6 for the redistribution phase and in Appendix 7 for the equilibrium phase.

Table 7 ^{15}N recoveries[a] (mean ± SE; $n = 3$) in different N pools in the redistribution phase (1 to 20 days) and equilibrium phase (20 to 355 days) following ^{15}N ($^{15}NH_4^+$ or $^{15}NO_3^-$) application. Values are for the top 5-cm depth, corresponding to the mineral soil for the forest at 1000 m elevation and organic layer for the forest at 3000 m elevation

N pools	Redistribution phase ^{15}N recovered/^{15}N applied (%)				Equilibrium phase ^{15}N recovered/^{15}N applied (%)			
	Control		N addition		Control		N addition	
	$^{15}NH_4^+$	$^{15}NO_3^-$	$^{15}NH_4^+$	$^{15}NO_3^-$	$^{15}NH_4^+$	$^{15}NO_3^-$	$^{15}NH_4^+$	$^{15}NO_3^-$
1000 m elevation								
NH_4^+	0.72 ± 0.13	0.75 ± 0.16	0.95 ± 0.15	0.73 ± 0.06	0.27 ± 0.06	0.35 ± 0.11	0.25 ± 0.07	0.40 ± 0.12
NO_3^-	0.11 ± 0.00	0.13 ± 0.02	0.13 ± 0.00	0.18 ± 0.05	0.04 ± 0.00	0.06 ± 0.01	0.02 ± 0.00	0.08 ± 0.02
Extractable organic N	0.88 ± 0.27	1.50 ± 0.21	0.98 ± 0.20	2.01 ± 0.39	0.57 ± 0.17	0.51 ± 0.06	0.56 ± 0.04	0.52 ± 0.04
Microbial N	23.24 ± 2.77	23.06 ± 1.93	15.89 ± 1.85	14.66 ± 1.64	8.24 ±1.42	8.38 ± 1.13	3.71 ± 0.55	4.25 ± 1.07
Fine roots	6.10 ± 1.02	6.89 ± 0.67	3.59 ± 0.47	3.88 ± 0.47	2.74 ± 0.27	2.63 ± 0.70	1.50 ± 0.10	1.99 ± 0.31
Soil organic N	56.80 ± 4.14	52.29 ± 4.14	42.52 ± 3.15	40.20 ± 2.54	60.69 ± 3.50	56.43 ± 5.13	37.99 ± 3.67	32.55 ± 2.03
Total N	87.75 ± 3.78	84.61 ± 4.15	63.66 ± 4.19	61.66 ± 3.48	72.42 ± 4.44	70.16 ± 5.19	44.03 ± 3.63	43.49 ± 2.11
3000 m elevation								
NH_4^+	0.64 ± 0.09	0.62 ± 0.09	0.63 ± 0.15	0.56 ± 0.11	0.12 ± 0.04	0.15 ± 0.06	0.13 ± 0.09	0.16 ± 0.05
NO_3^-	0.12 ± 0.01	0.12 ± 0.03	0.13 ± 0.01	0.15 ± 0.05	0.01 ± 0.00	0.04 ± 0.01	0.02 ± 0.00	0.03 ± 0.01
Extractable organic N	0.92 ± 0.35	1.40 ± 0.39	1.82 ± 0.21	1.67 ± 0.26	0.64 ± 0.26	0.58 ± 0.09	0.12 ± 0.05	0.19 ± 0.08
Microbial N	13.02 ± 1.28	11.61 ± 1.98	6.43 ± 0.75	9.82 ± 1.26	8.52 ± 0.63	11.40 ± 1.57	3.85 ± 0.20	3.25 ± 1.47
Fine roots	11.44 ± 1.33	14.50 ± 1.56	7.03 ± 0.81	8.18 ± 1.35	6.64 ± 1.06	6.38 ± 1.43	3.04 ± 0.70	3.79 ± 0.99
Soil organic N	61.79 ± 4.92	52.37 ± 1.98	45.73 ± 2.89	37.04 ± 0.92	64.19 ± 4.96	48.39 ± 1.56	37.21 ± 3.44	29.48 ± 4.95
Total N	87.64 ± 4.94	90.61 ± 2.90	61.67 ± 3.52	57.42 ± 1.53	75.12 ± 4.76	66.63 ± 3.41	43.47 ± 3.71	38.90 ± 3.81

[a] Statistical tests are given in Appendix 3 for the redistribution phase and in Appendix 5 for the equilibrium phase.

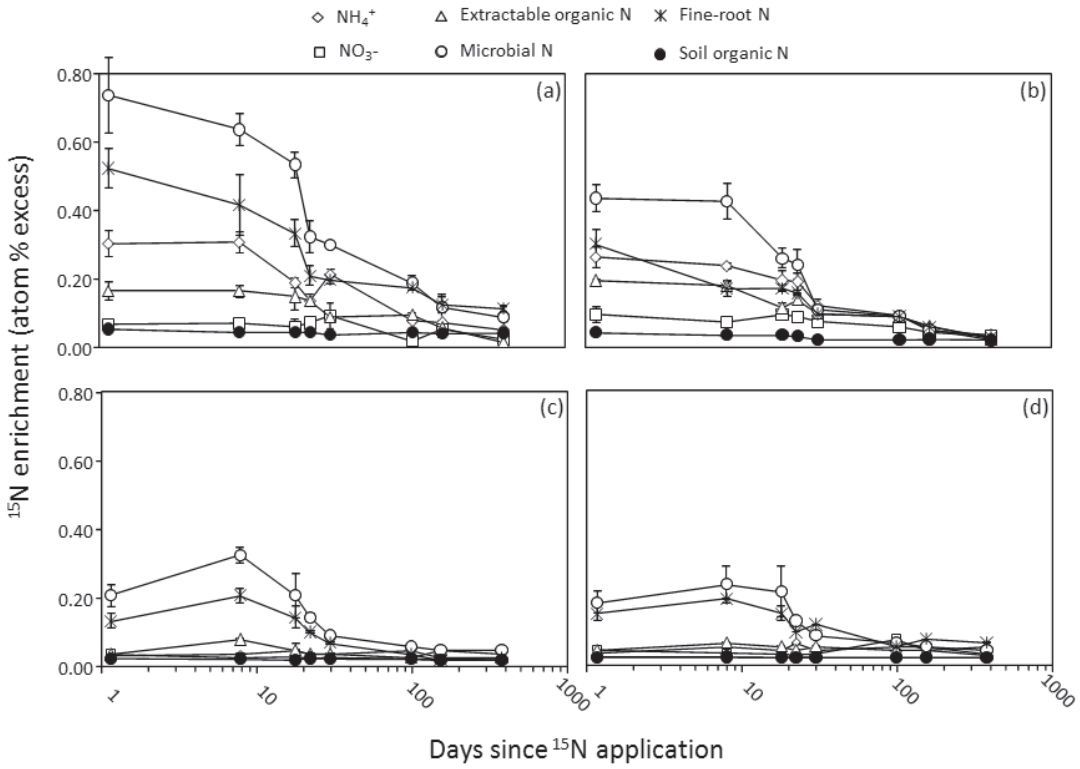


Fig. 7 ^{15}N (atom % excess; mean ± SE, n = 3) enrichments with time in different N pools (NH_4^+, NO_3^-, extractable organic N, microbial N, fine-root N and soil organic N) in the top 5-cm depth (mineral soil) of the forest at 1000 m elevation. $^{15}NH_4^+$ pulse chase in the (a) control and (b) N addition plots with 4 years of treatment; $^{15}NO_3^-$ pulse chase in the (c) control and (d) N-addition plots with 4 years of treatment

pool. At 1000 m, the gain of ^{15}N in soil organic N (0.68 ± 0.04 % d^{-1}) was paralleled by the loss of ^{15}N largely from microbial N (Fig. 9a); the cycle of ^{15}N through the microbial N pool was -0.66 ± 0.02 % d^{-1}. At 3000 m, the gain of ^{15}N in soil organic N (0.70 ± 0.09 % d^{-1}) was contributed both by the losses of ^{15}N from both microbial N (-0.31 ± 0.04 % d^{-1}) and fine roots (-0.17 ± 0.01 % d^{-1}) (Fig. 10a). The mean net rates of ^{15}N fluxes from the whole plots (i.e. sum of the measured component pools of soil N) were -0.34 ± 0.12 % d^{-1} at 1000 m (Fig. 9a) and -0.28 ± 0.03 % d^{-1} at 3000 m (Fig. 10a).

Equilibrium phase (20 – 355 days) of the ^{15}N tracers in the control forests

In either of the ^{15}N tracer source, the highest recoveries were still in soil organic N, microbial biomass and fine roots at 1000 m (Fig. 8a, c; Table 3) and 3000 m (Appendix 3a, c; Table 3). ^{15}N recoveries in extractable N pools further decreased from those at redistribution phase, ranging

from 0 to 0.6 % of the applied $^{15}NH_4^+$ or $^{15}NO_3^-$ at both elevations (Table 3). Similar to that in the redistribution phase, the forms of ^{15}N applied also did not influence the ^{15}N recoveries in the different N pools both at 1000 m and 3000 m (Table 3; all *F* and *P* values are reported in Appendix 5). ^{15}N recoveries in soil organic N and microbial N did not differ between elevations (Table 3; LME model; $F_{1,4} = 1.2$ to 2.1; $P = 0.22$ to 0.33) whereas recoveries in fine roots at 1000 m was lower than at 3000 m (Table 3; LME model; $F_{1,4} = 9.8$; $P = 0.04$). Total ^{15}N recoveries in the whole plot decreased from those in the redistribution phase, averaging 67-75 % of the added ^{15}N (Fig. 8a, 8c; Appendix 4a, c; Table 3).

In general, the gain of ^{15}N in soil organic N was smaller than that in redistribution phase both at 1000 m (0.003 ± 0.00 % d^{-1}; Fig. 9c) and 3000 m (0.002 ± 0.00 % d^{-1}; Fig. 10c) and there was a net loss of ^{15}N from the whole plot (-0.04 ± 0.00 % d^{-1} at 1000 m (Fig. 9c) and -0.05 ± 0.00 % d^{-1} at 3000 m (Fig. 10a)). These net losses of ^{15}N from the plots were paralleled by similar losses of ^{15}N from mainly microbial N pool at the 1000 m elevation (Fig. 9c) and from microbial N and fine-root N pools at the 3000 m elevation (Fig. 10c).

