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Canopy soil nutrient cycling and response to elevated nutrient levels along an elevation gradient of tropical montane forests

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“Die meisten Menschen wissen gar nicht, wie schön die Welt ist und wie viel Pracht in den kleinsten Dingen, in irgendeiner Blume, einem Stein, einer Baumrinde oder einem Birkenblatt sich offenbart. Die [meisten] erwachsenen Menschen, die Geschäfte und Sorgen haben und sich mit lauter Kleinigkeiten quälen, verlieren allmählich ganz den Blick für diese Reichtümer, welche die Kinder [und Wissenschaftler]...bemerken und mit dem ganzen Herzen lieben.”

Rainer Maria Rilke

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ZUSAMMENFASSUNG

Obwohl Böden des Kronendachs (*canopy soils*) deutlich zur oberiridischen labilen Biomasse beitragen können, werden sie oft in Studien über Nährstoffkreisläufe übersehen. In Wäldern mit einem großen Vorkommen an Böden im Kronendach, wie beispielsweise jene in tropischen Bergregionen, könnte dies zu einem unvollständigen Verständnis der Gesamt-Nährstoffprozesse des Waldes beitragen. Böden im Kronendach sind Ansammlungen organischen Materials, welche gewöhnlich auf Zweigen von Bäumen tropischer Wälder zu finden sind. Sie bestehen in erster Linie aus zersetztem epiphytischen Material aber umfassen auch herunterfallendes Laub, Staub, wirbellose Tiere, Pilze und Mikroorganismen. Es gibt nur eine Handvoll Studien, die Stickstoff (N) Kreisläufe und/oder Treibhausgas (THG) Flüsse in Böden des Kronendachs untersucht haben und keine hat versucht die tatsächlichen Feldraten zu bestimmen oder herauszufinden, wie sich diese Böden – welche besonders sensibel gegenüber atmosphärischen Prozessen sind – mit Nährstoffdeposition ändern könnten. Diese Dissertation stellt die Ergebnisse einer Forschungsstudie dar, welche N-Umsatzraten und THG Flüsse von Böden des Kronendachs quantifiziert und untersucht, wie diese Raten durch zunehmende Mengen an N und Phosphor (P) im Boden verändert werden.

In Gebieten mit atmosphärischer N- und P-Deposition, erhalten Böden des Kronendaches sowohl direkte als auch indirekte Nährstoffeinträge auf Grund von angereichertem Bestandsniederschlag und Pflanzenstreu. Es wurden folgende Umsatzraten in Böden des Kronendachs tropische Bergwälder entlang eines Höhengradienten (1000 m , 2000 m , 3000 m) gemessen: (1) asymbiotische biologische N_2-Fixierung, (2) Netto- und Brutto-N-Transformation, und (3) Kohlendioxid (CO_2), Methan (CH_4) und Lachgas (N_2O) Flüsse. Zudem wurden indirekte Auswirkungen von N-und P-Gaben, die auf dem Waldboden

ausgebracht wurden, untersucht. Umsatzraten der N_2-Fixierung, des N Kreislaufes und von THG Flüssen, welche in Böden des Kronendachs gemessen wurden, wurden mit denen vom Waldboden verglichen (entweder als Teil dieser Arbeit oder in parallelen Studien von zwei anderen Mitgliedern unserer Arbeitsgruppe), um die Aktivität von Böden des Kronendachs in den Kontext des gesamten Waldes zu stellen. N_2-Fixierung wurde mit der Acetylenreduktionsmethode, Netto-N-Umsatzraten wurden mittels *in situ* Inkubationen (*buried bag method*) und Brutto-N-Umsatzraten wurden mit der ^{15}N-Verdünnungsmethode (*^{15}N pool dilution technique*) bestimmt. Gasflüsse wurden sowohl unter Verwendung statischer Kammern gemessen, deren Sockel permanent im Boden angebracht waren, als auch unter Verwendung regelmäßig entfernter intakter Bodenproben, die zur Gasmessung in luftdichten Einweckgläsern inkubiert wurden. Messungen der N_2-Fixierung und des N Kreislaufes erfolgten während der Regen- und Trockenzeit im Feld unter Verwendung intakter Bodenproben. THG Messungen wurden fünf Mal während des Zeitraumes von einem Jahr durchgeführt. Der Waldboden unserer Standorte war 4 Jahre lang zweimal im Jahr mit moderaten Mengen an N (50 kg N ha^{-1} $Jahr^{-1}$) und P (10 kg P ha^{-1} $Jahr^{-1}$) gedüngt worden und umfasste folgende Behandlungen: Kontrolle, N-, P- und N+P-Zugaben.

Das Kronendach trug 7-13 % zur gesamten Boden N_2-Fixierung (Kronendach + Waldboden) bei, welche zwischen 0,8 und 1,5 kg N ha^{-1} $Jahr^{-1}$ lag. N_2-Fixierungsraten veränderten sich nur geringfüging mit der Höhenstufe, waren aber in der Trockenzeit deutlich höher als in der Regenzeit. N_2-Fixierung im Waldboden wurde in N-Parzellen im Vergleich zu Kontroll- und P-Parzellen gehemmt, währen sie in Böden des Kronendachs in P-Parzellen im Vergleich zu Kontrollparzellen stimuliert wurde. Böden des Kronendachs trugen bis zu 23% zur gesamten mineralischen N-Produktion (Kronendach + Waldboden) bei; Brutto-N-

Mineralisierung in Böden des Kronendachs lag zwischen 1,2 und 2,0 mg N kg^{-1} d^{-1}. In Kontrollparzellen nahmen Brutto-Umsatzraten von Ammonium (NH_4^+) mit zunehmender Höhe ab, wohingegen Brutto-Umsatzraten von Nitrat (NO_3^-) keinen klaren Trend mit der Höhenstufe aufwiesen, aber signifikant durch die Saison beeinflusst wurden. Effekte durch Nährstoff-Zugabe unterschieden sich je nach Höhenstufe, aber kombinierte N+P-Zugabe erhöhte in der Regel auf allen Höhenstufen die N-Umsatzraten. CO_2 Emissionsraten von Böden des Kronendachs berechnet auf der Basis der Fläche von Gaskammern (10,5 bis 109,5 mg CO_2-C m^{-2} h^{-1}) waren ähnlich denen vom Waldboden ähnlich und nahmen mit zunehmender Höhenstufe ab. Emissionen vom Kronendach, berechnet auf der Basis der Waldfläche (0,15 bis 0,51 Mg CO_2-C m^{-2} h^{-1}), machten jedoch nur 5-11% der gesamten Boden-CO_2 Emissionen (Kronendach + Waldboden) aus. CH_4 Flüsse (-0,07 bis 0,02 kg CH_4-C ha^{-1} $Jahr^{-1}$) und N_2O Flüsse (0,00 bis 0,01 kg N_2O-N ha^{-1} $Jahr^{-1}$) von Böden des Kronendachs machten weniger als 5% der Gesamtflüsse von Böden aus. P-Zugabe reduzierte CH_4 Emissionen in allen Höhenstufen, so dass Böden des Kronendachs als leichte CH_4 Senken agierten (-10,8 bis -2,94 µg CH_4-C m^{-2} h^{-1}). Nur in 2000 m wurden Böden des Kronendachs unter N Zugabe zu leichten N_2O Quellen (2,43 ± 3,72 µg N_2O-N m^{-2} h^{-1}), wohingegen P Zugabe die CO_2 emissionen um ungefähr 50% reduzierte.

Die Ergebnisse zeigen, dass Böden des Kronendachs eine aktive Mikrobengemeinschaft besitzen, welche wertvolle Dienstleistungen hinsichtlich von Nährstoffkreisläufen für das Ökosystem des Kronendachs erbringt. Zusätzlich, war der Nährstoffkreislauf der Böden des Kronendachs in unseren Wäldern eindeutig an die Nährstoffverfügbarkeit des Waldbodens gekoppelt, was im Gegensatz zu Theorien steht, die besagen dass Böden des Kronendachs vom Nährstoffkreislauf der Waldböden entkoppelt seien. Wir haben festgestellt, dass Böden

des Kronendachs in höheren Lagen eher einen wesentlichen Anteil des gesamten Wald-Nährstoffkreislaufes ausmachen; dies sollte in Studien berücksichtigt werden, die sich mit Nährstoffkreisläufen solcher Gegenden beschäftigen. Langfristige atmosphärische N- und P-Deposition verfügt über das Potenzial, die Dynamik von Nährstoffflüssen im Kronendach erheblich zu verändern. N-Deposition könnte die N_2-Fixierung hemmen, wobei "hotspots" weiterhin in Bereichen mit großen Mengen an P vorkommen. Interne N-Kreisläufe in Böden des Kronendachs werden wahrscheinlich durch N -und P-Deposition stimuliert werden, aber chronischen Nährstoffzugabe könnte auch zu erhöhten mineralischen N-Verlusten aus dem Bodensystem des Kronendachs führen. THG-relevante Prozesse in Böden des Kronendachs werden wahrscheinlich auch auf N- und P-Deposition reagieren, aber mit Ausnahme von CO_2-Emissionen ist es unwahrscheinlich, dass Gasflüsse von Böden des Kronendachs wesentlich zum gesamten THG-Budget des Waldes beitragen.

SUMMARY

Although canopy soils can contribute significantly to aboveground labile biomass, they are often overlooked in nutrient cycling studies. In forests with large accumulations of canopy soil, such as those found in tropical montane regions, this could contribute to an incomplete understanding of nutrient cycling in the overall forest. Canopy soils are collections of organic material commonly found on the branches of trees in humid forests; they are primarily made up of decomposed epiphytic material but also include intercepted litter, dust, invertebrates, fungi and microorganisms. There are only a handful of studies that have looked at nitrogen (N) cycling and/or greenhouse gas (GHG) flux in canopy soils and none have tried to assess the actual field rates or investigated how these soils - which are particularly sensitive to atmospheric processes - could change with nutrient deposition. This dissertation presents the results of a research study that quantified rates of canopy soil N cycling and GHG flux and assessed how these rates were affected by increased levels of N and phosphorus (P) in the soil.

In areas of atmospheric N and P deposition, canopy soils receive both direct inputs and indirect enrichment via enriched throughfall and plant litter. We measured rates of (1) free-living N_2 fixation, (2) net and gross mineral N cycling, and (3) carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) exchange, in canopy soils of tropical montane forests along an elevation gradient (1000 m, 2000 m and 3000 m) and assessed the indirect effects of N and P addition to the forest floor. Rates of N_2 fixation, N cycling and GHG flux measured in canopy soil were compared with those measured on the forest floor (either as a part of this work or in parallel studies by two other members of our working group), to put canopy soil activity in the context of the total forest. N_2 fixation was determined using the acetylene reduction assay, net N cycling rates were determined using the buried bag method and gross N cycling rates were

determined using ^{15}N pool dilution techniques. Gas fluxes were measured using static chambers with permanent bases in the soil, as well as intact soil cores sealed in jars. N_2 fixation and N cycling measurements took place in the field, in the wet and dry seasons, using intact cores of soil. GHG measurements were done five times during a one-year period. The forest floor of our study sites had been fertilized biannually with moderate amounts of N (50 kg N ha^{-1} yr^{-1}) and P (10 kg P ha^{-1} yr^{-1}) for 4 years; treatments included control, N, P and N+P.

The canopy contributed 7-13 % of total (canopy + forest floor) soil N_2 fixation, which ranged from 0.8 to 1.5 kg N ha^{-1} yr^{-1}. N_2 fixation rates exhibited little variation with elevation but were much higher in the dry season than the wet season. N_2 fixation was inhibited in forest floor N plots compared to control and P plots, and stimulated in canopy P plots compared to control. Canopy soils contributed up to 23% of total (canopy + forest floor) mineral N production; gross N mineralization in canopy soils ranged from 22.7 to 45.8 mg N kg^{-1} d^{-1} and gross nitrification ranged from 1.2 to 2.0 mg N kg^{-1} d^{-1}. In control plots, gross rates of ammonium (NH_4^+) transformations decreased with increasing elevation, whereas gross rates of nitrate (NO_3^-) transformations did not exhibit a clear elevation trend but were significantly affected by season. Nutrient-addition effects were different at each elevation, but combined N+P generally increased N cycling rates at all elevations. Rates of canopy CO_2 emissions based on chamber area (10.5 to 109.5 mg CO_2-C m^{-2} h^{-1}) were similar to those measured on the forest floor and decreased with increasing elevation. However, canopy emissions based on forest area (0.15 to 0.51 Mg CO_2-C ha^{-1} yr^{-1}) made up only 5-11% of total (canopy + forest floor) soil CO_2 emissions. Canopy soil CH_4 fluxes (-0.07 to 0.02 kg CH_4-C ha^{-1} yr^{-1}) and N_2O fluxes (0.00 to 0.01 kg N_2O-N ha^{-1} yr^{-1}) made up less than 5% of the total soil fluxes. P addition decreased net CH_4 emissions at all elevations, so that canopy soils acted as a slight

sink for CH_4 (-10.8 to -2.94 μg CH_4-C m^{-2} h^{-1}). At 2000 m only, canopy soils with N addition became a slight N_2O source (2.43 ± 3.72 μg N_2O-N m^{-2} h^{-1}), whereas P addition decreased CO_2 emissions by approximately 50%.

Results show that canopy soils have active microbial communities, which provide valuable nutrient cycling services to the canopy ecosystem. Additionally, in contrast to theories that canopy soil is decoupled from nutrient cycling in forest floor soil, nutrient cycling in the canopy soils of our forests was clearly linked to forest floor nutrient availability. We observed that canopy soils at higher elevations were more likely to make up a significant percentage of total forest nutrient cycling; this should be considered in nutrient cycling studies carried out in such areas. Long-term atmospheric N and P deposition has the potential to significantly change the dynamics of nutrient cycling in these canopies. N deposition may lead to inhibition of N_2 fixation, with hotspots still occurring in areas with higher amounts of P. Internal N cycling in canopy soils will likely be stimulated by N and P deposition, but chronic nutrient addition may also lead to increased mineral N losses from the canopy soil. GHG-related processes in canopy soils will likely also respond to N and P deposition, but with the exception of CO_2 emissions, fluxes in canopy soils are unlikely to significantly contribute to total forest GHG budgets.

Chapter 1

GENERAL INTRODUCTION

1.1 - Atmospheric deposition, tropical forests and canopy soil

Tropical regions are currently experiencing dramatic increases in nitrogen (N) and phosphorus (P) deposition as compared to historical levels and these increases are expected to continue (Boy et al. 2008; Galloway et al. 2004; Hietz et al. 2011; Mahowald et al. 2005, 2008). Deposition of N and P into otherwise undisturbed tropical forests could have a significant impact, as many of these forests are expected to be N and/or P limited (Elser et al. 2007; Vitousek et al. 2010). However, studies looking at nutrient cycling in tropical forests have shown that the heterogeneity of tropical forests makes it difficult to understand even current processes, much less predict how they could change (Townsend et al. 2008; 2011). One form of complexity that is often overlooked is the forest canopy. Despite the important role that canopies play in forest nutrient cycles, canopy-based processes are rarely included in studies of nutrient deposition.

The canopy of a forest is a complex ecosystem existing within the larger forest ecosystem (Nadkarni 1994; Ozanne et al. 2003); it includes not only plants and animals, but also wetlands (Martinson et al. 2010) and soil (Enloe et al. 2006). Canopies affect forest ecosystems in a number of vital ways, buffering extreme temperature changes through shading, altering hydrological conditions to reduce leaching and overland flow (Prescott 2002), providing a unique habitat for plant and animal 'canopy specialists' and acting as a storehouse/source of nutrients for the forest ecosystem (Nadkarni 1994, Nadkarni et al. 2002). Globally, forest canopies are thought to contain about 50% of the biodiversity of terrestrial ecosystems (as cited in Lowman and Schowalter 2012).

An important component of the canopy ecosystem is canopy soil, an accumulation of organic material primarily made up of decomposed material from epiphytes (Hietz et al.

2002), but also including intercepted litter, invertebrates, fungi and microorganisms (Nadkarni et al. 2002). Canopy soil performs several functions for the total forest ecosystem. First, it contributes to total canopy nutrient retention from precipitation (Umana and Wanek 2010). These nutrients can then be leached to the forest floor, adding to terrestrial soil nutrition (Zimmerman et al. 2007), or be taken up by epiphytes; epiphyte diversity has been observed to be higher in trees where canopy soil is present (Barthlott et al. 2001; Cardelus and Mack 2010). The soil found in the canopy can also be a reservoir for seeds (Nadkarni and Haber, 2009) and a habitat for a diverse community of invertebrates (Beaulieu et al. 2010; Yanoviak et al. 2007) - and these can, in turn, be a source of food for larger canopy dwellers. Birds, specifically, are known to forage in canopy soil, with some species foraging there almost exclusively (Nadkarni and Matelson 1989; Remsen and Parker 1984). Although there is clearly far less soil in the canopy than on the forest floor, it is not always an insignificant amount. Estimates of canopy soil biomass can range from only 1000 kg ha^{-1} up to 33,000 kg ha^{-1} (Chen et al. 2010; Freiberg and Freiberg 2000; Nadkarni et al. 2004; Vance & Nadkarni, 1990; Werner et al. 2012), becoming most significant in coastal rainforests or tropical montane forests (Coxson and Nadkarni 1995). Furthermore, while Nadkarni et al. (2004) showed that canopy soil made up only 6% of the aboveground biomass of a tropical forest, the canopy soil made up over 80% of the mass of labile (non-woody) components.

Studies looking at canopy soil have examined nutrient pools (Cardelus et al. 2009; Cardelus and Mack, 2010; Chen et al. 2010; Nadkarni et al. 2002, 2004; Soethe et al. 2008), net nutrient cycling (Clark et al. 1998, 2005), gross N cycling (Wanek et al. 2002), microbial biomass and potential microbial activity (Vance and Nadkarni, 1990) and decomposition rates (Cardelus 2010). Through such studies we know that, in comparison with forest floor soils (on

a mass-based scale), canopy soils can have similar or higher C:N ratios and cation exchange capacity (Cardelus et al. 2009, Nadkarni et al. 2002), similar (Vance and Nadkarni 1990) or higher (Cardelus et al. 2009) microbial biomass C and N, similar (Perez et al. 2005) or both higher and lower (Cardelus et al. 2009) net N cycling, and similar gross N cycling (Wanek et al. 2002). However, canopy soils are generally more acidic than forest floor soils (Cardelus et al. 2009; Vance and Nadkarni 1990), with significantly higher amounts of aluminum (Nadkarni et al. 2002). There is, however, still a paucity of data regarding field rates of N cycling and GHG flux in canopy soils of different regions.

1.2 - Deposition and the global N cycle

N is an indispensable element for all life on earth, forming an integral part of biomolecules such as proteins and DNA (Bernhard 2012). However, although N is ubiquitous worldwide in the form of dinitrogen gas (N_2), only a small fraction of global N is available for use by the majority of organisms. In order to become available, N_2 must be 'fixed' by one of the small number of bacteria or Achaea capable of breaking the triple bond between the two atoms and incorporating the N into a biologically available form (Bernhard 2012). Since this process has a very high energy requirement, N_2 fixation should only occur when no other form of N is available, and consequently the amount of reactive N in any given ecosystem should remain in check (Hedin et al. 2009). Historically, this was often the case, and N still limits primary production in the majority of undisturbed ecosystems (Vitousek and Howarth 1991). As global populations have increased in the last hundred years, however, there have been significant changes to this balance. Not only has the cultivation of N_2-fixing crops dramatically increased the fixation of N_2 through biological means, but fixation now also

occurs through fossil fuel combustion and the Haber-Bosch process (Boy et al. 2008; Galloway et al. 2004; Hietz et al. 2011). The latter is an industrial process created to generate synthetic fertilizer, which has allowed an exponential increase in the amount of reactive N entering ecosystems worldwide. It has been estimated that up to 80% of the N now found in human tissues was fixed through the Haber-Bosch process (Howarth 2008).

Once biologically-available N has been added to an ecosystem, it can have several fates: incorporation into organic matter, partitioning into inorganic N pools, leaching to groundwater or denitrification back to the atmosphere (Silver et al. 2001). In most undisturbed ecosystems, the N cycle is tightly controlled, but anthropogenic contributions to the amount of reactive N in an ecosystem can dramatically alter the N cycle and have serious consequences. In aquatic systems, excess N can cause eutrophication and declining habitat quality (Howarth et al. 2000; Schindler 2006; Smith et al. 2006). In forests, effects can include soil acidity (Hoegberg et al. 2006), decreases in biodiversity (Stevens et al. 2004; Vitousek et al. 1997) and losses in carbon storage (Cleveland and Townsend 2006). In populated regions, nitrate in drinking water can be a serious health issue (Townsend et al. 2003), and indirect effects of the changing N cycle could include an increased risk of parasites and infectious diseases among both humans and wildlife (Johnson et al. 2010). However, consequences are not limited to populated areas. Atmospheric processes allow reactive N to be transported and deposited long distances; the potential for N to be emitted, transported, deposited, re-emitted, etc. has been termed the ‘hopscotch’ of N around the world (Galloway et al. 1995). Galloway et al. (2003) describes the movement of N once it has been fixed into a reactive form as the *nitrogen cascade*. Briefly, once reactive N has entered an ecosystem it can travel through and affect the atmosphere, terrestrial ecosystems and aquatic ecosystems. The cascade only ends when the N

is converted back to unreactive N_2, but this may occur over very long time-scales and it is currently far outpaced by the production of reactive N, causing an accumulation of reactive N to occur globally.

1.3 - Deposition and the global P cycle

Like N, P is essential to most living things, as it is required for the formation of carbohydrate polymers, proteins and nucleic acids (Westheimer 1987). Unlike N, there is no biological mechanism to mobilize P; in undisturbed ecosystems, biologically available P must come from weathering of soil minerals or through atmospheric dust inputs (Chadwick et al. 1999). However, the global P cycle is changing. Worldwide, there is an ever-increasing demand for phosphate rock, which is used to produce fertilizer and other P-containing products (mostly detergents and animal feed) (Smil 2000). In order to meet this demand, extraction of phosphate rock has increased exponentially; between 1911 and 2011, worldwide production increased from 6 Mt yr^{-1} to 198 Mt yr^{-1} (Smil 2000; U.S. Geological Survey 2011).

In undisturbed ecosystems, the availability of P is highly dependent on soil weathering, so soils that have undergone more weathering are more prone to P limitation. Studies looking at soil chronosequences have found that younger soils tend to be limited by N, while mid-aged soils are co-limited by N and P, and older soils are limited by P (Harrington et al. 2001; Vitousek and Farrington 1997). This same theory has also been applied on a geographical scale, suggesting that P will be more available in high-latitude soils as compared to those of the lowland tropics, since the former have undergone more recent glaciation, which renews the supply of rock-based minerals in soils (Vitousek and Sanford 1986). Dust as a supplier of P becomes more important as available stocks of P through weathering are decreased

(Chadwick et al. 1999). Natural sources of aeolian P for weathered soils can be very geographically distinct. For example, Amazonian forests have been shown to be highly dependent on P deposition that originates from the Saharan desert (Okin et al. 2004; Swap et al. 1992). However, like N, anthropogenic activities can also be the cause of atmospheric mobilization of P. In fact, activities related to increased N in the environment (i.e. transformation of forested areas to pasture or farmland) can contribute P to the atmosphere, first through biomass burning (Mahowald et al. 2005; 2008) and then further through increased incidence of forest fires as a result of land clearing (Cochrane and Laurance 2008). In tropical areas such as the Amazon, slash-and-burn activities can result in combustion P losses greater than 20 kg ha^{-1}, not including subsequent wind and water erosion of P-containing ash (Kauffman et al. 1993).

Regardless of the P source, once soil mineral P has been mobilized by weathering, it can have three general fates: sorption (adsorption onto secondary clay minerals or being bound as aluminum or iron phosphates), losses through hydrological processes, or immobilization by microbes or plants; P returned as organic P can also be sorbed or leached, or mineralized back to the available inorganic P pool (Reed et al. 2011). Sorption is an important controller of P availability in soils, particularly highly-weathered tropical soils, as it can result in sorbed inorganic P concentrations in soil being several orders of magnitude higher than available P concentrations (as cited in Reed et al. 2011). Hydrological P losses are also an important controller of P retention; although it has been shown that P losses decrease with increasing P limitation in soils, small but significant losses continue regardless of the extent of P limitation (Hedin et al. 2003). P enrichment of ‘downstream’ ecosystems through atmospheric and hydrological processes has fewer known negative consequences as compared to N enrichment,

but it is a major driver of aquatic eutrophication (Carpenter et al. 1998; Schindler 2006; Smith et al.2006) and has been linked to carbon storage losses in terrestrial ecosystems (Cleveland and Townsend 2006).

1.4 - Experimental set-up and study objectives

This study took place in three forest sites, which formed an elevation gradient (1000 m, 2000 m and 3000 m asl) in the Andes of southern Ecuador. The forests in this area are considered diversity hot-spots for vascular plants (Barthlott et al. 2007; Brummitt and Lughadha 2003) and birds (Orme et al. 2005). The study sites were in or adjacent to Podocarpus National Park (Figure 1.1), a primary forest covering an area of approximately 1450 km² on the border of the provinces of Loja and Zamora Chinchipe (Ministerio del Ambiente, no date). At each elevation, four replicate blocks were laid out, with each replicate block containing a control plot and three treatment plots: added N, added P and the combination of added N and P (Figure 1.2). More detailed site information is included in the following chapters, and the study area is also extensively reviewed by Richter et al. (2013).