N addition effects on the soil N pools and the dynamics of ^{15}N tracers

Similar to the control forests, the NH_4^+ and NO_3^- concentrations before and 1-day after application of either of the ^{15}N tracers did not differ (Paired T test; $P = 0.15$ to 0.43) as well as the forms of ^{15}N applied did not change the sizes of the extractable N pools across all sampling days at both 1000 m and 3000 m elevations (Table 1; LME model; $F_{1,4} = 1.80$ to 2.29; $P = 0.20$ to 0.25). At both elevations, four years of N addition increased the NH4+ levels and decreased microbial C and microbial C:N ratios; NO_3^- levels only increased with N addition at 1000 m but not at 3000 m (Table 1).

We observed similar patterns of the dynamics of ^{15}N tracers as those in the control plots: large fluxes and fast transfers of ^{15}N among N pools in the redistribution phase, and small fluxes and stable transfers of ^{15}N among N pools in the equilibrium phase (Fig. 7b, 7d; Appendix 2b, 2d). At both elevations, similar to the control plots, the addition $^{15}NH_4^+$ tracer showed that atom percent excess ^{15}N was largest in microbial biomass followed by fine roots, NH_4^+ and extractable

organic N pools (Fig. 7b, Appendix 2b; Table 2 for the averages of atom percent excess ^{15}N in the redistribution and equilibrium phases); the addition of $^{15}NO_3^-$ tracer also showed that atom

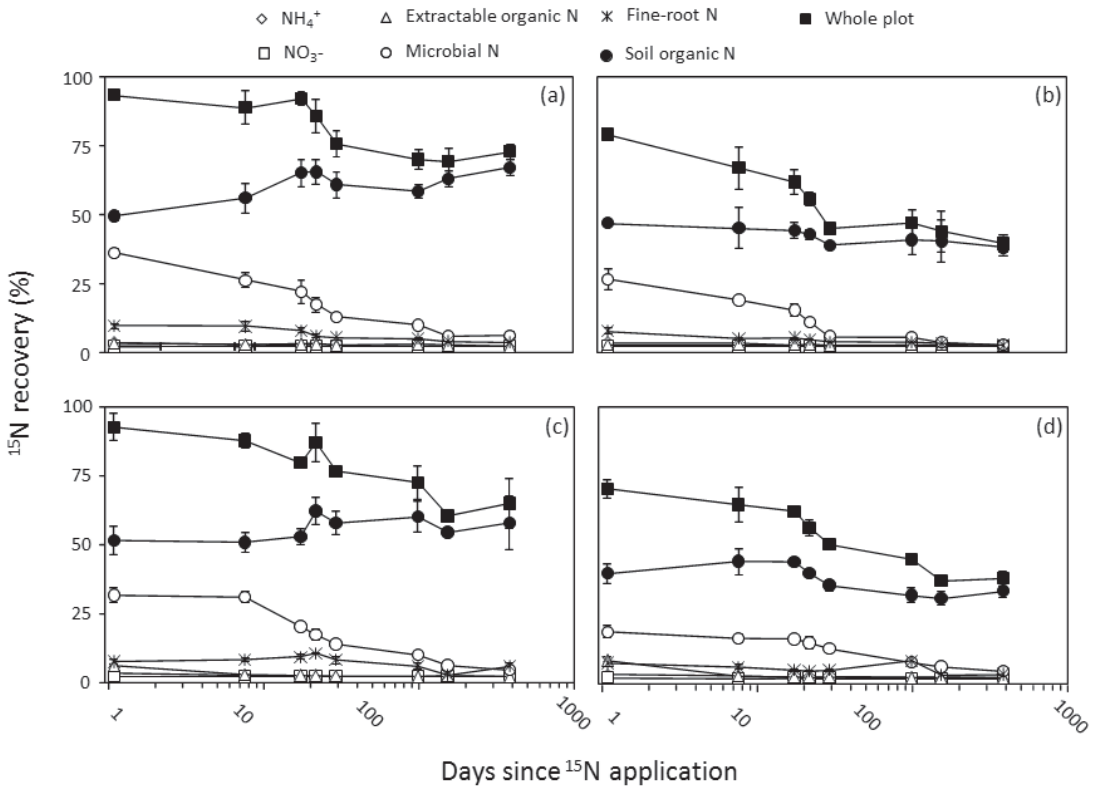


Fig. 8 ^{15}N % recovery (^{15}N recovered in a N pool ÷ ^{15}N applied x 100; mean ± SE, n = 3) with time in different N pools (NH_4^+, NO_3^-, extractable organic N, microbial N, fine-root N and soil organic N) in the top 5-cm depth (mineral soil) of the forest at 1000 m elevation. $^{15}NH_4^+$ pulse chase in the (a) control and (b) N addition plots with 4 years of treatment; $^{15}NO_3^-$ pulse chase in the (c) control and (d) N-addition plots with 4 years of treatment

percent excess ^{15}N was largest in microbial biomass and fine roots (Fig. 7d; Appendix 2d; Table 2). It should be noted, however, that while the patterns of ^{15}N tracer dynamics were similar as those in the control plots, the levels of ^{15}N enrichments were reduced in plots with four years of N addition compared to the controls at both elevations, particularly in microbial biomass, fine roots and organic N pool in the redistribution phase (Table 2; all *F* and *P* values are reported in Appendix 6) and equilibrium phase (Table 2; all *F* and *P* values are reported in Appendix 7). Also, the atom percent excess ^{15}N of N pools with $^{15}NH_4^+$ addition was larger than those with $^{15}NO_3^-$ addition (Table 2; all *F* and *P* values are reported in Appendices 6 and 7), which as mentioned above necessitates the use the ^{15}N recoveries (to account for the differences in the sizes of N pools) to trace the changes in paths, fluxes and fates of $^{15}NH_4^+$ and $^{15}NO_3^-$ tracers as affected by chronic N addition.

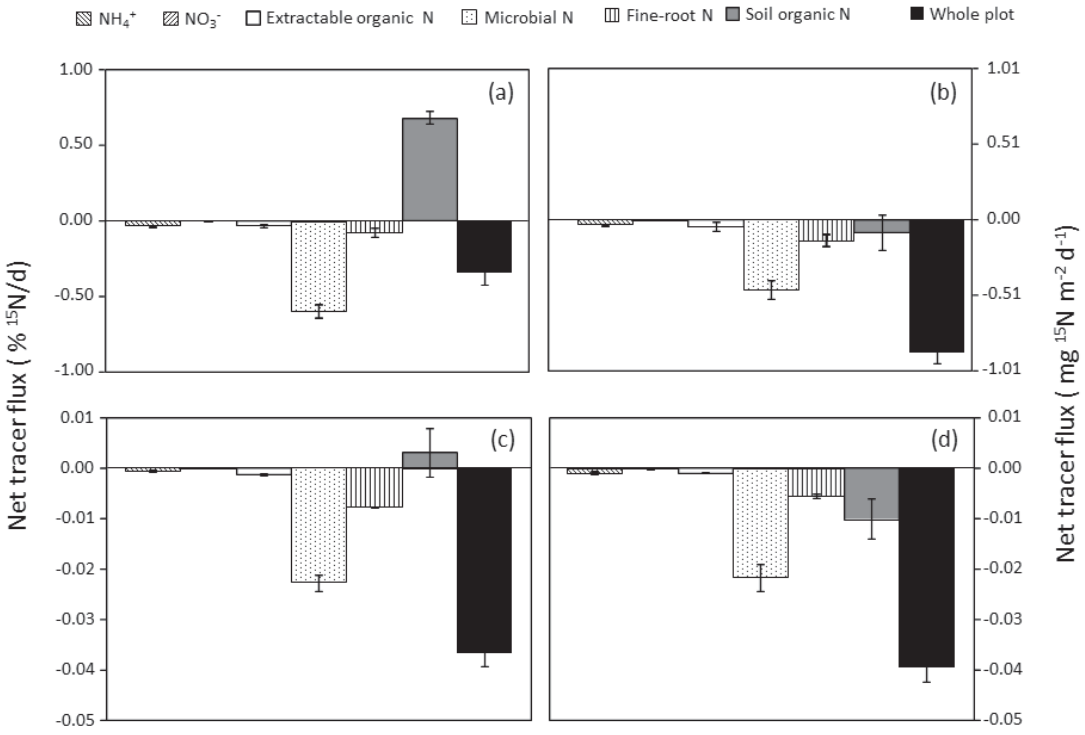


Fig. 9 Net ^{15}N tracer fluxes in different N pools ((NH_4^+, NO_3^-, extractable organic N, microbial N, fine-root N and soil organic N) in the top 5-cm depth (mineral soil) of the forest at 1000 m elevation during the redistribution phase (1 to 20 days) in the (a) control and (b) N addition plots with 4 years of treatment and during the equilibrium phase (20 to 355 days) in the (c) control and (d) N addition plots with 4 years of treatment. Net tracer fluxes are expressed in two equivalent units on the y-axes: left axis is percentage of initially added ^{15}N cycled per day; right axis is ^{15}N fluxes as milligrams of added ^{15}N cycled per square meter per day. Bars are averages of both ^{15}N sources ($^{15}NH_4^+$ and $^{15}NO_3^-$) and are the mean $\pm$ SE, n = 3

N addition effects on the redistribution phase (1 – 20 days) of the ^{15}N tracers

Similar to the control plots, the addition of either $^{15}NH_4^+$ or $^{15}NO_3^-$ tracers showed the highest ^{15}N recoveries in the soil organic N followed by microbial N and fine roots at 1000 m (Fig. 8b, 8d; Table 3) and 3000 m (Appendix 4b, 4d; Table 3). ^{15}N recoveries in extractable N pools were only 0.1-2.0 % of the applied $^{15}NH_4^+$ or $^{15}NO_3^-$ (Table 3). Although the trends of ^{15}N recoveries among these N pools were similar to those in the control plots, the magnitudes of ^{15}N recoveries differed. Four years of N addition decreased the ^{15}N recoveries in soil organic N, microbial N and fine roots from the applied $^{15}NH_4^+$ or $^{15}NO_3^-$ compared to the control plots (Table 3; Appendix 3

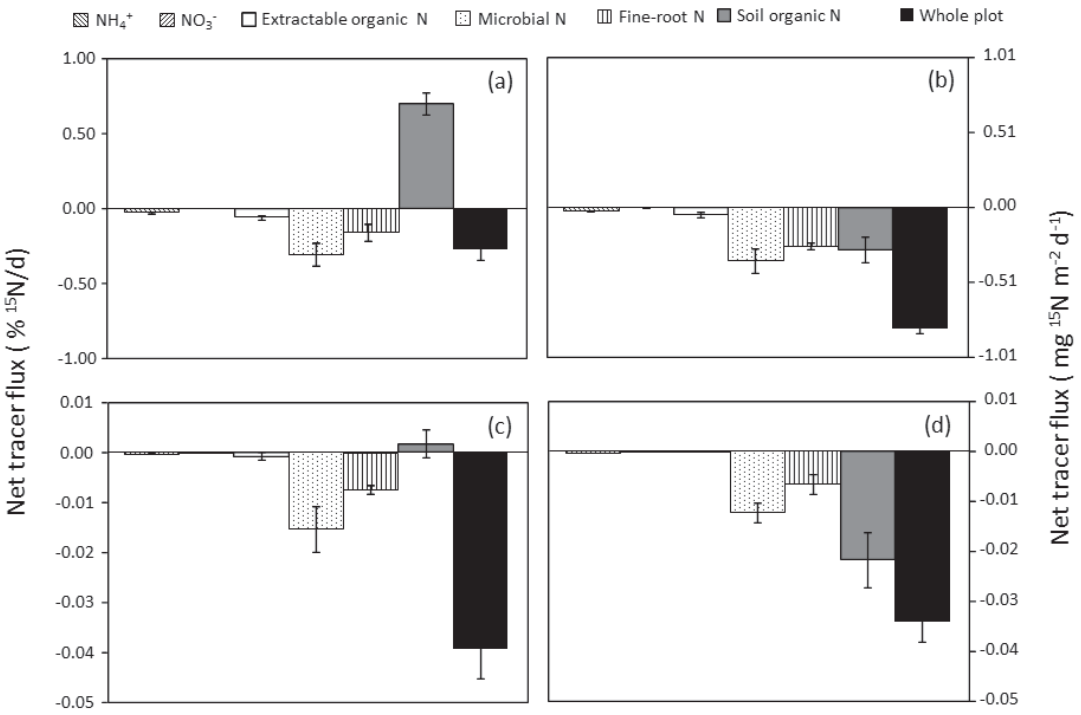


Fig. 10 Net ^{15}N tracer fluxes in different N pools ((NH_4^+, NO_3^-, extractable organic N, microbial N, fine-root N, and soil organic N) in the top 5-cm depth (organic layer) of the forest at 3000 m elevation during the redistribution phase (1 to 20 days) in the (a) control and (b) N addition plots with 4 years of treatment and during the equilibrium phase (20 to 355 days) in the (c) control and (d) N addition plots with 4 years of treatment. Net tracer fluxes are expressed in two equivalent units on the y-axes: left axis is percentage of initially added ^{15}N cycled per day; right axis is ^{15}N fluxes as milligrams of added ^{15}N cycled per square meter per day. Bars are averages of both ^{15}N sources ($^{15}NH_4^+$ and $^{15}NO_3^-$) and are the mean $\pm$ SE, n = 3

for *F* and *P* values). Consequently, total ^{15}N recoveries in the whole plots were also lower than those in the control plots at both elevations, averaging 57-64 % of the added ^{15}N (Table 3; Appendix 3 for *F* and *P* values). The form of ^{15}N tracer also did not have an effect on the ^{15}N recoveries in the different N pools and in the whole plot (all *F* and *P* values are reported in Appendix 3) at both elevations, and thus ^{15}N recoveries from both $^{15}NH_4^+$ and $^{15}NO_3^-$ tracers were combined in calculating the net tracer flux in each N pool.

At 1000 m, as opposed to the control plots, there was a loss of ^{15}N from the soil organic N (-0.18 $\pm$ 0.12 % d^{-1}) and this was paralleled by the loss of ^{15}N largely from the microbial N (-0.46 $\pm$ 0.06 % d^{-1}) (Fig. 9b). At 3000 m, there was also a loss of ^{15}N from the soil organic N (-0.29 $\pm$ 0.09 % d^{-1}) and this was paralleled by the losses of ^{15}N from both the microbial N (-0.36 $\pm$ 0.04 % d^{-1}) and fine roots (-0.26 $\pm$ 0.01 % d^{-1}) (Fig. 10b). The mean net rates of ^{15}N fluxes from the

whole plots were -0.88 ± 0.07 % d^{-1} at 1000 m (Fig. 9b) and -0.81 ± 0.03 % d^{-1} at 3000 m (Fig. 10b), which were larger (in terms of absolute values) than the mean net rates of whole-plot ^{15}N fluxes in the corresponding control plots (Figs. 9a and 10a).

N addition effects on the equilibrium phase (20 – 355 days) of the ^{15}N tracers

In either of the ^{15}N tracer source, the highest ^{15}N recoveries were still in soil organic N followed by microbial biomass and fine roots both at 1000 m (Fig. 8b, 8d; Table 3) and 3000 m (Appendix 4b, 4d; Table 3). ^{15}N recoveries in extractable N pools further decreased from those in the redistribution phase, ranging from 0.02 to 0.6 % of the applied $^{15}NH_4^+$ or $^{15}NO_3^-$ at both elevations (Table 3). Similarly, four years of N addition decreased the ^{15}N recoveries in soil organic N, microbial N and fine roots from the applied $^{15}NH_4^+$ or $^{15}NO_3^-$ compared to the control plots (Table 3; Appendix 5 for *F* and *P* values). Thus, total ^{15}N recoveries in the whole plots were lower than those in the control plots at both elevations, averaging 39 - 44 % of the added ^{15}N (Table 3; Appendix 5 for *F* and *P* values). Similar to that in the redistribution phase, the forms of ^{15}N applied also did not influence the ^{15}N recoveries in the different N pools both at 1000 m and 3000 m (Table 3; all *F* and *P* values are reported in Appendix 5).

As opposed to the control plots, there were losses of ^{15}N from the soil organic N but these losses were smaller than those in the redistribution phase (-0.01 ± 0.00 % d^{-1} at 1000 m, Fig. 9d; -0.02 ± 0.00 % d^{-1} at 3000 m, Fig. 10d). Consequently, there were net losses of ^{15}N from the whole plot (-0.04 ± 0.00 % d^{-1} at 1000 m, Fig. 9d; -0.03 ± 0.00 % d^{-1} at 3000 m, Fig. 10d). These net losses of ^{15}N from the plots were paralleled by similar losses of ^{15}N from mainly the microbial N pool at the 1000 m (Fig. 9d) and from the microbial N and fine-root N pools at the 3000 m (Fig. 10d).

Discussion

Studies that trace the fates of inorganic N in forest ecosystems are rare in the tropics and we only know so far of one that was conducted in Puerto Rico (Templer et al. 2008). Our ^{15}N recoveries in different N pools (e.g. microbial biomass, fine roots and soil organic N) and whole-

plot ^{15}N recoveries were comparable with those from an unpolluted temperate forest in Chile (Perakis and Hedin 2001), which had similar experimental set-up as our study (i.e. ^{15}N applied onto the plots and traced in the same soil N pools at several periods in a year or two), but higher (particularly for recoveries in microbial biomass) than those from a tropical montane forest in Puerto Rico (Templer et al. 2008). This study in Puerto Rico differed from ours, aside from differences in vegetation and soil types (i.e. lower montane forest dominated by one species on a Cambisol soil in Puerto Rico), in terms of climate (4.5 m $year^{-1}$ rainfall), experimental design and the duration of the experiment; they used a 10-cm in-growth soil cores into which the ^{15}N tracer was injected and the tracing was done until 7 days after ^{15}N application. Templer and colleagues (2012), in their meta-analysis of tracing studies, found that several factors such as ecosystem type, vegetation, mycorrhiza, soil C:N, disturbance history and even the form of ^{15}N applied and its mode of application influence ecosystem retention of ^{15}N. In summary, inorganic N tracing studies are very limited in tropical forest ecosystems and different factors influence the amount of ^{15}N retained in ecosystem pools, making comparisons not easy.

Fluxes and fates of mineral N in control forests

Pulse-chase ^{15}N tracer methods allow us to understand the short-term mechanism (days to weeks) of competition and retention of soil mineral N and how these translate into longer term (months) patterns of storage, loss and recycling of N. The ^{15}N-labelled mineral N pools (NH_4^+ or NO_3^-) did not show the highest ^{15}N enrichments even after 1 day of ^{15}N application but instead showed rapid transfer of ^{15}N into soil organic N, microbial biomass and fine roots. Such rapid turnover supported our measured mean residence times (i.e. NH_4^+ or NO_3^- ÷ gross N mineralization or nitrification rates; Chapter 2) of NH_4^+ (0.8 day at 1000 m and 2.7 days at 3000 m) and NO_3^- pools (1.4 days at 1000 m and 3.2 days at 3000 m). These indicate that added or produced mineral N in the soil turned over into other N pools in about 1-3 days. Thus, we saw in the redistribution phase fast transfers of the added ^{15}N into N pools other than the labelled mineral N pools. The similar $^{15}NH_4^+$ and $^{15}NO_3^-$ recoveries in the soil organic N, microbial biomass and fine roots over short-term and long-term time scales (Table 3) demonstrated that

in these montane forest soils both forms of inorganic N were equally used relative to their internal supply, indicating a lack of preferential retention of either form of mineral N. The low N availability in these forest soils (i.e. low gross rates of N cycling compared to the gross N-cycling rates of montane forests on Andosol soils (Arnold et al. 2009; Corre et al. 2013)) might have induced plant roots, microbes and, also possibly, direct reactions to soil organic N to utilize equally either form of mineral N. This suggests that patterns of N uptake by microbes and plant roots might be influenced more by low N availability rather than the energetic advantages associated with using more reduced forms of N for growth. Similar results were reported for unpolluted temperate forest in Chile with low N availability (Perakis and Hedin 2001). We suggest that the much higher NH_4^+ than NO_3^- production (i.e. larger gross rates of N mineralization than nitrification; see Chapter 2), combined with non-preferential uptake of mineral N, leads to the characteristic dominance of NH_4^+ and NO_3^- observed from tropical montane forest soils (Silver et al. 2001; Arnold et al. 2009; Corre et al. 2013).