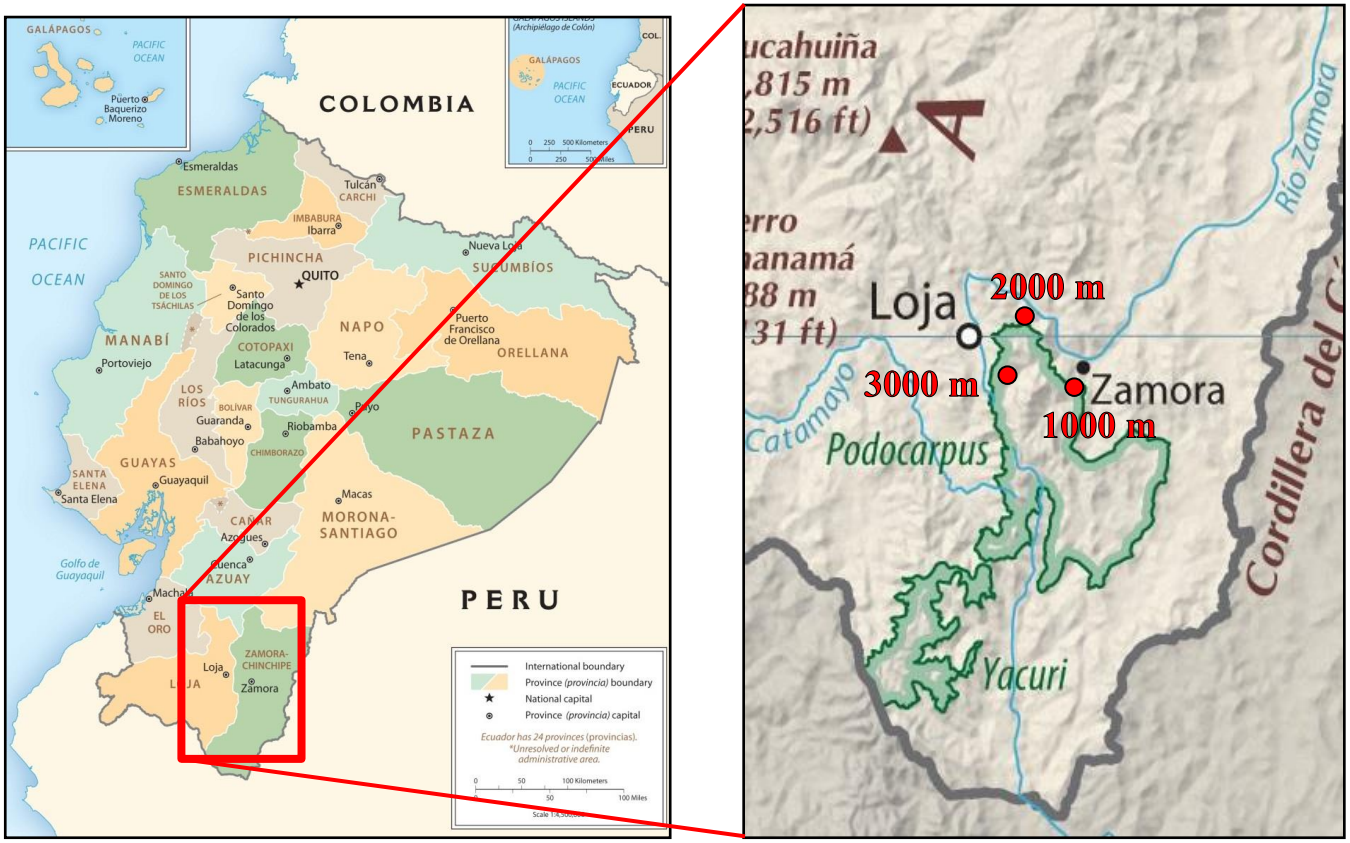

Figure 1.1 Map of Ecuador (left) and the area around Loja and Zamora (right) showing the approximate locations of the 1000 m, 2000 m and 3000 m study sites. Pictures adapted from: https://www.cia.gov/library/publications/cia-maps-publications/Ecuador.html (left) and https://www.cia.gov/library/publications/cia-maps-publications/Ecuador.html (right).

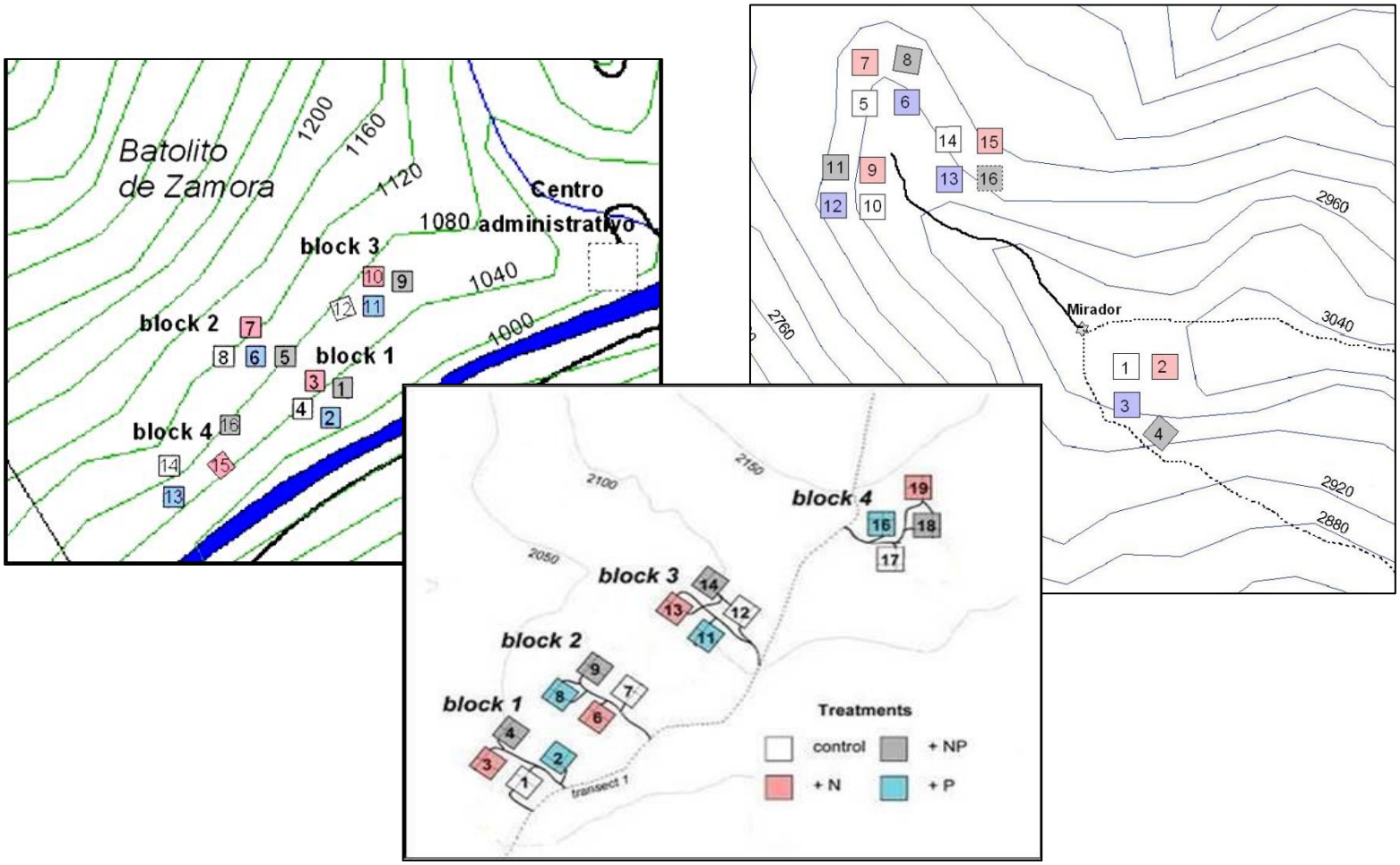

Figure 1.2 Topographic maps showing the layout of the nutrient manipulation experiment (NUMEX) plots at 1000 m (left), 2000 m (middle) and 3000 m (right) (diagrams adapted from J. Homeier, 2010).

Our objectives were to quantify rates of (1) N_2 fixation, (2) internal N cycling, and (3) carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) flux, for canopy soils along an elevation gradient of tropical montane forests, and put these measurements in context of the total forest by comparing them with measurements from the forest floor. In addition, we assessed, in the context of the above-mentioned measurements, the sensitivity of the canopy soils to four years of moderate nutrient addition to the forest floor. Due to the short duration of the nutrient manipulation experiment before we began our measurements, and the low amount of fertilizer added to the forest floor, we anticipated only small changes as a result of fertilizer addition, but were looking for confirmation of the following hypotheses (the detailed justification for which is outlined in the introductory sections of Chapters 2, 3 and 4):

(1) N_2 fixation would be inhibited in N and N+P plots but enhanced in P plots (we expected this to be significant on the forest floor but to see only trends in the canopy).
(2) N cycling rates would increase as a result of all three treatments, since both nutrients should be limiting activity in canopy soils.
(3) N and P would stimulate CH_4 uptake and improve litter quality, increasing CO_2 emissions. There would be no change in N_2O flux, as the canopy should be N-limited and therefore have a very conservative N cycle.

1.5 - References

Barthlott W, Schmit-Neuerburg V, Nieder J, Engwald S (2001) Diversity and abundance of vascular epiphytes: a comparison of secondary vegetation and primary montane rain forest in the Venezuelan Andes. Plant Ecology 152:145–156

Barthlott W, Hostert A, Kier G, Kuper W, Kreft H, Mutke J, Rafiqpoor D, Sommer JH (2007) Geographic patterns of vascular plant diversity at continental to global scales. Erdkunde 61:305–315

Beaulieu F, Walter DE, Proctor HC, Kitching RL (2010) The canopy starts at 0.5 m: predatory mites (Acari: Mesostigmata) differ between rain forest floor soil and suspended soil at any height. Biotropica 42:704–709

Bernhard A (2012) The nitrogen cycle: processes, players, and human impact. Nature Education Knowledge 3:25

Boy J, Rollenbeck R, Valarezo C, Wilcke W (2008) Amazonian biomass burning-derived acid and nutrient deposition in the north Andean montane forest of Ecuador. Global Biogeochem Cycles 22: GB4011

Brummitt N, Lughadha EN (2003) Biodiversity: where's hot and where's not. Conserv Biol 17:1442–1448

Cardelús CL (2010) Litter decomposition within the canopy and forest floor of three tree species in a tropical lowland rain forest, Costa Rica. Biotropica 42:300–308

Cardelus CL, Mack MC (2010) The nutrient status of epiphytes and their host trees along an elevational gradient in Costa Rica. Plant Ecol 207:25–37

Cardelús CL, Mack MC, Woods CL, DeMarco J. Treseder, K (2009) Nutrient cycling in canopy and forest floor soils in a lowland tropical wet forest, Costa Rica. Plant Soil 318:47–61

Carpenter SR, Caraco NF, Correll DL, Howarth RW, Sharpley AN, Smith VH (1998) Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecol Appl 8:559–568

Chadwick OA, Derry LA, Vitousek PM, Huebert BJ, Hedin LO (1999) Changing sources of nutrients during four million years of ecosystem development. Nature 397:491–497

Chen L, Liu WY, Wang GS (2010) Estimation of epiphytic biomass and nutrient pools in the subtropical montane cloud forest in the Ailao Mountains, southwestern China. Ecol Res 25:315–325

Clark KL, Nadkarni NM, Gholz HL (1998) Growth, net production, litter decomposition, and net nitrogen accumulation by epiphytic bryophytes in a tropical montane forest. Biotropica 30:12–23

Clark KL, Nadkarni NM, Gholz HL (2005) Retention of inorganic nitrogen by epiphytic bryophytes in a tropical montane forest. Biotropica 37:328–336

Cleveland CC, Townsend AR (2006) Nutrient additions to a tropical rain forest drive substantial soil carbon dioxide losses to the atmosphere. PNAS 103:10316-10321

Cochrane MA, Laurance WF (2008) Synergisms among fire, land use, and climate change in the Amazon. Ambio 37:522–527

Coxson DS and Nadkarni NM (1995) Ecological Role of Epiphytes in Nutrient Cycles. *in* Lowman MD, Nadkarni NM (eds.), Forest Canopies. Academic Press, New York, pp. 495–543

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol Lett 10:1135–1142

Enloe HA, Graham RC, Sillett SC (2006) Arboreal Histosols in old growth redwood forest canopies, northern California. Soil Sci Soc Am J 70:408–418

Freiberg M, Freiberg E (2000) Epiphyte diversity and biomass in the canopy of lowland and montane forests in Ecuador. Journal of Tropical Ecology 16:673–688

Gaige E, Dail DB, Hollinger DY, Davidson EA, Fernandez IJ, Sievering H, White A, Halteman W (2007) Changes in canopy processes following whole-forest canopy nitrogen fertilization of a mature spruce-hemlock forest. Ecosystems 10:1133–47

Galloway JN, Aber JD, Erisman JW, Seitzinger SP, Howarth RW, Cowling EB, Cosby BJ (2003) The Nitrogen Cascade. BioScience 53:341–356

Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, Karl DM, Michaels AF, Porter JH, Townsend AR, Vörösmarty CJ (2004) Nitrogen cycles: past, present, and future. Biogeochemistry 70:153–226

Galloway JN, Schlesinger WH, Levy H, Michaels A, Schnoor JL (1995) Nitrogen fixation: anthropogenic enhancement—environmental response. Global Biogeochem Cycles 9:235–252

Harrington RA, Fownes JH, Vitousek PM (2001) Production and resource use efficiencies in N and P-limited tropical forests: a comparison of responses to long-term fertilzation. Ecosystems 4:646–657

Hedin LO, Brookshire ENJ, Menge DNL, Barron AR (2009) The nitrogen paradox in tropical forest ecosystems. Annu Rev Ecol Evol S 40:613–635

Hedin LO, Vitousek PM, Matson PA (2003) Nutrient losses over four million years of tropical forest development. Ecology 84:2231–2255

Hietz P, Wanek W, Wania R, Nadkarni NM (2002) Nitrogen-15 natural abundance in a montane cloud forest canopy as an indicator of nitrogen cycling and epiphyte nutrition. Oecologia 131:350–355

Hietz P, Turner BL, Wanek W, Richter A, Nock CA, Wright SJ (2011) Long-term change in the nitrogen cycle of tropical forests. Science 334:664–666

Hoegberg P, Fan H, Quist M, Binkley D, Tamm CO (2006) Tree growth and soil acidification in response to 30 years of experimental nitrogen loading on boreal forest. Global Change Biology 12:489–499

Howarth RW (2008) Coastal nitrogen pollution: A review of sources and trends globally and regionally. Harmful Algae 8:14–20

Howarth RW, Anderson D, Cloern J, Elfring C, Hopkinson C, Lapointe B, Malone T, Marcus N, McGlathery K, Sharpley A, Walke D (2000) Nutrient pollution of coastal rivers, bays, and seas. Issues Ecol 7:1–15

Kauffman JB, Sanford RL, Cummings DL, Salcedo IH, Sampaio EVSB (1993) Biomass and nutrient dynamics associated with slash fires in neotropical dry forests. Ecology 74:140–151

Johnson PTJ, Townsend AR, Cleveland CC, Glibert PM, Howarth RW, et al. (2010) Linking environmental nutrient enrichment and disease emergence in humans and wildlife. Ecol. Appl. 20:16–29

Jørgensen PM, Leon-Yanez S (eds) (1999) Catalogue of the vascular plants of Ecuador. Monographs in systematic botany of the Missouri Botanical Garden, 75. Missouri Botanical Garden, St Louis.

Lowman MD, Schowalter TD (2012) Plant science in forest canopies – the first 30 years of advances and challenges (1980–2010). New Phytologist 194:12–27

Mahowald, NM, Artaxo P, Baker AR, Jickells TD, Okin GS, Randerson JT, Townsend AR (2005) Impacts of biomass burning emissions and land use change on Amazonian atmospheric phosphorus cycling and deposition. Global Biogeochem Cycles 19:GB4030

Mahowald N, Jickells TD, Baker AR, Artaxo P, Benitez-Nelson CR, Bergametti G, Bond TC, Chen Y, Cohen DD, Herut B, Kubilay N, Losno R, Luo C, Maenhaut W, McGee KA, Okin GS, Siefert RL, Tsukuda S (2008) Global distribution of atmospheric phosphorus sources, concentrations and deposition rates, and anthropogenic impacts. Global Biogeochem Cycles 22:GB4026

Martinson GO, Werner FA, Scherber C, Conrad R, Corre MD, Flessa H, Wolf K, Klose M, Gradstein SR, Veldkamp E (2010) Methane emissions from tank bromeliads in neotropical forests. Nat Geosci 3:766–769

Ministerio del Ambiente (n.d.) Parque Nacional Podocarpus *in* Ministerio del Ambiente: Programas/Servicios, accessed March 2014, at http://www.ambiente.gob.ec/parque-nacional-podocarpus/

Nadkarni NM (1994) Diversity of species and interactions in the upper tree canopy of forest ecosystems. American Zoologist 34:321–330

Nadkarni NM, Haber WA (2009) Canopy seed banks as time capsules of biodiversity in pasture-remnant tree crowns. Conservation Biology 23:1117–1126

Nadkarni NM, Matelson TJ (1989) Bird use of epiphyte resources in neotropical trees. Condor 69:891–907

Nadkarni NM, Schaefer DA, Matelson TJ, Solano R (2002) Comparison of arboreal and terrestrial soil characteristics in a lower montane forest, Monteverde, Costa Rica. Pedobiologia 46:23–33

Nadkarni NM, Schaefer DA, Matelson TJ, Solano R (2004) Biomass and nutrient pools of canopy and terrestrial components in a primary and a secondary montane cloud forest, Costa Rica. For Ecol Manag 198:223–236

Okin GS, Mahowald N, Chadwick OA, Artaxo P (2004) Impact of desert dust on the biogeochemistry of phosphorus in terrestrial ecosystems. Glob Biogeochem Cycles 18:GB2005

Orme CDL, Davies RG, Burgess M, Eigenbrod F, Pickup N, Olson VA, Webster AJ, Ding T-S,Rasmussen PC, Ridgely RS, Stattersfield AJ, Bennett PM, Blackburn TM, Gaston KJ,

Owens IPF (2005) Global hotspots of species richness are not congruent with endemism or threat. Nature 436:1016–1019

Ozanne CM, Anhuf PD, Boulter SL, Keller M, Kitching RL, Körner C, Meinzer FC, Mitchell AW, Nakashizuka T, Dias PLS, Stork NE, Wright SJ, Yoshimura M (2003) Biodiversity meets the atmosphere: a global view of forest canopies. Science 301:183–186

Perez CA, Guevara R, Carmona MR, Armesto JJ (2005) Nitrogen mineralization in epiphytic soils of an old-growth *Fitzroya cupressoides* forest, southern Chile. Ecoscience 12:210–215

Prescott CE (2002) The influence of the forest canopy on nutrient cycling. Tree Physiology 22:1193–1200

Reed SC, Townsend AR, Taylor PG, Cleveland CC (2011) Phosphorus cycling in tropical forests growing on highly-weathered soils. *in* Buneman E, Oberson A, Frossard E (eds) Phosphorus in action--biological processes in soil phosphorus cycling. Springer Publishing Co pp. 339–369

Remsen JV, Parker TA (1984) Arboreal dead-leaf-searching birds of the Neotropics. Condor 86:36–41

Richter M, Beck E, Rollenbeck R, Bendix J (2013) The study area. *in* Bendix J, Beck E, Bräuning A, Makeschin F, Mosandl R, Scheu S, Wilcke W. (eds) Ecosystem Services, Biodiversity and Environmental Change in a Tropical Mountain Ecosystem of South Ecuador. Ecological Studies 221:3–18

Schindler DW (2006) Recent advances in the understanding and management of eutrophication. Limnol Oceanogr 51:356–363

Silver WL, Herman DJ, Firestone MK (2001) Dissimilatory nitrate reduction to ammonium in upland tropical forest soils. Ecology 82:2410–2416

Smil V (2000) Phosphorus in the environment: natural flows and human interferences. Annu Rev Energy Environ 25:53–88

Smith VH, Joye SB, Howarth RW (2006) Eutrophication of freshwater and marine ecosystems Limnol Oceanogr 51:351–355

Soethe N, Lehmann J, Engels C (2008) Nutrient availability at different altitudes in a tropical montane forest in Ecuador. Journal of Tropical Ecology 24:397–406

Stevens CJ, Dise NB, Mountford O, Gowing DJ (2004) Impact of nitrogen deposition on the species richness of grasslands. Science 303:1876–1879

Swap R, Garstang M, Greco S, Talbot R, Kallberg P (1992) Saharan dust in the Amazon basin. Tellus B Chem Phys Meteorol 44:133–1149

Townsend AR, Asner GP, Cleveland CC (2008) The biogeochemical heterogeneity of tropical forests. Trends Ecol Evol 23:424–431

Townsend AR, Cleveland CC, Houlton BZ, Alden CB, White JWC (2011) Multi-element regulation of the tropical forest carbon cycle. Front Ecol Environ 9:9–17

Townsend AR, Howarth RH, Bazzaz FA, Booth MS, Cleveland CC, Collinge SK, Dobson AP, Epstein PR, Holland EA, Keeney DR, Mallin MA, Rogers CA, Wayne P, Wolfe AH (2003) Human health effects of a changing global nitrogen cycle. Front Ecol Environ 1:240–246

Umana NH, Wanek W (2010) Large canopy exchange fluxes of inorganic and organic nitrogen and preferential retention of nitrogen by epiphytes in a tropical lowland rainforest. Ecosystems 13:367–381

U.S. Geological Survey (2011) Phophate rock statistics *in* Kelly TD, and Matos GR, (comps.) Historical statistics for mineral and material commodities in the United States: U.S. Geological Survey Data Series 140, accessed March 2014, at http://pubs.usgs.gov/ds/2005/140/

Vance E, Nadarni NM (1990) Microbial biomass and activity in canopy organic matter and the forest floor of a tropical cloud forest. Soil Biol Biochem 22:677–684

Vitousek P, Aber J, Howarth R, Likens G, Matson P, Schindler D, Schlesinger W, Tilman G (1997) Human Alteration of the Global Nitrogen Cycle: Causes and Consequences. Issues in Ecology 1:1–16

Vitousek PM, Farrington H (1997) Nutrient limitation and soil development: Experimental test of a biogeochemical theory. Biogeochemistry 37:63–75

Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea: How can it occur? Biogeochemistry 13:87–115

Vitousek PM, Porder S, Houlton BZ, Chadwick OA (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. Ecol Appl 20:5–15

Vitousek PM, Sanford RL (1986) Nutrient Cycling in Moist Tropical Forest. Annual Review of Ecology and Systematics 17:137–167

Wanek W, Arndt SK, Huber W, Popp M (2002) Nitrogen nutrition during ontogeny of hemiepiphytic *Clusia* species. Functional Plant Biology 29:733–740

Westheimer FH (1987) Why nature chose phosphates. Science 235:1173–1178

Werner FA, Homeier J, Oesker M, Boy J (2012) Epiphytic biomass of a tropical Andean forest varies with topography. J Trop Ecol 28:23–31

Yanoviak SP, Nadkarni NM, Solano JR (2007) Arthropod assemblages in epiphyte mats of Costa Rican cloud forests. Biotropica 39:202–210

Zimmermann A, Wilcke W, Elsenbeer H (2007) Spatial and temporal patterns of throughfall quantity and quality in a tropical montane forest in Ecuador. Journal of Hydrology 343:80–96

Chapter 2

RESPONSE OF FREE-LIVING NITROGEN FIXATION TO ELEVATED NUTRIENT INPUTS IN TROPICAL MONTANE FOREST FLOOR AND CANOPY SOILS OF SOUTHERN ECUADOR

2.1 - Abstract

Although the canopy can play an important role in forest nutrient cycles, canopy-based processes are often overlooked in studies on atmospheric deposition. In areas of nitrogen (N) and phosphorus (P) deposition, canopy soils receive both direct atmospheric inputs and indirect enrichment via enriched throughfall and plant litter. We measured rates of free-living N_2 fixation along an elevation gradient (1000, 2000 and 3000 m) of tropical montane canopy soils, compared these to rates measured in the top 5 cm of forest floor soils, and assessed the indirect effects from elevated nutrient inputs to the forest floor. N_2 fixation was measured using the acetylene reduction assay. Measurements took place in the field, in the wet and dry seasons, using intact cores of soil. The forest floor had been fertilized biannually with moderate amounts of N and P for 4 years; treatments included control, N, P and N+P. The canopy contributed 7-13 % of free-living soil N_2 fixation, which ranged from 0.8 to 1.5 kg N ha^{-1} yr^{-1}. N_2 fixation rates exhibited little variation with elevation but were much higher in the dry season than the wet season. Fixation activity was inhibited in forest floor N plots compared to control and P plots, and stimulated in canopy P plots compared to control. Results suggest that N_2 fixation is an active process in canopy soils, but is extremely variable across seasons and sensitive to changes in nutrient availability. Long-term atmospheric N and/or P deposition has the potential to significantly change the dynamics of soil N cycling in these canopies.

2.2 - Introduction

Tropical regions have experienced dramatic increases in anthropogenic nitrogen (N) and phosphorus (P) deposition in recent decades – mainly as a result of increased fertilizer use, fossil fuel use and biomass burning – and these increases are expected to continue (Boy et al. 2008; Galloway et al. 2004; Hietz et al. 2011; Mahowald et al. 2005, 2008). It has been projected that almost two-thirds of N fertilizer use and energy-related N inputs worldwide will be occurring in the tropics and subtropics by 2020 (Matthews 1994; Galloway et al. 1994). Although these inputs are the by-product of necessary activities required to sustain a growing global population, reactive N is prone to moving into neighboring, undisturbed areas through hydrological and atmospheric processes (Galloway et al. 2003). Additionally, many of the activities related to increased N in the environment (i.e. transformation of forested areas to pasture or farmland) contribute nutrients such as P to the atmosphere, first through biomass burning (Mahowald et al. 2005; 2008) and then further through increased incidence of forest fires as a result of land clearing (Cochrane and Laurance 2008). Deposition of N and P into otherwise undisturbed tropical forests could have a significant impact, as many of these forests are expected to be N and/or P limited (Elser et al. 2007; Vitousek et al. 2010). However, the long-term effect that deposition of these nutrients will have on tropical forests is still uncertain.