The similarity in retention of both forms of ^{15}N tracers over short-term and long-term time scales further suggests similar mechanisms of uptake and redistribution in the plant-soil-microbe system. The dominant fate of either ^{15}N tracer in the soil organic N at both elevations, which was observed already after 1 day of ^{15}N addition, suggests both biotic (microbial and plant uptake) and possibly abiotic reactions of mineral N with soil organic matter. Our study could not verify the contribution of abiotic reactions of NH_4^+ and NO_3^- with soil organic matter as such analysis needs storage of soil samples and chemical inhibitions of biotic processes (e.g. Johnson et al. 2000; Davidson et al. 2003; Fitzhugh et al. 2003), which would contrast our in-situ measurements of the different N pools to minimize disturbance. Nonetheless, similar findings as ours on high ^{15}N recoveries in organic N pool after 0.1 and 1 day of either $^{15}NH_4^+$ or $^{15}NO_3^-$ additions were reported for unpolluted temperate forest in Chile (Perakis and Hedin 2001). Based on the net ^{15}N trace flux, the short-term fate (i.e. redistribution phase) of mineral N at the 1000 m elevation showed that its sink in the soil organic N pool was largely contributed by microbial turnover of N and less by fine roots (Fig. 3; Table 3). At this forest site, the fine root density (Leuschner et al. 2007) and fine-root N in the top 5 cm of soil (Table 1) were low

whereas microbial N was large (Table 1), suggesting that microbes may be better competitors for available N than the fine roots. At 3000 m, the sink of mineral N in the soil organic N was contributed by the turnover of N both through the microbial biomass and fine roots (Fig. 9; Table 3). At this site, the fine root density at the top 5 cm was higher than at 1000 m elevation (Leuschner et al. 2007) and there was also larger fine-root N than microbial N (Table 1). These suggest that fine roots may be able to compete with microbes for the much lower supply of available N at this site (i.e. lower gross rates of N mineralization and nitrification than at 1000 m; Chapter 2). The turnover times of microbial N (i.e. microbial N ÷ $NH_4^+ + NO_3^-$ immobilization rates; Chapter 2) in these forests were on average 17 days at 1000 m and 19 days at 3000 m, indicating that the mineral N immobilized by the microbial biomass had turned over at least once during the redistribution phase. Fine-root turnover time at these sites is about one year (Graefe et al. 2008), but transfer of N from root uptake to soil organic N may not only be through root turnover but also through sloughing off of root cells, root exudates (Jones et al. 2009) and root-mycorrhizal association (e.g. Hobbie et al. 2007; Jones et al. 2009). In summary, the whole-plot ^{15}N recoveries indicated complete recovery of the added ^{15}N during the redistribution phase, and the net ^{15}N tracer flux signified the movement of ^{15}N from the microbial pool (i.e. 1000 m elevation) and fine root together (i.e. 3000 m elevation) to the soil organic N pool. This suggests that microbial turnover (e.g. mineral soils with low fine-root density) and fine-root release of N (e.g. organic layer with high fine-root density) are important controls on the short-term retention of N in the soil organic N pool.

The long-term fate (i.e. equilibrium phase) of mineral N showed that about 25 % of the added ^{15}N was not recovered in the N pools measured at least at the top 5 cm. Based on the ^{15}N tracer fluxes, the removal of ^{15}N from microbial pool (i.e. 1000 m; Fig. 9c) and fine roots together (i.e. 3000 m; Fig. 10c) was not compensated by the gain of ^{15}N in soil organic N pool, resulting in the net removal of ^{15}N from the whole plot. We interpret the net removal of ^{15}N from the whole plot as movement of N into other pools which we did not measure. N might have moved into the deeper soil layers (Emmett and Qarmby 1991), surface litter (Downs et al. 1996), other forest floor components like woody debris (Currie et al. 2002) and aboveground parts of plants (as

was implied by Perakis and Hedin 2001). If we take the total fine litter production of these forest sites (reported by Wolf et al. 2011) and multiplied by the net ^{15}N tracer flux from the whole plot, about one-fourth (0.01 mg N m^{-2} d^{-1}) of the ^{15}N unaccounted or removed from the whole plot (Figs. 9c and 10c) could be in the fine litterfall. Another sink that is difficult to measure in situ is N_2 flux from the soil. From another study conducted in the same sites, denitrification was shown as the dominant process of N_2O production (Müller et al. unpublished data) which could imply N_2 losses. The N that is released from microbial biomass and taken up by the roots may have been redistributed to unmeasured aboveground sink in plants and surface litter layer. Nevertheless, it should be pointed out that the ~75 % of ^{15}N tracer recovered was largely retained in the soil organic N with only negligible turnover into the extractable N pools (Table 3). A strong demand for N in these forests with low N availability may have resulted in efficient short- and long-term retention of N.

Rates of net ^{15}N tracer flux in the equilibrium phase (Figs. 9c and 10c) were an order of magnitude lower than in the redistribution phase (Figs. 9a and 10a). If we assume that any newly recycled ^{15}N in the equilibrium phase behaves similarly as the initial ^{15}N additions in the redistribution phase, then the relatively slow rates of net tracer flux during the equilibrium phase suggests that recycling of N through inorganic N pools and microbes was small at least throughout the period of our ^{15}N tracing experiment. Also, the consistent flux of N into slowly cycling pools of soil organic N suggests that internal soil N cycling may not be largely dependent on the recycling of recently incorporated N in soils. Similar to tracing studies conducted in other forests (e.g. Emmett and Quarmby 1991; Nadelhoffer et al. 1994; Buchmann et al. 1996; Downs et al. 1996; Magill et al. 1997; Perakis and Hedin 2001; Templer et al. 2008; Krause et al. 2012) and in a meta-analysis of ^{15}N tracing studies (Templer et al. 2012), the forest floor, specifically the soil organic matter, seemed to be the largest sink of the ^{15}N applied in our study. Our results suggest that the long-term fate of mineral N in the soil organic N pool was due to the rapid influx of both forms of inorganic N through microbes and fine roots.

Changes in fluxes and fates of N with four years of N addition

In the redistribution phase, although the paths of either ^{15}N tracer in the plots with 4 years of N addition were similar as those in the control plots, the fluxes were different. The short-term fate of ^{15}N showed that only about two-thirds of the added ^{15}N was recovered in the measured N pools, with most of it in the soil organic N (maximally 46 %) followed by microbial N and fine-root N pools (Table 3). The net tracer flux indicated that the loss of ^{15}N from the organic N pool and the whole plot was largely due to the fluxes of ^{15}N from the microbial biomass for the 1000 m elevation (Fig. 9b) and also with equal importance due to ^{15}N fluxes from the fine root for the 3000 m (Fig. 10b). Aside from the fact that there were increases in gross rates of N transformations in these N-addition plots, which may have increased transfers of N to other pools which we did not measure (as mentioned above), the net loss of ^{15}N from microbial and fine root-related N release could also be due to increases in N losses due to leaching and denitrification. Supports for these claims were first the indications from a forest site at 2000 m elevation (also part of the NUMEX) that transfers of N to above-ground plant parts and surface litter have increased even only after one year of N addition (Homeier et al. 2012). They observed that litterfall N concentration and N return with litterfall have significantly increased compared to the control plots. A similar finding was reported by Perakis et al. (2005) from an unpolluted temperate forest in Chile where the relative importance of soil organic N as ^{15}N sink decreased whereas ^{15}N sink in coarse roots increased across increasing rates of N additions, suggesting that shifts in the importance of forest floor components as potential N sinks may occur with chronic N addition. Second, we found that microbial N retention and production was uncoupled after 3-4 years of N addition (i.e. microbial N immobilization was less than microbial production of mineral N; Chapter 2), suggesting increased possibility for N losses or for plant uptake due to possible reduction in competition between microbes and plants for the increased available N. The turnover times of microbial N pool were also more than those in the control plots (32 and 23 days for the sites at 1000 m and 3000 m, respectively; Chapter 2). Thus, the reduced microbial N immobilization and increased turnover time may have resulted to the decreased

transfer of N into the soil organic N pool. Third, N leaching below the organic layer has increased in the first year of N addition to the forest soil at 2000 m elevation, although the leached N from the organic layer is all retained in the mineral soil (Wullaert et al. 2010). Increase in leaching of mineral N could happen in our sites especially that NH_4^+ (both at 1000 m and 3000 m; Table 1) and NO_3^- levels (at 1000 m) had increased with N addition. Lastly, soil N_2O fluxes at 1000 m have increased rapidly and fairly largely in the first two years of N addition whereas only small increases were observed at 3000 m in the second year of treatment (Martinson et al. 2013); in the third and fourth year of treatments, soil N_2O emissions from these N-addition plots did not anymore differ from the control plots (Müller et al. unpublished data). N loss as N_2 through denitrification (i.e. microbial consumption of N_2O to N_2) is difficult to measure in situ (Yang et al. 2010); this was not yet estimated for our study sites although from a separate study on ^{15}N tracing to N_2O, denitrification was the dominant process producing N_2O from our soils (Müller et al. unpublished data). Altogether, our results clearly show that chronic N addition resulted in reduced retention of the added ^{15}N because the cycling of N through microbial biomass (at 1000 m) and fine roots together (at 3000 m) did not result to its accumulation in the soil organic N pool but a net removal from this pool and thus the whole plot. Thus, the controls exerted by the microbial biomass and fine roots on the short-term retention of N may have lessened under elevated N input in these montane forests.