The major non-anthropogenic pathway of N input to an ecosystem is N_2 fixation. Since it has a very high energy requirement, N_2 fixation should, theoretically, down-regulate as other sources – such as atmospheric deposition – increase N availability in the soil. However, in what has been termed the *nitrogen paradox* (Hedin et al. 2009), this is not always the case in tropical forests, where N supply often seems to exceed biological demand. The majority of N_2

fixed worldwide is through symbiotic bacteria in root nodules (Cleveland et al. 1999), but in tropical forests a significant amount of N_2 can also be fixed by asymbiotic or 'free-living' microbes in litter or soil (Cleveland et al. 1999, Jordan et al. 1982, Maheswaran and Gunatilleke 1990, Reed et al. 2007). However, the distribution and controls of free-living N_2 fixation are still not well understood and documented in tropical regions. Hedin et al. (2009) suggest in their theory to explain the nitrogen paradox that N_2 fixation (and especially that from free-living N_2 fixers) might occur in zones of N deficiency, which are spatially separated from areas of N abundance, allowing N_2 fixation to continue despite the ecosystem being N-rich as a whole. The two areas that they suggest are the surface of forest floor soils and the forest canopy.

Despite the important role that canopies play in forest nutrient cycles, canopy-based processes are often overlooked in studies on nutrient cycling. Canopies affect forest ecosystems in a number of vital ways, buffering extreme temperature changes through shading, altering hydrological conditions to reduce leaching and overland flow (Prescott 2002), providing a unique habitat for plant and animal 'canopy specialists' and acting as a storehouse/source of nutrients for the forest ecosystem (Nadkarni 1994, Nadkarni et al. 2002). Often envisioned as just the uppermost part of the trees in a forest, the canopy is, in fact, a complex ecosystem existing within the larger forest ecosystem (Nadkarni 1994; Ozanne et al. 2003); it includes not only plants and animals, but also wetlands (Martinson et al. 2010) and soil (Enloe et al. 2006). In tropical montane forests, a major component of canopy functioning is canopy soil, an accumulation of organic matter found on branches and tree junctions. Canopy soil is mainly comprised of decomposed material from epiphytes (Hietz et al. 2002), but also includes intercepted litter, invertebrates, fungi and microorganisms (Nadkarni et al.

2002). Studies have shown that canopy soil has many similarities to tropical forest floor litter (Cardelus et al. 2009; Nadkarni et al. 2002; Vance and Nadkarni 1990), but due to its isolation from the mineral soil, it could continue to be nutrient limited – and therefore active in fixing N_2 – even as N accumulated on the forest floor. However, to date, N_2 fixation studies in tropical forests that include the canopy have done so in only a few compartments: canopy leaves (Bentley 1987; Carpenter 1992; Cusack et al. 2009; Freiberg 1998; Fürnkranz et al. 2008; Goosem and Lamb 1986; Reed et al. 2008), bryophytes (Cusack et al. 2009; Matzek and Vitousek 2003) and lichens (Benner et al. 2007; Cusack et al. 2009; Forman 1975; Matzek and Vitousek 2003). Potential N_2 fixation rates from these canopy compartments vary, but are suggested to be up to 8 kg N ha^{-1} yr^{-1} (Forman 1975). In terms of nutrient response, several studies observed a positive effect of P (or low N:P ratios) on N_2 fixation rates (Benner et al. 2007; Bentley 1987; Matzek and Vitousek 2003; Reed et al. 2008), but often mixed effects with N. Cusack et al. (2009) observed that added N decreased N_2 fixation rates in the canopy and on the forest floor, but effects were only significant on the forest floor. The results of these studies indicate that the canopy could remain a zone of N deficiency as N accumulated elsewhere in the forest. However, while all of these studies provided estimates and information about different compartments in the canopy, several were lab-based studies and/or provided only potential values, and none of them included canopy soil.

Although canopy soil may not be a significant part of all forest ecosystems, it can be a significant part of some; estimates of canopy soil biomass range from 1000 to 33000 kg ha^{-1} (Vance and Nadkarni, 1990; Nadkarni et al. 2004; Chen et al. 2010; Werner et al. 2012). Furthermore, Nadkarni et al. (2004) showed that while canopy soil made up only 6 % of the aboveground biomass of a tropical montane forest in Costa Rica, when one focused on the

biomass of just labile (non-woody) components, canopy soil made up over 80 % of the total. Therefore, in order to understand tropical montane forest ecosystems and predict changes due to disturbances such as nutrient deposition, we need to better understand how canopy soil functions and how it compares to forest floor soil.

In this study, we measured rates of N_2 fixation along an elevation gradient of tropical montane forests in intact cores of canopy and forest floor soil (*forest floor soil* is defined as the top 5 cm of material - excluding coarse litter - found on the forest floor). Cores were taken from plots with and without fertilization of N and P. In order to avoid short-term effects from adding fertilizer directly to canopy soils, we used a pre-existing experimental setup where N and P had been added to the forest floor for four years. Our objectives were to: (1) determine and compare rates of free-living N_2 fixation in canopy and forest floor soil and (2) assess whether these rates were affected by indirect enrichment from moderate nutrient inputs to the forest floor. We hypothesized that N_2 fixation would be inhibited in the N-fertilized forest floor soils but not in their corresponding canopy soils. Similarly, we expected N_2 fixation to increase in P-fertilized forest floor soils but not in the corresponding canopy soils.

2.3 - Materials and Methods

2.3.1 Study sites

This study was carried out along an elevation gradient in and adjoining Podocarpus National Park, a tropical montane forest in the Andes of southern Ecuador. The gradient included three study areas: 1000 m (4.115° S, 78.968° W; ranging from 990-1100 m), 2000 m (3.982° S, 79.083° W; ranging from 1950-2100 m) and 3000 m (4.110° S, 79.178° W; ranging from 2900-3050 m) (Martinson et al. 2013). Details about general climate, soil parameters and

vegetative cover are included in Table 2.1. Although we attribute differences between the three study areas to the combination of climatic, vegetation and soil factors associated with elevation, it is notable that the elevations themselves were not replicated, so these results cannot be said to represent these elevations in tropical montane forests as a whole.

Table 2.1 Site and soil characteristics along an elevation gradient from 1000 m to 3000 m, in a tropical montane forest of southern Ecuador. Soil characteristics (mean (SE); n=4) were measured from the top 5 cm of soil on the forest floor (mineral soil at 1000 m and organic soil at 2000 m and 3000 m) and on branches in the upper and lower canopy. Previously published material: temperature, rainfall (Moser et al. 2007), vegetation type (Homeier et al. 2010), stand height, tree density, forest floor organic layer, soil type and forest floor total phosphorus (Martinson et al. 2013).

	1000 m	**2000 m**		**3000 m**	
Annual temperature (°C)	19.4	15.7		9.4	
Annual rainfall (mm)	2230	1950		4500	
Vegetation type	Premontane	Lower montane		Upper montane	
Stand height (m)	20-25	10-14		6-8	
Tree density (trees ha^{-1})	747.5	1142.5		1305.0	
Forest floor organic layer (cm)	0	10-30		10-40	
Soil type	Dystric Cambisol	Stagnic Cambisol		Stagnic Histosol	
Canopy soil (0-5 cm)	*Upper*	*Upper*	*Lower*	*Upper*	*Lower*
$\delta^{15}N$	1.2 (0.4)	-0.03 (0.7)	-0.8 (0.2)	0.1 (0.5)	-0.2 (0.3)
Total carbon (%)	48.9 (0.9)	48.0 (0.6)	47.8 (0.4)	48.9 (1.5)	49.3 (1.8)
Total nitrogen (%)	2.4 (0.3)	1.7 (0.1)	1.9 (0.1)	1.5 (0.2)	1.7 (0.3)
C/N ratio	20.8 (2.2)	28.7 (1.6)	25.4 (1.6)	34.4 (2.3)	30.5 (3.8)
pH (1:4 soil-to-H_2O)	4.2 (0.2)	3.7 (0.3)	3.4 (0.1)	3.8 (0.2)	4.3 (0.5)
Total magnesium (mg Mg g^{-1})	0.9 (0.2)	0.6 (0.2)	0.3 (0.1)	1.3 (0.2)	2.3 (1.4)
Total phosphorus (mg P g^{-1})	0.9 (0.2)	0.5 (0.1)	0.5 (0.0)	0.5 (0.0)	0.6 (0.1)
Forest floor soil (0-5 cm)					
$\delta^{15}N$[a]	4.1 (0.6)	0.3 (1.0)		0.2 (1.0)	
Total carbon (%)[a]	5.7 (1.7)	47.5 (0.7)		47.4 (2.0)	
Total nitrogen (%)[a]	0.4 (0.1)	1.9 (0.1)		1.4 (0.1)	
C/N ratio[a]	13.7 (1.2)	26.2 (2.2)		34.7 (1.4)	
pH (1:4 soil-to-H_2O)[a]	4.3 (0.2)	4.0 (0.1)		3.7 (0.0)	
Total magnesium (mg Mg g^{-1})[a]	-	0.4 (0.0)		0.1 (0.1)	
Total phosphorus (mg P g^{-1})	0.1 (0.0)	0.5 (0.0)		0.7 (0.0)	

[a] Unpublished data used with the permission of A. Baldos

2.3.2 Nutrient addition

The individual study sites were plots of the nutrient manipulation experiment (NUMEX) project (fully described in Martinson et al. 2013), in which 20 x 20 m plots had been fertilized biannually with moderate amounts of N (urea at 50 kg N ha^{-1} yr^{-1}) and P (analytical grade monosodium phosphate at 10 kg P ha^{-1} yr^{-1}) since 2008. In comparison, between 1998 and 2012, ambient deposition near our 2000 m site ranged from 14 to 45 kg N ha^{-1} yr^{-1} and 0.4 to 4.9 kg P ha^{-1} yr^{-1} (Homeier et al. 2012). Rates of nutrient addition were deliberately chosen to be low, as compared to other nutrient manipulation experiments (Crews et al. 2000; Benner et al. 2007; Hall and Matson 2003; Koehler et al. 2009; Reed et al. 2007), in order to more accurately mimic projected atmospheric deposition rates (Galloway et al. 2004; Phoenix et al. 2006). Treatments plots (control, N, P and N+P) were grouped into blocks, with a minimum distance of 10 m between each plot. Each elevation had four replicate blocks, making a total of 16 plots per elevation. Solid fertilizer was applied to the forest floor by hand; in 2011 and 2012, fertilization at all elevations occurred once between February and April, and once in August or September.

Sampling sites for canopy soil were chosen in trees within the NUMEX plots which fulfilled certain criteria. First, we looked for individuals with the presence of canopy soil ≥ 5 cm in depth, and enough volume to take the required number of samples (between 4 and 6 cores, depending on the treatment). Then, we excluded any trees in which canopy soil was in an area that was inaccessible using rope techniques or a ladder. Finally, to avoid edge effects, we chose from the remaining trees, the individual that was the furthest possible distance from the edge of the plot. The original intent was to limit the study to specific species, but there was no species that had individuals in every plot, which had an adequate volume of canopy soil in

an accessible location. We chose, instead, to see whether we could observe general canopy soil trends despite any variability caused by tree species.

2.3.3 N_2 fixation

N_2 fixation was measured once in the dry season (November 2011) and once in the wet season (June 2012), using the acetylene reduction assay (Hardy et al. 1968); climate parameters for these specific dates are shown in Table 2.2. Pairs of intact soil cores were taken from the forest floor (near trees where canopy samples were taken) and canopy, in each treatment plot; cores were 4.7 cm deep with a volume of 78.1 cm^3. In all cores (forest floor and canopy), twigs and leaves were removed before sampling, but moss, lichens and litter that was at least partially decomposed was included. Cores from the forest floor at 1000 m were a mix of organic matter and mineral soil, whereas the thick organic layer at 2000 m and 3000 m (see Table 2.1) made those cores entirely organic. At 2000 m and 3000 m, samples were taken in all four blocks, from both upper canopy (near the top of a tree in an area relatively open to sun/wind/rain) and lower canopy (mid to lower area of a tree, with less exposure to sun/wind/rain), as well as the forest floor, making 6 cores per plot (2-forest floor, 4-canopy). At 1000 m, there was little canopy soil in the lower-canopy region of trees, so sampling occurred only in upper canopy and forest floor soil, making 4 cores per plot (2-forest floor, 2-canopy).

Table 2.2 Soil moisture (mean (SE); n=4) and climatic parameters along a montane forest elevation gradient, measured during the dry season (November 2011) and wet season (May/June 2012), on days when N_2 fixation was determined. All parameters differed between the two seasons ($P \leq 0.09$ for climate station data and $P \leq 0.08$ for soil moisture) except relative humidity at 1000 m ($P = 0.12$) and solar radiation at 2000 m ($P = 0.22$).