The long-term fate of mineral N showed that more than half of the added ^{15}N was not recovered in the N pools measured at the top 5 cm. Based on the net ^{15}N tracer fluxes, the transfer of N out of the microbial biomass (at 1000 m) and fine roots together (at 3000 m) were mirrored in the loss of ^{15}N out of the soil organic N pool, which resulted in the net removal of ^{15}N from the whole plot. Although the paths of ^{15}N transfers in the different N pools were similar as those in the redistribution phase, the net ^{15}N flux rates were an order of magnitude lower (Figs. 9d and 10d), signifying that there was very little recycling between microbe-soil-plant system of the newly incorporated ^{15}N in the soil at least within one year. Even though the ^{15}N recoveries have decreased with N addition, about a third of the added ^{15}N still remained in the soil organic N pool. Assuming that this long-term fate of ^{15}N holds true for the added N fertilizer, we

calculated that out of the applied 50 kg N ha^{-1} $year^{-1}$ about 17 kg N ha^{-1} $year^{-1}$ will stay in the organic N pool which would mean a sequestration of 238 kg C ha^{-1} $year^{-1}$ for the total soil C:N ratio of 14 at 1000 m and 476 kg C ha^{-1} $year^{-1}$ of sequestered C for the total soil C:N ratio of 28 at 3000m (Appendix 1). These C fluxes, however, are very small to detect and are only within the standard errors of the annual soil CO_2 fluxes (8.2 $\pm$ 0.3 and 3.9 $\pm$ 1.0 Mg C ha^{-1} $year^{-1}$ at 1000 m and 3000 m, respectively; Müller et al. unpublished data). Furthermore, the soil CO_2 fluxes at 1000 m did not differ between the control and N addition plots during the first five years of treatment. At 3000 m, N addition significantly increased soil CO_2 fluxes by 1.5 Mg C ha^{-1} $year^{-1}$ more than those from the control plots (Müller et al. unpublished data); if we assume that at least half of these CO_2 fluxes is from heterotrophic respiration (Zimmermann et al. 2010; van Straaten et al. 2011), the increase in heterotrophic respiration would be 750 kg C ha^{-1} $year^{-1}$, which would offset the sink of C in the soil organic pool at this site. Thus, N and C would possibly be retained in the soil organic pool at 1000 m but maybe removed at the 3000 m at time scales of decades. In summary, while it is difficult to speculate the fates of N that moved out of the pools we measured, it is clear that N and C could still be retained in the soil organic pool of these forest sites with 4 years of low N-addition rate although at reduced rates than in the control plots. Whether such reduced rates of N and C retention with N addition would result in overall reduction of N and C retention in the ecosystem would depend on whether or not such reductions are compensated by increases in plant-derived N and C inputs and in plant growth.

Conclusions

The low N availability in the control forest soils might have induced the lack of preferential retention for either NH_4^+ or NO_3^- and the efficient retention of both added ^{15}N tracers. Over short- and long-term temporal scales, the mechanisms of mineral N uptake and redistribution among of different N pools were similar in both forest sites, which were largely through microbial turnover and plant root uptake. Although N addition was only for 4 years with a total of 200 kg N ha^{-1}, we have observed a clear reduction in the N retention in the whole plot which was because the fluxes of N out of the microbial and fine-root N pools did not result in its

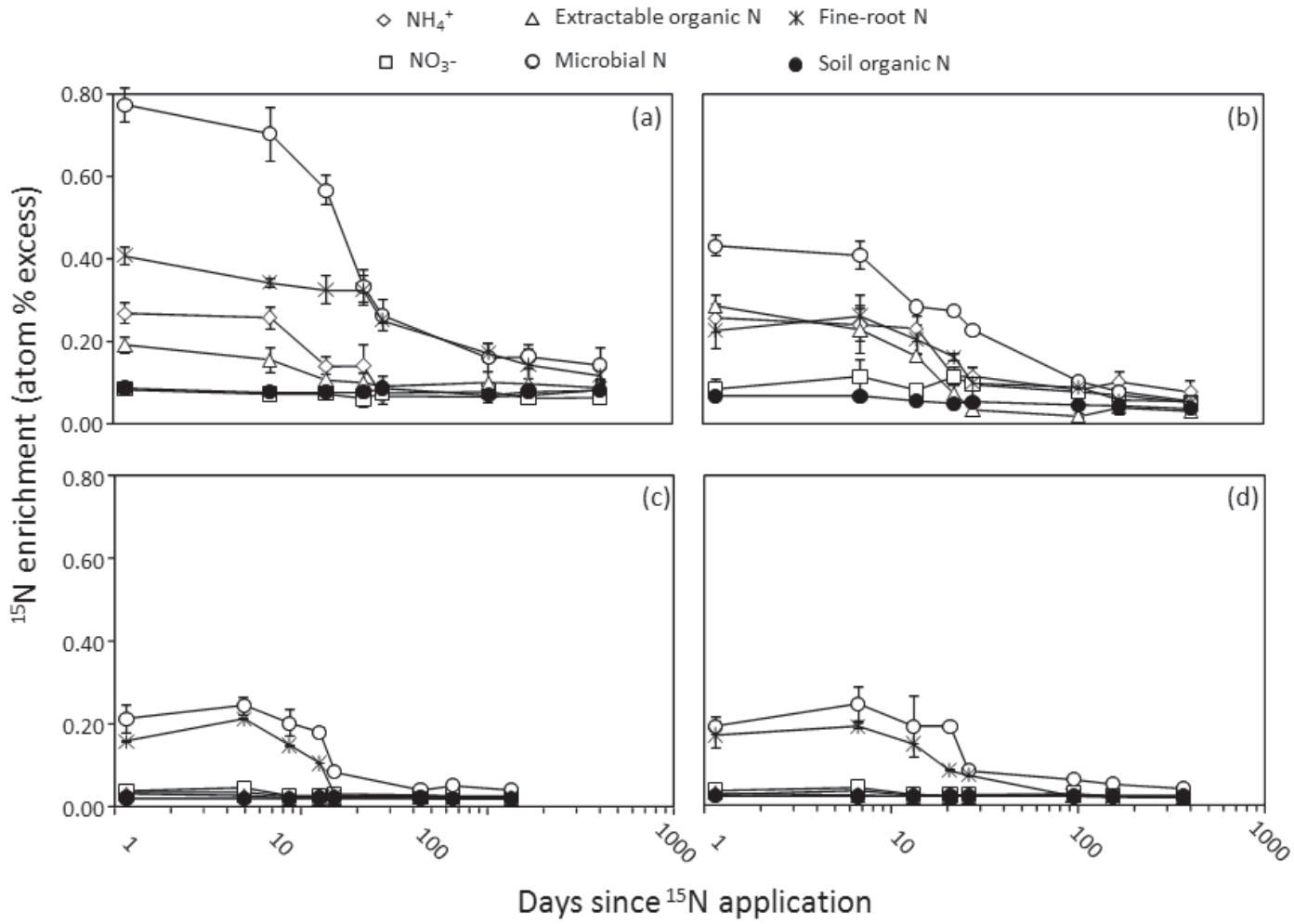
accumulation in the soil organic N pool. These results robustly show that these Andean montane forests are highly sensitive to changes in nutrient deposition. Therefore, greater attention should be paid to the biological implications of increased N deposition with the consequent decrease in N retention in these forests. As our present study could not ascertain the fates of N that moved out of the pools we measured, future studies should include tracing ^{15}N in aboveground N pools such as surface litter, above-ground plant biomass and in other forest floor components including deeper soil layers to further our understanding on the fates of added N. Whether in the long run soil organic N and C at the lower montane forest would continue to be retained, despite at lower rates than the control, and the soil N and C at the upper montane forest would be removed with chronic N addition necessitate complementary studies on the dynamics of different C fractions in soil organic matter in order to predict how the ecosystem C balance would be affected by the advent of increase in nutrient deposition in this Andean tropical montane forest region.

Appendices to Chapter III

Appendix 1 Soil characteristics (mean ± SE, n = 4)[a] in montane forests at 1000 m and 3000 m elevations after 4 years of N addition at 50 kg urea-N ha^{-1} $year^{-1}$ at each elevation, no significant differences in these soil biochemical characteristics were detected between the control and N addition plots at all depths (Independent T test at $P = 0.29$ to 0.65)