	1000 m	**2000 m**	**3000 m**
November 2011 (7:00-19:00)			
Air temperature (°C)[a]	23.6[a]	17.6[b]	10.2[c]
Relative humidity (%)[a]	74.3[a]	69.2[a]	67.5[a]
Solar radiation (W m^{-2})[a]	422[a]	404[a]	400[a]
Soil moisture (% dry wt) – Upper canopy	160 (22)[Aa]	171 (23)[Aa]	193 (49)[Aa]
Soil moisture (% dry wt) – Lower canopy	-	225 (32)[Aa]	236 (62)[Aa]
Soil moisture (% dry wt) – Forest floor	49.0 (10.1)[Bb]	268 (32)[Aa]	268 (27)[Aa]
May/June 2012 (7:00-19:00)			
Air temperature (°C)[a]	21.7[a]	14.8[b]	6.7[c]
Relative humidity (%)[a]	81.8[a]	80.3[a]	99.9[b]
Solar radiation (W m^{-2})[a]	283[ab]	322[a]	187[b]
Soil moisture (% dry wt) – Upper canopy	290 (21)[Aa]	263 (18)[Ba]	323 (31)[Ba]
Soil moisture (% dry wt) – Lower canopy	-	396 (64)[Aa]	388 (38)[ABa]
Soil moisture (% dry wt) – Forest floor	55.0 (15.2)[Bc]	368 (32)[ABb]	481 (12)[Aa]

[a] Unpublished data used with the permission of T. Peters

[*] Values with different lowercase letters indicate significant differences between elevations ($P < 0.03$ for climate station data and $P < 0.03$ for soil moisture).

[**] Values with different uppercase letters indicate significant differences in moisture within each elevation and season ($P < 0.04$).

To measure N_2 fixation, cores were sealed in 500 mL glass mason jars fitted with septa for gas sampling. In one set of cores, 10 % of the headspace air was removed and replaced with acetylene (scrubbed according to Hyman and Arp 1987); the other set was incubated without acetylene to measure background ethylene (C_2H_4) production rates. The jars were then partially buried – so that they were still exposed to light while keeping the cores at a realistic field temperature – and incubated in the field for 24 hours, during which 4 samples of headspace air were taken. Samples (15 mL) were taken using a 20-mL syringe and injected into 12-mL Labco Exetainer® (Labco Limited, Lampeter, UK) evacuated tubes. Each time an

air sample was removed from a jar, an equal volume of ambient air was injected in order to prevent an under-pressure in the jar. This dilution was accounted for during calculations using values from an empty jar which was also incubated with 10 % acetylene and sampled exactly like the others. Tubes with gas samples were shipped to Germany for analysis. The soil from each core was returned to the field station and dried to a constant weight, to measure dry soil mass and gravimetric water content.

Gas samples were analyzed for C_2H_4 concentrations using a gas chromatograph (Shimadzu GC-14B, Duisburg, Germany) with a flame ionization detector (FID). Operating conditions for the GC were: 65 °C injector temperature, 80 °C oven temperature and 290 °C FID temperature, with N_2 as the carrier gas. C_2H_4 separations used a Heyesep T column (5.0 m length x 0.2 cm inside diameter). Total C_2H_4 produced was plotted for each time step and the C_2H_4 production rate was calculated as the linear slope of the resulting best-fit line through these points.

2.3.4 N_2 fixation conversion factor

In 2012, six additional cores per elevation (taken only from control plots) were incubated with ^{15}N-enriched N_2 for 12-13 days (based on previous measurements of how long oxygen concentration remained above 10% in the jars' headspace), in order to determine the correct conversion factor for the measured C_2H_4 production rates to N_2 fixation. Due to the costs associated with this method, we could only do the conversion factor in one season, and chose to do it in the wet season (June 2012), assuming it would be the season with the most activity. The calculated C_2H_4:N_2 conversion factors were: (a) 2.1 at 3000 m, (b) 1.2 at 2000 m, and (c) 0.11 at 1000 m.

Given that N_2 fixation can be sensitive to increased soil N availability (i.e. Cusack et al. 2009), the reliability of the $^{15}N_2$-incubation could be highly dependent on mineral N availability in the soil. Thus, we also compared net nitrification rates in our soil cores to the C_2H_4:N_2 conversion factors and found a negative relationship between net nitrification rates and C_2H_4:N_2 conversion factors along the elevation gradient (not shown). This indicated that increased N availability in the cores may have inhibited N_2 fixation and resulted in a lower C_2H_4:N_2 conversion factor than was actually occurring in the field. In retrospect, the $^{15}N_2$ calibration could have been made more robust by incubating additional cores in jars with an unenriched headspace, and then assessing C_2H_4 production several times throughout the $^{15}N_2$ incubation. Given that we did not do so, we chose to use the theoretical ratio of 3:1 (Hardy et al. 1968), which is the other common practice used in N_2 fixation studies (i.e. Cusack et al. 2009 & Reed et al. 2008 [1:3]; Benner et al. 2007 & Matzek and Vitousek 2003 [1:3.1]).

2.3.5 Soil analyses

In 2011, additional soil samples to those used in the incubations were taken from the canopy soil in each plot and used to measure other soil parameters. $\delta^{15}N$ signatures of the soils were measured using IRMS (Delta Plus, Finnigan MAT, Bremen, Germany). Total C and N were measured by dry combustion in a CN analyzer (Elementar Vario EL; Elementar Analysis Systems GmbH, Hanau, Germany). Total elemental concentration of P, Fe, Mg, Mn, Na, S, Fe, K and Al were determined using pressure digestion of the sample in concentrated nitric acid and then analysis of the resulting solution with an inductively coupled plasma-atomic emission spectrometer (Spectroflame, Spectro Analytical Instruments, Kleve, Germany). Soil

pH (H_2O) was analyzed in a 1:4 soil-to-water ratio. The forest floor parameters shown in Table 2.1 were from a 2010 analysis of our study sites (Baldos et al. unpublished data).

2.3.6 Statistics and calculations

Results were analyzed using the R (version 2.15.3) open source software. N_2 fixation rates were significantly non-normal, according to both Kolmogorov-Smirnov and Shapiro-Wilk tests, exhibiting a zero-inflated Poisson distribution. We tested control plots for differences between elevations and positions using a Kruskal-Wallis H test followed by a pairwise Mann-Whitney U test with Holm's correction for multiple comparisons. Seasons were compared using a paired Mann-Whitney U test and correlations with soil parameters were tested using Spearman rank correlations. The climate data at each elevation were tested for differences between seasons using a linear mixed effect model. To test for treatment effects, the data was modeled using the Tweedie compound Poisson linear model (cplm) R package (Zhang 2012). In our model, N_2 fixation was the response variable, with season, block (replicate) and position (canopy or forest floor) as random effects, and treatment and elevation as fixed effects. We also made smaller models for each position and season, with block as the only random effect. We used the Tukey HSD test to assess the differences between fixed effects. We accepted *P* values of $P < 0.10$ as significant.

We report N_2 fixation rates both in units of mg kg^{-1} d^{-1} as well as kg ha^{-1} yr^{-1}. In order to calculate the latter, we used data from Werner et al. (2012). They found a total of 3877 kg ha^{-1} canopy soil (which they termed 'dead organic matter') on trees from mid-slope positions at 2000 m, in the same forest (although a different area) where we worked. We then made a rough approximation based on our field observations, and adjusted the amount of canopy soil

to half that amount for the plots at 1000 m and twice that amount for those at 3000 m. In order to upscale to an annual rate, we assumed that the wet and dry season each lasted for six months.

2.4 - Results

2.4.1 Control plots (canopy vs. forest floor, elevation, seasonality and controlling soil factors)

N_2 fixation was an active process in our montane forests, occurring in both canopy and forest floor soils at all elevations (Table 2.3). N_2 fixation rates from control plots were not significantly different between canopy (upper canopy at 1000 m and both upper and lower canopy at 2000 m and 3000 m) and forest floor soils, in the dry season (*P = 0.25* at 1000 m, *P = 0.11* at 2000 m and *P = 0.62* at 3000 m) or the wet season (*P = 0.15* at 1000 m, *P = 0.87* at 2000 m and *P = 0.70* at 3000 m). Although N_2 fixation rates in the canopy soils in the dry season appeared to decrease with elevation (Table 2.3), this trend was not statistically significant in the dry season (*P = 0.28* for the upper canopy and *P = 0.15*, for the lower canopy) or the wet season (*P = 0.11* for the upper canopy and *P = 0.72* for the lower canopy) due to the variation within the replicates (i.e. large standard errors). There were also no differences between elevations in the forest floor soils in the dry (*P = 0.87*) or wet season (*P = 0.12*).

Table 2.3 N_2 fixation rates (mean (SE); n=4) along a montane forest elevation gradient in southern Ecuador, measured in the dry season (November 2011) and the wet season (May/June 2012). Measurements were taken from the top 5 cm of soil from control plots on the forest floor (mineral soil at 1000 m and organic soil at 2000 m and 3000 m) and in the canopy.

Elevation (m)	Dry season (mg N kg^{-1} d^{-1})		Wet season (mg N kg^{-1} d^{-1})		Annual (kg N ha^{-1} yr^{-1})[a]	
	Forest floor	Canopy	Forest floor	Canopy	All soil	Canopy contribution
1000	0.08 (0.05)[a]	0.30 (0.16)[a]	0.00 (0.00)[a]	0.00 (0.00)[a]	1.5 (0.8)	7%
2000	0.10 (0.03)[a]	0.20 (0.11)[a]	0.04 (0.03)[a]	0.01 (0.01)[ab]	1.2 (0.8)	12%
3000	0.09 (0.05)[a]	0.05 (0.03)[a]	0.01 (0.01)[a]	0.02 (0.01)[b]	0.8 (0.5)	13%

[a] Annual fixation rates are calculated as the combined fixation occurring in forest floor and canopy soil at each elevation, on a per area basis (see *Materials and Methods: Statistics and Calculations*).

[*] For each column, means with different letters indicate significant differences between elevations (*P = 0.09*).

N_2 fixation rates in the dry season were markedly higher than those in the wet season (Fig. 1a and 1b). Comparing across elevations, rates of N_2 fixation in the control plots were significantly higher in the dry season than the wet season in the upper canopy (*P = 0.02*), lower canopy (*P = 0.02*) and forest floor (*P = 0.01*). In addition, we observed significant differences in climatic factors between the two seasons. Our measured soil moisture contents, as well as air temperature, relative humidity and solar radiation measured from climate stations installed in the forests where we worked – and taken from only the dates when N_2 fixation was determined – showed clear differences between seasons (Table 2.2). At all elevations, the wet season was cooler, moister and darker as compared to the dry season (*P < 0.03* for climate station data and *P < 0.08* for soil moisture), with only two exceptions (relative humidity at 1000 m and solar radiation at 2000 m), where the trend was the same but the differences were not significant (*P = 0.12* for humidity and *P = 0.22* for radiation).

Soil properties for canopy and forest floor soil at each elevation are shown in Tables 2.1 and 2.2. In the dry season, lower canopy rates were negatively correlated with $\delta^{15}N$ (*P < 0.01*)

but no significant correlations were observed for the upper canopy. In the wet season, upper canopy N_2 fixation rates were positively correlated with the C:N ratio ($P = 0.03$) and total Mg ($P = 0.05$) and negatively correlated with total N ($P = 0.05$), total S ($P < 0.01$) and $\delta^{15}N$ ($P = 0.02$), but no significant correlations were observed for the lower canopy. There were no significant correlations with N_2 fixation rates in the forest floor soils (not including 1000 m, since those were mineral soils and the others were organic) in either season.

2.4.2 Nutrient-addition effects (canopy vs. forest floor, seasonality and elevation)

Data for all three elevations is combined when reporting treatment effects (Fig. 2.1). Comparing across treatments, in the dry season there were no significant elevation effects on N_2 fixation, while in the wet season, the only significant elevation effect was N_2 fixation rates on the forest floor at 1000 m, which were lower (close to zero) than at 2000 m and 3000 m ($P < 0.01$ for both).

Looking at N_2 fixation rates on the forest floor across elevations, the control plots were higher than the N treatment ($P = 0.02$ wet season, $P = 0.09$ dry season) but not the N+P treatment ($P = 0.23$ wet season, $P = 0.16$ dry season) or P treatment ($P = 1.00$ in both seasons). The P treatment was higher than the N treatment ($P = 0.06$ in both seasons) but not the N+P treatment ($P = 0.48$ wet season, $P = 0.10$ dry season). There was no significant difference between the N and N+P treatments ($P = 0.89$ wet season, $P = 0.99$ dry season).

Looking at N_2 fixation rates in the canopy across elevations, the control plots were lower than the P treatment ($P = 0.04$ wet season, $P = 1.00$ dry season) but not different from the N ($P = 0.97$ wet season, $P = 0.72$ dry season) or N+P ($P = 0.98$ wet season, $P = 0.27$ dry season) treatments. The P treatment was higher than both the N ($P = 0.01$ wet season, $P =$

0.78 dry season) and N+P (*P = 0.09* wet season, *P = 0.32* dry season) treatments. There was no significant difference between the N and N+P treatments (*P = 0.83* wet season, *P = 0.87* dry season).