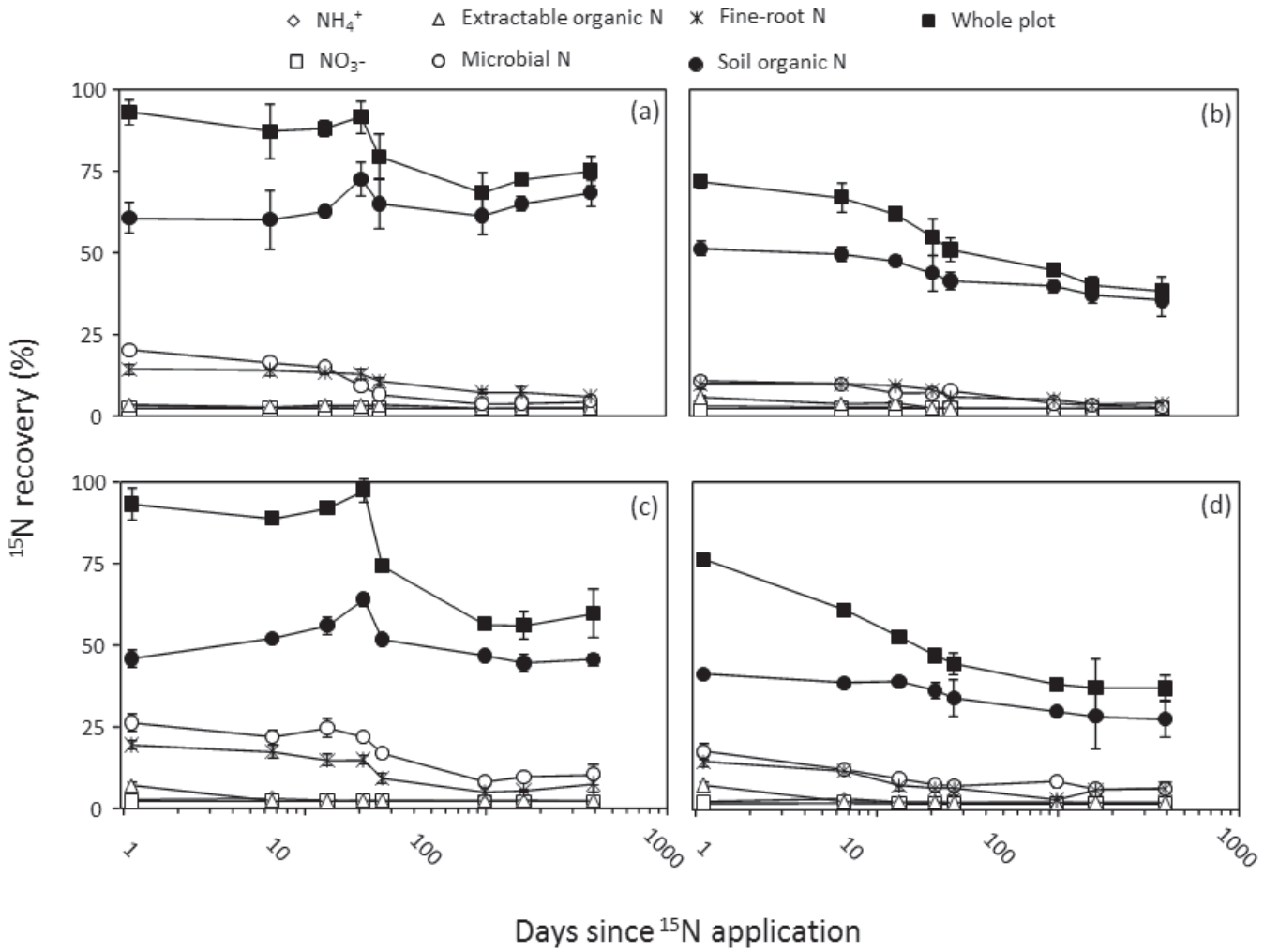
Elevation, treatment, depth	Total C (mg C g^{-1})	Total N (mg N g^{-1})	C:N ratio	pH-H_2O	Effective cation exchange capacity ($mmol_c$ kg^{-1})	Base saturation (%)
1000 m above sea level (asl)						
Control						
0-5 cm	57.3 ± 17.0	3.9 ± 0.8	13.7 ± 1.2	4.3 ± 0.2	74.8 ± 18.1	45.3 ± 8.0
5-10 cm	33.3 ± 4.2	2.7 ± 0.2	12.1 ± 0.8	4.1 ± 0.1	59.3 ± 8.1	12.6 ± 1.9
10-25 cm	21.2 ± 3.5	1.7 ± 0.1	11.8 ± 1.1	4.1 ± 0.1	43.1 ± 5.8	7.1 ± 1.1
25-50 cm	9.1 ± 1.7	0.8 ± 0.1	10.9 ± 1.3	4.6 ± 0.1	26.2 ± 4.2	11.1 ± 3.3
Nitrogen						
0-5 cm	59.5 ± 14.6	4.1 ± 0.6	14.1 ± 1.4	3.9 ± 0.2	68.5 ± 7.8	37.8 ± 10.2
5-10 cm	58.0 ± 24.9	3.8 ± 1.35	13.9 ± 1.3	4.0 ± 0.1	77.1 ± 23.9	21.8 ± 14.1
10-25 cm	35.7 ± 8.2	2.7 ± 0.5	13.3 ± 1.0	4.2 ± 0.1	58.8 ± 9.9	17.0 ± 5.2
25-50 cm	24.2 ± 12.5	1.8 ± 0.8	12.7 ± 1.5	4.4 ± 0.2	40.9 ± 10.1	10.7 ± 5.0
3000 m asl						
Control						
0-5 cm	474.0 ± 20.3	13.7 ± 0.7	34.7 ± 1.4	3.7 ± 0.0	-	-
5-10 cm	437.9 ± 50.9	13.5 ± 1.5	32.6 ± 2.3	4.0 ± 0.3	-	-
10-25 cm	438.7 ± 46.4	16.1 ± 1.2	27.1 ± 1.2	3.7 ± 0.2	-	-
25-50 cm	117.3 ± 40.5	5.2 ± 1.7	21.7 ± 0.8	3.6 ± 0.1	-	-
Nitrogen						
0-5 cm	455.4 ± 26.5	16.4 ± 1.0	28.3 ± 3.0	3.9 ± 0.2	-	-
5-10 cm	447.5 ± 27.6	16.8 ± 0.7	27.0 ± 2.5	3. 8 ± 0.2	-	-
10-25 cm	440.7 ± 32.7	16.6 ± 0.7	26.5 ± 1.1	3.9 ± 0.2	-	-
25-50 cm	69.3 ± 14.2	3.4 ± 0.3	20.0 ± 2.3	3.7 ± 0.1	-	-

Appendix 2 ^{15}N (atom % excess; mean ± SE, n = 3) enrichments with time in different N pools (NH_4^+, NO_3^-, extractable organic N, microbial N, fine roots and soil organic matter) in the top 5-cm depth (organic layer) of the forest at 3000 m elevation. $^{15}NH_4^+$ pulse chase in the (a) control and (b) N addition plots with 4 years of treatment; $^{15}NO_3^-$ pulse chase in the (c) control and (d) N-addition plots with 4 years of treatment



Appendix 3 Statistical analysis (*F* and *P* values, $n = 3$) to test the differences in ^{15}N recoveries of N pools in the redistribution phase (1 to 20 days) between ^{15}N sources ($^{15}NH_4^+$ vs $^{15}NO_3^-$) for each treatment and between treatments (control vs N addition) for each ^{15}N source using linear mixed effects model

N pools	Control $^{15}NH_4^+$ vs $^{15}NO_3^-$		N-addition $^{15}NH_4^+$ vs $^{15}NO_3^-$		$^{15}NH_4^+$ enriched Control vs N addition		$^{15}NO_3^-$ enriched Control vs N addition	
	$F_{1,4}$	P	$F_{1,4}$	P	$F_{1,4}$	P	$F_{1,4}$	P
1000 m elevation								
NH_4^+	0.50	0.52	0.55	0.50	2.48	0.19	0.23	0.66
NO_3^-	0.41	0.56	0.23	0.66	2.75	0.17	2.60	0.18
Extractable organic N	0.55	0.50	0.39	0.57	1.40	0.30	2.18	0.21
Microbial N	0.01	0.92	0.06	0.82	17.73	0.01	40.45	0.00
Fine roots	1.60	0.27	1.61	0.27	18.09	0.01	51.89	0.00
Soil organic N	1.39	0.30	1.19	0.33	28.02	0.00	22.80	0.01
Total N	0.98	0.38	1.03	0.37	60.16	0.00	61.17	0.00
3000 m elevation								
NH_4^+	2.99	0.16	1.23	0.33	0.79	0.42	1.24	0.33
NO_3^-	0.53	0.51	0.37	0.57	1.49	0.29	0.94	0.39
Extractable organic N	1.30	0.32	0.73	0.44	0.49	0.52	0.94	0.44
Microbial N	0.11	0.76	0.77	0.43	59.09	0.00	89.38	0.00
Fine roots	0.00	0.82	0.73	0.46	34.54	0.00	30.90	0.01
Soil organic N	1.08	0.36	1.28	0.32	27.11	0.01	36.88	0.00
Total N	1.34	0.31	0.90	0.39	70.42	0.00	47.97	0.00



Appendix 4 ^{15}N % recovery (^{15}N recovered in a N pool ÷ ^{15}N applied x 100; mean ± SE, $n = 3$) with time in different N pools (NH_4^+, NO_3^-, extractable organic N, microbial N, fine roots and soil organic matter) in the top 5-cm depth (organic layer) of the forest at 3000 m elevation. $^{15}NH_4^+$ pulse chase in the (a) control and (b) N addition plots with 4 years of treatment; $^{15}NO_3^-$ pulse chase in the (c) control and (d) N-addition plots with 4 years of treatment

Appendix 5 Statistical analysis (F and P values, $n = 3$) to test the differences in ^{15}N recoveries of N pools in the equilibrium phase (20 to 355 days) between ^{15}N sources ($^{15}NH_4^+$ vs $^{15}NO_3^-$) for each treatment and between treatments (control vs N addition) for each ^{15}N source using linear mixed effects model

N pools	Control $^{15}NH_4^+$ vs $^{15}NO_3^-$		N-addition $^{15}NH_4^+$ vs $^{15}NO_3^-$		$^{15}NH_4^+$ enriched Control vs N addition		$^{15}NO_3^-$ enriched Control vs N addition	
	$F_{1,4}$	P	$F_{1,4}$	P	$F_{1,4}$	P	$F_{1,4}$	P
1000 m elevation								
NH_4^+	1.37	0.31	0.67	0.46	0.51	0.51	0.28	0.62
NO_3^-	0.66	0.46	1.86	0.24	0.95	0.38	0.08	0.79
Extractable organic N	0.05	0.83	0.97	0.38	0.36	0.58	0.45	0.54
Microbial N	0.01	0.92	0.52	0.51	33.06	0.00	21.17	0.01
Fine roots	0.67	0.46	0.25	0.64	61.53	0.00	15.36	0.02
Soil organic N	2.22	0.21	0.63	0.47	91.25	0.00	82.76	0.00
Total N	0.50	0.52	0.06	0.82	108.49	0.00	112.76	0.00
3000 m elevation								
NH_4^+	0.23	0.66	1.29	0.32	0.20	0.68	0.19	0.68
NO_3^-	0.61	0.48	0.82	0.42	0.78	0.43	0.52	0.51
Extractable organic N	1.12	0.35	0.27	0.63	0.49	0.52	2.81	0.17
Microbial N	0.36	0.58	0.74	0.44	12.41	0.02	18.26	0.01
Fine roots	2.31	0.20	1.29	0.32	29.68	0.01	14.07	0.02
Soil organic N	0.36	0.61	0.51	0.51	95.73	0.00	43.00	0.00
Total N	0.87	0.40	0.18	0.69	128.32	0.00	70.75	0.00