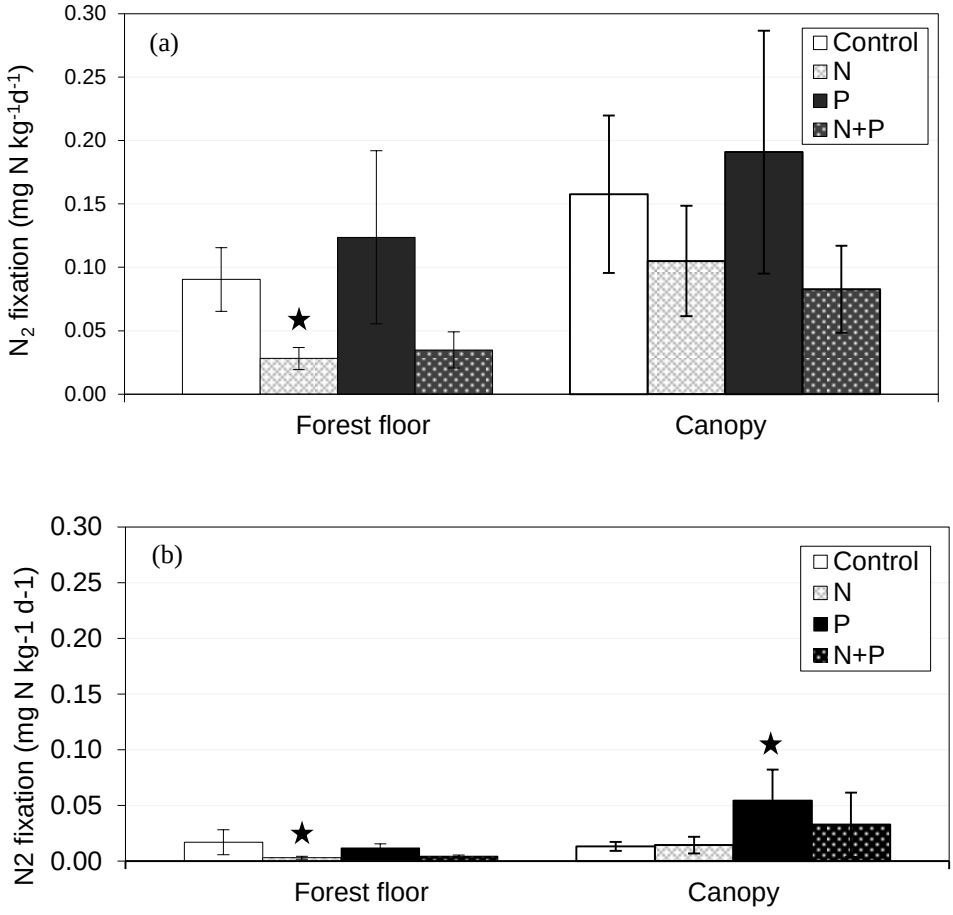

Figure 2.1 N_2 fixation rates (mg N $kg^{-1}d^{-1}$) along a montane forest elevation gradient (1000 m, 2000 m and 3000 m) in southern Ecuador. Dry season measurements (a) were taken in November 2011. Wet season measurements (b) were taken in June 2012. Values for each treatment are the average of 4 replicates taken from 3 elevations (n=12); in the dry season there were no significant difference between elevations and in the wet season, forest floor values at 1000 m were significantly lower (close to zero) than those at the higher elevations (*P < 0.01*). Treatments (applied only to the forest floor) started in 2008 and include: control, nitrogen (N), phosphorus (P) and combined N+P; stars indicate that a treatment is significantly different from the control

2.5 - Discussion

2.5.1 Canopy vs. forest floor soils

Our results show that N_2 fixation was an active process in canopy soils, with mass-based rates similar to those found in forest floor soils and area-based rates comprising 7-13 % of total (canopy and the top 5 cm of forest floor) free-living soil N_2 fixation (Table 2.3). N_2 fixation rates in canopy soils (0.10 to 0.15 kg N ha^{-1} yr^{-1}) fall within the lower end of the 0.02 to 8 kg N ha^{-1} yr^{-1} range observed and/or predicted for other tropical forest canopy compartments (Benner et al. 2007; Carpenter 1992; Cusack et al. 2009; Forman 1975; Freiberg 1998; Fürnkranz et al. 2008; Matzek and Vitousek 2003; Reed et al. 2008). Summed N_2 fixation rates for canopy and forest floor soils (0.8 to 1.5 kg N ha^{-1} yr^{-1}) also fall within the lower end of the 0.1 to 60 kg N ha^{-1} yr^{-1} range reported for free-living N_2 fixation in tropical forest floor soils (Reed et al. 2011). Our lower rates are unsurprising, given that several of the other canopy studies were based on laboratory incubations, which would be anticipated to have higher rates than those observed in the field (Keuter et al. 2014). Additionally, in both canopy and forest floor studies, most measurements come from lowland or premontane forests, whereas our sites span premontane to upper montane forests.

Observations regarding the relative importance of mass-based N_2 fixation rates in the canopy as compared to forest floor soil are inconsistent. When there is an abundance of N_2-fixing bryophytes and/or lichens found in the canopy, the rate of N_2 fixation can be equivalent to (Cusack et al. 2009; Matzek and Vitousek 2003), or much higher than (Benner et al. 2007), the rate in forest floor soils. When only considering N_2 fixation on leaf surfaces, rates in the canopy are smaller than those in the forest floor (Reed et al. 2008). In our study, we only considered the top 5 cm of forest floor soil, and included a combination of all of the above-

mentioned fractions, in that our intact core samples included moss, lichens and partially-decomposed leaf litter as well as the organic soil itself. Therefore, it is unsurprising that overall, we did not detect a significant difference between the canopy and forest floor.

2.5.2 Seasonality (soil moisture, solar radiation, temperature and soil properties)

Seasonal patterns in soil N_2 fixation can be controlled by soil moisture. Since the nitrogenase enzyme is sensitive to O_2 (Hicks et al. 2003; Nohrstedt 1983) and increased soil moisture decreases soil O_2 concentrations (Silver et al. 1999), rates of N_2 fixation in the wet season are often higher than those in the dry season (Hofmockel and Schlesinger 2007; Reed et al. 2007). However, although the wet season in our study had higher soil moisture (Table 2.2), we observed significantly lower N_2 fixation rates in the wet season as compared to the dry season. It is notable, though, that although moisture increased in the wet season, it was (except the forest floor at 1000 m) well over 100 % dry weight in both seasons. Given that cyanobacteria, for example, can fix N_2 at moisture levels as low as 6 % dry weight and in one study reached maximum N_2 fixation rates at soil moistures between 22 % and 42 % (Jones 1977), it is possible that seasonal changes in moisture are not directly responsible for seasonal variations in N_2 fixation in our soils. In contrast, although our soils were moist, the water-filled pore space remained below 60 %, even in the wet season, due to the low bulk density of organic soils. This combination of high moisture and highly porous soils could have led to higher soil nitrification rates (which we observed in another set of canopy soils during the wet season; Matson et al. unpublished data). Although nitrification is an aerobic process that often decreases in soils during the wet season (Breuer et al. 2002), an increase in soil moisture may

increase nitrification in organic soils (as seen by Ingwersen et al. 1999 in the organic layer of the forest floor), with the increased mineral N then inhibiting N_2 fixation.

Seasonal patterns in N_2 fixation can also be controlled by solar radiation, if the N_2-fixing microbial community is predominantly made up of photoautotrophic organisms, such as cyanobacteria, which are dependent on photosynthetically active radiation (PAR). A positive correlation of N_2 fixation with PAR has been observed several times for tropical forest canopy leaves (Bentley 1987; Freiberg 1998; Reed et al. 2008). In low PAR conditions, N_2 fixation can continue in cyanobacteria, but normally at a reduced rate, which is limited in duration by the organism's carbon stores (Jones 1977, Millbank 1978; Rai et al. 1981). Although we did not measure PAR in this study, we did observe more cloud cover and significantly less solar radiation (Table 2.2) in the wet season as compared to the dry season.

Given adequate moisture and light, the other most common factor affecting seasonal patterns in N_2 fixation is temperature (Belnap and Lange 2001). In our study, temperature as a possible driver of seasonal differences in N_2 fixation was possible at 1000 m. It has been shown that at temperatures above 22 °C, the activation energy for nitrogenase is 0.65 eV, but that once the temperature drops below that threshold, the required activation energy increases to 2.18 eV (Vitousek et al. 2013). The average temperature at our 1000 m site (the elevation that exhibited the most dramatic change in N_2 fixation rates between seasons) was above this threshold during the dry season sampling, but below it during the wet season sampling. However, an increase in the required activation energy does not explain the significant decreases in N_2 fixation rates at 2000 m or 3000 m in the wet season. At those elevations, both temperatures in the dry and wet seasons were well below 22 °C. In addition, the similar N_2 fixation rates at all three elevations indicated that N_2 fixation was not responding strongly to

elevation-related differences in air temperature. Nevertheless, if N_2-fixing organisms at each elevation were adapted to their specific environmental niche, changes from the dry to wet season could have affected N_2-fixers at each elevation in a similar manner, so that N_2 fixation rates remained similar across elevations.

Finally, seasonal patterns in N_2 fixation could also be controlled by season-related differences in microbial diversity. Seasonal changes have been shown to affect microbial diversity in soil (Smit et al. 2001), and microbial community shifts have been shown to at least partially explain observed differences in N_2 fixation in a tropical rain forest (Reed et al. 2010). Although we did not measure microbial diversity, we observed far more correlations with soil properties in the wet season as compared to the dry season. In the wet season, N_2 fixation rates were more affected by N (as shown by the negative correlation with total N and positive correlation with C:N ratio), more affected by P (as shown by the positive effect of the P addition treatment), and were also correlated with other soil nutrients, namely total Mg and total S.

2.5.3 Canopy response to forest floor nutrient addition

Although many studies have shown and/or postulated that the canopy is largely decoupled from forest floor nutrient pools in lowland and lower-montane tropical forests (Hedin et al. 2009; Hietz et al. 2002; Stewart et al. 1995; Tozer et al. 2005; Wania et al. 2002), our study shows that this may not always be the case in lower- to upper-montane tropical forests. The NUMEX experiment had only been active for four years when we initiated our study, and our rates of nutrient addition to the forest floor were quite low as compared to other fertilization studies (Benner et al. 2007; Crews et al. 2000; Reed et al. 2007). Moreover, we knowingly

allowed an additional source of variation into our measurements by including different tree species. Therefore, although there were no significant N-addition effects on N_2 fixation in the canopy soil, the trend towards N_2 fixation being inhibited in N and N+P plots (Fig. 2.1a), as well as the significant positive effect of P addition in the wet season (Fig. 2.1b), suggest that, contrary to the hypothesis that the canopy is decoupled from nutrients in forest floor soil, it may actually be sensitive to relatively small changes in forest floor nutrient availability.

Studies from temperate forests have shown that nutrients leaching from host tree leaves and bark can affect epiphytic lichens (Gauslaa 1995; Goward and Arsenault 2000; Hauck 2003; Hauck and Runge 2002), and in a Hawaiian montane forest, Benner et al. (2007) and Benner and Vitousek (2007) reported that 14 years of high (100 kg ha^{-1} yr^{-1}) P addition to the forest floor, resulted in a significant increases in epiphyte abundance and diversity - in particular N_2-fixers. Although their study did not report when effects first became visible, in our study area the effects of forest floor fertility on canopy processes seemed to occur very rapidly. Not only did we see significant changes in canopy soil N_2 fixation after four years of forest floor fertilization, but in 2008 and 2009, Wullaert et al. (2010) had already observed significant changes in throughfall nutrients in the NUMEX plots at 2000 m. Although the canopy in all plots still exhibited net retention of N and P deposited from the atmosphere, there was already a decrease in N and P retention (i.e. higher throughfall N and P fluxes) in N-, P- and combined N+P-fertilized plots as compared to control plots after the second and third fertilization (Wullaert et al. 2010).

2.5.4 Forest floor and canopy soil response to N and P addition

N_2 fixation in our forest floor soils was significantly inhibited by N addition (Fig. 2.1), an outcome which has been observed in previous tropical forest studies (Barron et al. 2008; Crews et al. 2000; Cusack et al. 2009). However, a surprising number of studies have also observed no significant effect of N on N_2 fixation (Benner et al. 2007; Matzek and Vitousek 2003; Reed et al. 2007), which is generally attributed to a study area being N-limited or having N_2-fixers that are not sensitive to N supply. In our study, the inhibitory effect of N on the forest floor was not as strong in the N+P plots as it was in the N plots. If soils and/or plants in our forest stands were co-limited by N and P (Homeier et al. 2012; Wullaert et al. 2010), the addition of both nutrients together may have facilitated nutrient immobilization, so that the added N had less of an inhibitory effect on N_2 fixation. Although the P plots were not significantly different than the control on the forest floor, an alternate explanation could be that combined N+P addition may have been simultaneously decreasing (in the case of N) and increasing (in the case of P) N_2 fixation so that the net effect was not significantly different from the control.