Appendix 6 Statistical analysis (F and P values, $n = 3$) to test the differences in ^{15}N enrichments of N pools in the redistribution phase (1 to 20 days) between ^{15}N sources ($^{15}NH_4^+$ vs $^{15}NO_3^-$) for each treatment and between treatments (control vs N addition) for each ^{15}N source using linear mixed effects model

N pools	Control $^{15}NH_4^+$ vs $^{15}NO_3^-$		N-addition $^{15}NH_4^+$ vs $^{15}NO_3^-$		$^{15}NH_4^+$ enriched Control vs N addition		$^{15}NO_3^-$ enriched Controll vs N addition	
	$F_{1,4}$	P	$F_{1,4}$	P	$F_{1,4}$	P	$F_{1,4}$	P
1000 m elevation								
NH_4^+	60.61	0.00	71.36	0.00	0.00	0.48	0.11	0.76
NO_3^-	78.05	0.00	28.96	0.00	0.78	0.43	0.00	0.42
Extractable organic N	66.16	0.00	53.83	0.00	0.04	0.85	0.61	0.48
Microbial N	66.46	0.00	26.66	0.00	29.88	0.00	20.96	0.01
Fine roots	16.76	0.01	87.64	0.00	20.05	0.01	25.06	0.01
Soil organic N	43.16	0.00	37.05	0.00	15.62	0.02	17.60	0.01
3000 m elevation								
NH_4^+	30.82	0.00	59.35	0.00	0.17	0.70	0.38	0.57
NO_3^-	84.89	0.00	14.60	0.02	0.69	0.45	0.80	0.42
Extractable organic N	25.19	0.01	33.48	0.00	0.46	0.53	0.32	0.60
Microbial N	48.92	0.00	88.64	0.00	70.25	0.00	60.92	0.00
Fine roots	43.00	0.00	23.68	0.01	34.87	0.00	20.89	0.01
Soil organic N	68.05	0.00	47.46	0.00	27.39	0.01	66.04	0.00

Appendix 7 Statistical analysis (F and P values, n = 3) to test the differences in ^{15}N enrichments of N pools in the equilibrium phase (20 to 355 days) between ^{15}N sources ($^{15}NH_4^+$ vs $^{15}NO_3^-$) for each treatment and between treatments (control vs N addition) for each ^{15}N source using linear mixed effects model

N pools	Control $^{15}NH_4^+$ vs $^{15}NO_3^-$		N-addition $^{15}NH_4^+$ vs $^{15}NO_3^-$		$^{15}NH_4^+$ enriched Control vs N addition		$^{15}NO_3^-$ enriched Control vs N addition	
	$F_{1,4}$	P	$F_{1,4}$	P	$F_{1,4}$	P	$F_{1,4}$	P
1000 m elevation								
NH_4^+	90.71	0.00	37.30	0.00	0.73	0.44	0.15	0.72
NO_3^-	55.26	0.00	70.44	0.00	0.53	0.51	1.29	0.32
Extractable organic N	36.23	0.00	70.46	0.00	0.22	0.66	0.27	0.63
Microbial N	41.75	0.00	53.47	0.00	32.37	0.00	30.14	0.00
Fine roots	38.68	0.00	50.04	0.00	19.28	0.01	17.10	0.01
Soil organic N	29.00	0.00	34.87	0.00	35.73	0.00	33.67	0.00
3000 m elevation								
NH_4^+	37.89	0.00	14.27	0.02	1.43	0.29	0.11	0.76
NO_3^-	78.05	0.00	28.73	0.01	2.29	0.20	0.35	0.58
Extractable organic N	61.80	0.00	37.23	0.00	1.97	0.23	0.12	0.75
Microbial N	16.46	0.01	33.17	0.00	26.25	0.01	26.90	0.01
Fine roots	18.94	0.01	61.38	0.00	36.66	0.00	10.85	0.03
Soil organic N	53.74	0.00	39.43	0.00	66.12	0.00	51.42	0.00

CHAPTER IV Synthesis of results and implications of thesis

Soil N availability in montane forests in the Ecuadorian Andes

The tropics are increasingly being exposed to elevated inputs of nutrients mainly through atmospheric deposition. In the montane forests of Ecuador, the major source of atmospheric nutrients deposited is by biomass burning in the Amazon indicating that this environmental change can impact areas far from its source. Several studies have linked increased N inputs with increases in soil N-oxide (NO + N_2O) emissions, nitrate (NO_3^-) leaching, decrease in microbial N immobilization, and differential changes in plant productivity, soil carbon dioxide (CO_2) fluxes and soil carbon (C) dynamics (e.g. Hall and Matson 2003; Lohse and Matson 2005; Koehler et al. 2009a, 2009b; Corre et al. 2010, 2013; Cusack et al. 2011a; Wright et al. 2011) suggesting deleterious consequences on the environment and changes in soil C storage. These processes are strongly controlled by internal transformations of N in the soil, and because N cycle is closely linked with C cycle the fates of N in the soil influence the ability of the soil to sequester C (Cusack et al. 2011b; Templer et al. 2012).

Tropical montane forests are relatively not well-studied in part because of the difficulty in accessing these sites. These ecosystems are important because it stores more soil C per unit area than tropical lowland forests (Girardin et al. 2010), and the possibility that these ecosystems are limited in nutrients, as indicated by thick organic layers. In the eastern Andean tropical montane forests, organic layer thickness increase with elevation (Wilcke et al. 2002), and this has been tied to soil N availability of this ecosystem (Wolf et al. 2011). Results of this thesis show that in pristine tropical montane forest soils in Ecuador, gross rates of soil N cycling decreased with elevation increase, and microbial N retention rates were tightly coupled with mineral N production rates. Gross rates of mineral N production were closely coupled with microbial retention of N indicating that in these montane forests with low N availability, soil N cycling is tight. The decreasing gross rates of soil N cycling with increasing elevation strongly signified a decreasing soil N availability along our elevation gradient. Unfavorable soil biochemical conditions, decreasing substrate quantity and quality, increasing organic layer thickness, and decreasing temperatures were important controls that contributed to the

decreasing soil N cycling rates along our elevation gradient. Soil N cycling rates have been linked with other ecosystem characteristics like ^{15}N natural abundance signatures of soil or litterfall, forest productivity and soil N-oxide emissions. This opens the possibility of using easily-measurable proxy variables (e.g. stand basal area increment, litter production or ^{15}N signatures of soil or litterfall) for estimating large-scale N-oxide fluxes since there is evidence that in tropical montane forests forest productivity is limited by N and that N-oxide fluxes depend on N cycling rates and N availability (Corre et al. 2010; Wolf et al. 2011). In addition, this thesis illustrate the importance of quantifying gross rates of soil N cycling, which separate mineral N production processes from microbial N retention processes, in order to understand the mechanisms of how changes in soil N-cycling rates with elevated N input result in changes in N losses.

Elevated N inputs decoupled microbial N production from N retention, indicating a leaky soil N cycle which creates a potential for N losses that may lead to deleterious environmental consequences. The increases in gross rates of N mineralization were influenced more by the increases in substrate quality and quantity and less likely by the amount of microbial biomass since microbial C content decreased in these treatment plots. On the other hand, the decrease in microbial immobilization of NH_4^+ and NO_3^- could be attributed to the decrease in microbial C in these plots at all elevations. We speculate that the increased N addition had possibly shifted to a more bacterial-dominated community with reduction in labile C and, together with the changes in soil chemical characteristics, resulted in reduced microbial biomass C and consequently reduced microbial N immobilization.

These montane forests along the elevation gradient all showed similar responses to four years of low N addition despite their initial differences in soil N availability. This suggests that the factors that regulate soil N-cycling rates in control plots seem to be overridden by increased N input. This further implies that tropical montane forests are strongly vulnerable to increases in N deposition, regardless of differences in the soil N cycling rates before nutrient manipulation. In addition, the effects of environmental change (e.g. increasing nutrient deposition) on the

biogeochemistry of tropical montane forest ecosystems are of great importance in developing future climate change scenarios.

Gross fluxes of internally produced N in montane forests in the Ecuadorian Andes

The fates of inorganic N in an ecosystem can be characterized in terms of the dynamics of N fluxes in the plant-soil-microbe system over time. Short term-fluxes are N fluxes that occur within days to weeks without recycling among plants, microbes, and soil while long-term N fluxes are those that happen over months to years with recycling (Kaye and Hart 1997). Studies assessing short-term N cycles mostly exclude competition from plant uptake while long-term N cycling involves several short-term N-cycling events. Hence, the recycling of N within the plant-soil-microbe system links the short-term fates of N into long-term patterns of retention in an ecosystem. The long-term fates of inorganic N in tropical forest soils are not well known because the studies conducted so far have only traced ^{15}N within days (e.g. Templer et al. 2008). How increase in N deposition affects the long-term fates of soil mineral N in tropical montane forest soils remain poorly understood.

Fluxes of inorganic nitrogen into microbial, fine roots (as proxy for plants), and soil organic N pools were calculated by weighting the gross N mineralization rates or gross nitrification rates by the net ^{15}N % tracer flux of the above-mentioned N pools. Gross flux is the amount of tracer that transfers into/out of an N pool per m^{-2} per day. This provides an approximation of relative amounts of either NH_4^+ or NO_3^- that each N pool assimilates (positive flux) or turns-over (negative flux) in a given period of time. The higher gross N mineralization rates over gross nitrification rates (Chapter 2) resulted in higher NH_4^+ over NO_3^- fluxes into/out of the microbial, plant, and organic N pools of our montane sites at 1000 m and 3000 m elevations (Table 8). In addition, the similarity of the ^{15}N recoveries in the microbial, plant, and organic N pools illustrate the lack of preference for either form of inorganic N (Chapter 3). The higher NH_4^+ than NO_3^- production rates plus the lack of preferential uptake might possibly lead to a characteristic dominance of NH_4^+ over NO_3^- in these montane forests. This mechanism has

ecosystem implications especially in these N-poor montane forests that are increasingly exposed to anthropogenic N input. A strong dominance of NH_4^+ and its storage in soil (if it is not taken up by microbes or plants, or if it is not retained in recalcitrant pools of the soil organic matter) might lead to its oxidation into NO_3^-, a process that is considered a gateway for losses because NO_3^- is more mobile and may be leached, and/or NO3- can be further oxidized to other forms of N leading to gaseous losses.

Under elevated N inputs, there was transfer of NH_4^+ and NO_3^- out of the organic N pool. Also, the gross fluxes of inorganic N out of the microbial and fine root pools were higher than those in the control plots. This suggests that the rate with which the microbe-plant-soil system assimilate and redistribute NH_4^+ and NO_3^- in these montane forests has changed under elevated N inputs. Since microbes and plants were observed to exert control on the transfer of inorganic N to organic N pools under pristine conditions (Chapter 3), changes in the rates of assimilation and redistribution might lead to changes in N availability, gaseous losses of N from the ecosystem, and leaching losses which also impact streams and rivers and affect water quality, nutrient imbalance in vegetation, changes in forest health, and declines in biodiversity. Whether such reduced rates of N and C retention with N addition would result in overall reduction of N and C retention in the ecosystem would depend on whether or not such reductions are compensated by increases in plant-derived N and C inputs and in plant growth.

N budget of a montane forest in the Ecuadorian Andes

Figure 11 shows a relatively complete budget for soil N cycling in a montane forest in the Ecuadorian Andes. A balanced N budget is not easy to attain because N has numerous valence states and gaseous forms that escape notice and travel long distances in the atmosphere. The atmosphere contains the vast majority of the Earth's nitrogen (e.g. Chapin et al. 2011). N fixation by lightning (from annual global estimate; Schlesinger 2008) is relatively very small compared to the annual estimate for biological N fixation (forest floor and canopy at the 2000 m elevation of the NUMEX in Ecuador; Matson et al. unpublished data). A major source of new N input in this tropical montane forest at 2000 m elevation (part of the NUMEX sites in Ecuador) is deposition

Tabelle 8 Gross fluxes of $^{15}NH_4^+$ and $^{15}NO_3^-$ in the microbial, plant, and organic N pools

	Gross $^{15}NH_4^+$ flux ($mg\ ^{15}NH_4^+\ m^{-2}\ day^{-1}$)			Gross $^{15}NO_3^-$ flux ($mg\ ^{15}NO_3^-\ m^{-2}\ day^{-1}$)		
	Microbial N	Fine root N	Soil organic N	Microbial N	Fine root-N	Soil organic N
			1000 m			
Control	-1.55 ± 0.05	-0.21 ± 0.07	1.60 ±0.10	-0.58 ± 0.00	-0.08 ± 0.00	0.60 ± 0.00
4-yr N addition	-2.32 ± 0.00	-0.71 ± 0.00	-0.91 ± 0.01	-0.53 ± 0.00	-0.16 ± 0.00	-0.21 ± 0.01
			3000 m			
Control	-0.20 ± 0.00	-0.10 ± 0.00	0.42 ± 0.01	-0.08 ± 0.00	-0.04 ± 0.00	0.18 ± 0.00
4-yr N addition	-0.45 ± 0.01	-0.24 ±0.00	-1.01 ± 0.03	-0.11 ± 0.00	-0.08 ± 0.00	-0.09 ± 0.00

via bulk precipitation (including in this estimate is the contribution by biomass burning in the Amazon, the major source of anthropogenic N deposited in this forest; Boy et al. 2008; Wullaert et al. 2010). Estimates of the annual soil internal N cycling in this montane forest (2000 m elevation) is ~ 20-fold higher than the annual inputs of new N. The soil N cycle of this forest is tight and the major fate of N is in microbes. Plant N uptake estimate is taken from another tropical montane forest (Puerto Rico; Templer et al. 2008) because we do not have this data from our site. Annual total fine litterfall N production (Wullaert et al. 2010) is similar to the annual N deposition in this forest. Leaching losses (Wullaert et al. 2010) are quite small in this montane forest (the leached N from the top layer moves down to the mineral soil depths) while the annual N_2O emission (Martinson et al. 2013) is a very small fraction of the annual N deposited. In this forest, denitrification has been found to be the dominant process (Müller et al. unpublished data) producing N_2O. This process not only produces N_2O but NO and N_2 as well. We do not have measurements of how much N_2 is produced via denitrification. Denitrification is a process of great environmental importance but is difficult to study in terrestrial ecosystems since methods for quantifying the process are problematic, variability in activity is high, and temporal and spatial scaling challenges are extreme (Groffman 2012). Most fundamentally, it is very difficult to quantify the dominant end-product (N_2) of denitrification given its high background concentration in the atmosphere.

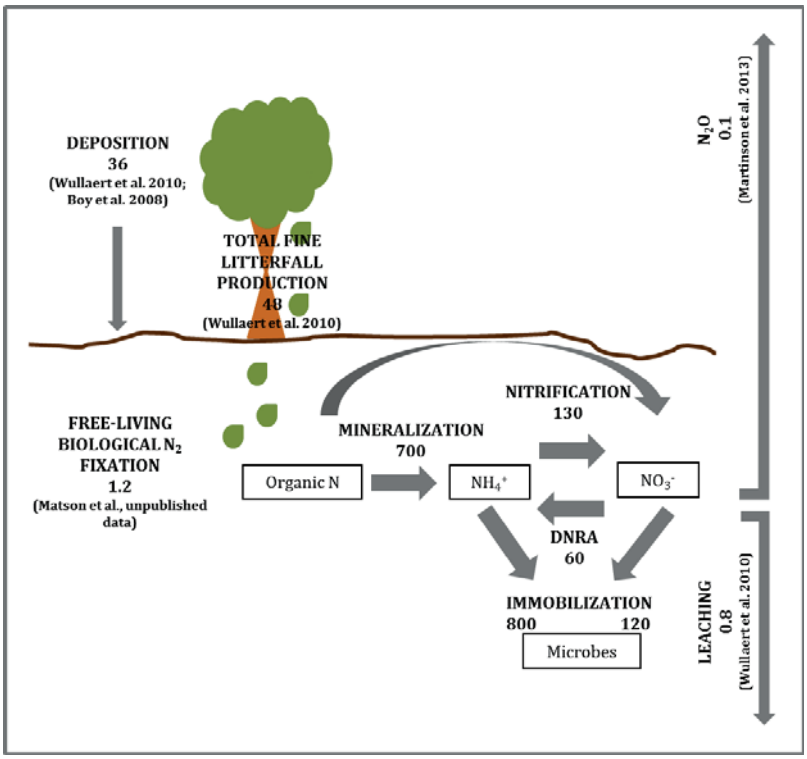


Fig. 11 The N cycle in the montane forest at 2000 m elevation. Inputs and soil N cycling rates (soil N cycling rates were measured at the top 5-cm depth) are in kg N ha^{-1} $year^{-1}$.

Implications of results under increasing anthropogenic nutrient deposition

The decrease in soil N availability along the elevation gradient also reflected the pattern of forest productivity and soil N-oxide emissions (Wolf et al. 2011; Martinson et al. 2013). The link between soil N-cycling rate, natural abundance ^{15}N signature of soil or litterfall, forest productivity and soil N-oxide emission opens the possibility of using easily-measurable proxy variables like stand basal area increment, litterfall, or $\delta^{15}N$ signature of soil or litterfall for estimating large-scale N-oxide fluxes from these montane forest soils in southern Ecuador.

Since soil N availability of these montane forests was generally low, increases in soil N cycling rates may also benefit forest productivity, if increases in N availability will not limit other nutrients. The relatively rapid response of the internal soil N cycling to even lower rates of N addition, which was within the expected N deposition rate in the region (Homeier et al. 2012), suggests that greater attention should be paid to the biological implications of increased soil N

availability and decreased microbial N retention in response to an increase in atmospheric N deposition onto montane forests. Many of these responses may be only observed after several years. Our results support the importance of long-term manipulation experiments at a large scale. Further investigations on the 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 community structure, function and enzyme activity will increase our understanding of how increases in N deposition affect the biogeochemistry of tropical montane forests

Atmospheric deposition to terrestrial ecosystems is confirmed to be a major source of above and below ground biodiversity reduction in temperate and boreal ecosystems and is likely to be so across many world regions with excess nitrogen. At the same time, only a small fraction of the nitrogen from humans and livestock systems entering water systems is treated, leading to serious losses of biodiversity in freshwater, estuarine and coastal marine systems. Nearly all of our information on the effects of reactive nitrogen on biodiversity is derived from studies on temperate ecosystems. More studies on tropical terrestrial and aquatic/marine ecosystems are needed.

There is a pressing need to develop more-integrated, rigorous and multi-disciplinary approaches for the management of sources, sinks, flows and effects of nitrogen and other nutrients at the local and national level. This includes ways to optimize the efficient use of inorganic and organic fertilizers world-wide, and to facilitate enhanced access and sustainable use of N inputs in the predominantly N-deficient soils of Africa and parts of Latin America and Asia. These approaches must be based on consolidation and synthesis of existing data, identification of gaps to undertake necessary research, a comprehensive global assessment for policy makers, and the use of information to promote appropriate practices and technologies.

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DECLARATION OF ORIGINALITY AND CERTIFICATE OF OWNERSHIP

I, Angelica P. Baldos, hereby declare that I am the sole author of this dissertation entitled "**Soil Nitrogen Cycling and Long-term Fates of Nitrogen in Montane Forests Along a 3000-m Elevation Gradient in the Ecuadorian Andes**". All references and data sources that were used in this dissertation have been appropriately acknowledged. I furthermore declare that this work has not been submitted elsewhere in any form as part of another dissertation procedure.

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