The effect of N addition on N_2 fixation was not significant in the canopy soils, which is unsurprising given that we expected the canopy to be N-limited (Hedin et al. 2009). However, the fact that the N_2 fixation rates in control canopy soil were negatively related to $\delta^{15}N$ enrichment (which can be an indication of more N-rich soils, as shown by Arnold et al. 2009), were positively related to soil C:N ratios in the wet season, and exhibited a tendency to be inhibited by N in the dry season (Fig. 2.1), indicates that with time, chronic N addition will likely inhibit N_2 fixation in canopy soils as it did in the forest floor soils. We saw a positive effect of P addition on N_2 fixation in our canopy soils, which has been observed in the canopy

(Benner et al. 2007; Bentley 1987; Matzek and Vitousek 2003; Reed et al. 2008) and on the forest floor (Crews et al. 2000; Reed et al. 2007; Vitousek and Hobbie 2000). While the canopy soils in the P plots exhibited higher N_2 fixation rates than all other treatment plots, the N+P plots were not significantly different from the control and only marginally smaller than the P plots. Similar to the N and N+P effects on the forest floor, this also suggests that a co-limitation of N and P may have facilitated the immobilization of these nutrients when both were added together, or that the counteracting effects of these two nutrients on N_2 fixation led to no net change. Reed et al (2010) found that P fertilization increased both diversity and abundance of N_2-fixers in tropical forest soils. If there was an increased diversity of N_2-fixers in the canopy soil due to P addition, only some of which were inhibited by additional N in the N+P plots, this could also explain why we saw no net effect in these plots. Similarly, it could explain why, during the wet season, when N_2 fixation in all our soils seemed to be suppressed, we observed higher N_2 fixation in the canopy soils of P plots than in all other plots.

2.5.5 Conclusion

Tropical montane forest soils in Ecuador can fix 0.8-1.5 kg N ha^{-1} yr^{-1} through free-living soil N_2 fixation, up to 13 % of which may be a result of fixation in canopy soils. However, N_2 fixation in these soils can be subject to high seasonal variation and the specific environmental factors causing this warrant further study. Atmospheric N and P deposition could change the dynamics of soil N_2 fixation in these forests; additional N may cause significant decreases in N_2-fixing activity, while hotspots occur in areas with additional P. However, future research should focus on the link between canopy and forest-floor soil fertility, as this will impact how nutrient deposition affects N_2 fixation in the two different soils.

2.6 - References

Arnold J, Corre MC, Veldkamp E (2009) Soil N cycling in old-growth forests across an Andosol toposequence in Ecuador. For Ecol Manag 257:2079-2087

Barron AR,Wurzburger N, Bellenger JP,Wright SJ, Hedin LO (2008) Molybdenum limitation of asymbiotic nitrogen fixation in tropical forest soils. Nat Geosci 2:42-45

Belnap J, Lange OL (2001) Biological soil crusts: structure, function and management. Ecol Stud 150:241-261

Benner JW, Conroy S, Lunch CK, Toyoda N, Vitousek PM (2007) Phosphorus fertilization increases the abundance and nitrogenase activity of the cyanolichen Pseudocyphellaria crocata in Hawaiian montane forests. Biotropica 39:400-405

Benner JW, Vitousek PM (2007) Development of a diverse epiphyte community in response to phosphorus fertilization. Ecology Letters 10:628-636

Bentley BL (1987) Nitrogen fixation by epiphylls in a tropical rainforest. Ann Missouri Bot Gard 74:234-241

Boy J, Rollenbeck R, Valarezo C, Wilcke W (2008) Amazonian biomass burning-derived acid and nutrient deposition in the north Andean montane forest of Ecuador. Global Biogeochem Cycles 22: GB4011

Breuer L, Kiese R, Butterbach-Bahl K (2002) Temperature and moisture effects on nitrification rates in tropical rain-forest soils. Soil Sci Soc Am J 66:834-844

Cardelús CL, Mack MC, Woods CL, DeMarco J. Treseder, K (2009) Nutrient cycling in canopy and forest floor soils in a lowland tropical wet forest, Costa Rica. Plant Soil 318:47-61

Carpenter EJ (1992) Nitrogen-fixation in the epiphyllae and root-nodules of trees in the lowland tropical rainforest of Costa Rica. Acta Oecol 13:153-160

Chen L, Liu WY, Wang GS (2010) Estimation of epiphytic biomass and nutrient pools in the subtropical montane cloud forest in the Ailao Mountains, southwestern China. Ecol Res 25:315-325

Cleveland CC, Townsend AR, Schimel DS, Fisher H, Hedin LO, Perakis SS, Latty EF, Von Fischer JC, Elseroad A, Wasson MF (1999) Global patterns of terrestrial biological nitrogen (N2) fixation in natural ecosystems. Global Biogeochem Cycles 13:623-645

Cochrane MA, Laurance WF (2008) Synergisms among fire, land use, and climate change in the Amazon. Ambio 37:522-527

Crews TE, Farrington H, Vitousek PM (2000) Changes in asymbiotic, heterotrophic nitrogen fixation on leaf litter of Metrosideros polymorpha with long-term ecosystem development in Hawaii. Ecosystems 3:386-395

Cusack DF, Silver WL, McDowell WH (2009) Biological nitrogen fixation in tropical forests: spatial variation and effects of nitrogen deposition. Ecosystems 12:1299-1315

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol Lett 10:1135-1142

Enloe HA, Graham RC, Sillett SC (2006) Arboreal Histosols in old growth redwood forest canopies, northern California. Soil Sci Soc Am J 70:408-418

Forman RT (1975) Canopy lichens with blue-green algae: a nitrogen source in a Colombian rain forest. Ecology 56:1176-1184

Freiberg E (1998) Microclimatic parameters influencing nitrogen fixation in the phyllosphere of a Costa Rican premontane rain forest. Oecologia 17:9-18

Fürnkranz M, Wanek W, Richter A, Abell G, Rasche F, Sessitsch A (2008) Nitrogen fixation by phyllosphere bacteria associated with higher plants and their colonizing epiphytes of a tropical lowland rainforest of Costa Rica. The ISME Journal 2:561-570

Galloway JN, Levy HII, Kasibhatla PS (1994) Year 2020: Consequences of population growth and development on the deposition of oxidized nitrogen. Ambio 23:120-123

Galloway JN, Aber JD, Erisman JW, Seitzinger SP, Howarth RW, Cowling EB, Cosby BJ (2003) The Nitrogen Cascade. BioScience 53:341-356

Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, Karl DM, Michaels AF, Porter JH, Townsend AR, Vörösmarty CJ (2004) Nitrogen cycles: past, present, and future. Biogeochemistry 70:153-226

Gauslaa Y (1995) The Lobarion, an epiphytic community of ancient forests threatened by acid rain. Lichenologist 27:59-76

Goosem S, Lamb D (1986) Measurements of phyllosphere nitrogen fixation in a tropical and two sub-tropical rain forests. J Trop Ecol 2:373-376

Goward T, Arsenault A (2000) Cyanolichen distribution in young unmanaged forests: a dripzone effect? Bryologist 10:28-37

Hall SJ, Matson PA (2003) Nutrient status of tropical rain forests influences soil N dynamics after N additions. Ecol Monogr 73:107-129

Hardy RWF, Holsten RD, Jackson EK, Burns RC (1968) The acetylene-ethylene assay for N_2 fixation: laboratory and field evaluation. Plant Physiol 43:1185-207

Hart SC, Stark JM, Davidson EA, Firestone MK (1994) Nitrogen mineralization, immobilization, and nitrification. In: Weaver R (ed) Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties. SSSA Book Series No. 5, American Society of Agronomy, Madison, WI, pp 985-1019

Hauck M (2003) Epiphtic lichen diversity and forest dieback: the role of chemical site factors. Bryologist 106:257-269

Hauck M, Runge M (2002) Stemflow chemistry and epiphytic lichen diversity in dieback-affected spruce forest of the Harz Mountains, Germany. Flora 197:250-261

Hedin LO, Brookshire ENJ, Menge DNL, Barron AR (2009) The nitrogen paradox in tropical forest ecosystems. Annu Rev Ecol Evol S 40:613-635

Hicks WT, Harmon ME, Griffiths RP (2003) Abiotic controls on nitrogen fixation and respiration in selected woody debris from the Pacific Northwest, U.S.A. Ecoscience 10:66-73

Hietz P, Wanek W, Wania R, Nadkarni NM (2002) Nitrogen-15 natural abundance in a montane cloud forest canopy as an indicator of nitrogen cycling and epiphyte nutrition. Oecologia 131:350-355

Hietz P, Turner BL, Wanek W, Richter A, Nock CA, Wright SJ (2011) Long-term change in the nitrogen cycle of tropical forests. Science 334:664-666

Hofmockel KS, Schlesinger WH (2007) Carbon dioxide effects on heterotrophic dinitrogen fixation in a temperate pine forest. Soil Sci Soc Am J 71:140-44

Holst J, Butterbach-Bahl K, Liu C, Zheng X, Kaiser AJ, Schnitzler JP, Zechmeister-Boltenstern S, Brüggemann N (2009) Dinitrogen fixation by biological soil crusts in an Inner Mongolian steppe. Biol Fertil Soils 45:679-690

Homeier J, Breckle SW, Gunter S, Rollenbeck RT, Leuschner C (2010) Tree diversity, forest structure and productivity along altitudinal and topographical gradients in a species-rich Ecuadorian montane rain forest. Biotropica 42:140-148

Homeier J, Hertel D, Camenzind T et al. (2012) Tropical Andean forests are highly susceptible to nutrient inputs—rapid effects of experimental N and P addition to an Ecuadorian montane forest. PLoSONE doi:10.1371/journal.pone.0047128

Hyman MR, Arp DJ (1987) Quantification and removal of some contaminating gases from acetylene used to study gas-utilizing enzymes and microorganisms. Appl Environ Microbiol 53:298-303

Ingwersen A, Butterbach-Bahl K, Gasche R, Richter O, Papen H (1999) Barometric process separation: new method for quantifying nitrification, denitrification, and nitrous oxide sources in soils. Soil Sci Soc Am J 63:117-128

Jones K (1977) The effects of moisture on acetylene reduction by mats of blue-green algae in sub- tropical grassland. Ann Bot 41:801-806

Jordan C, Caskey WW, Escalante G, Herrera R, Montagnini F, Todd R, Uhl C (1982) The nitrogen cycle in a 'Terra Firme' rain forest on oxisol in the Amazon Territory of Venezuela. Plant Soil 67:325-332

Keuter A, Veldkamp E, Corre MD. (2014) Asymbiotic biological nitrogen fixation in a temperate grassland as affected by management practices. Soil Biol Biochem 70:38-46

Koehler B, Corre MD, Veldkamp E, Wullaert H, Wright SJ (2009) Immediate and long-term nitrogen oxide emissions from tropical forest soils exposed to elevated nitrogen input. Glob Change Biol 15:2049-2066

Maheswaran J, Gunatilleke IAUN (1990) Nitrogenase activity in soil and litter of a tropical lowland rain forests and an adjacent fernland in Sri Lanka. J Trop Ecol 6:281-89

Mahowald, NM, Artaxo P, Baker AR, Jickells TD, Okin GS, Randerson JT, Townsend AR (2005) Impacts of biomass burning emissions and land use change on Amazonian atmospheric phosphorus cycling and deposition. Global Biogeochem Cycles 19:GB4030

Mahowald N, Jickells TD, Baker AR, Artaxo P, Benitez-Nelson CR, Bergametti G, Bond TC, Chen Y, Cohen DD, Herut B, Kubilay N, Losno R, Luo C, Maenhaut W, McGee KA, Okin GS, Siefert RL, Tsukuda S (2008) Global distribution of atmospheric phosphorus sources,

concentrations and deposition rates, and anthropogenic impacts. Global Biogeochem Cycles 22:GB4026

Martinson GO, Werner FA, Scherber C, Conrad R, Corre MD, Flessa H, Wolf K, Klose M, Gradstein SR, Veldkamp E (2010) Methane emissions from tank bromeliads in neotropical forests. Nat Geosci 3:766-769

Martinson GO, Corre MD, Veldkamp E (2013) Responses of nitrous oxide fluxes and soil nitrogen cycling to nutrient additions in montane forests along an elevation gradient in southern Ecuador. Biogeochemistry 112:625-636

Matthews E (1994) Nitrogenous fertilizers: global distribution of consumption and associated emissions of nitrous oxide and ammonia. Global Biogeochem Cycles 8:411-439

Matzek VA, Vitousek PM (2003) Nitrogen fixation in bryophytes, lichens, and decaying wood along a soil age gradient in Hawaiian montane rainforest. Biotropica 35:12-19

Millbank JW (1978) The contribution of nitrogen fixing lichens to the nitrogen status of their environment: environmental role of nitrogen-fixing blue-green algae and asymbiotic bacteria Ecological Bulletins 26:260-265

Moser G, Hertel D, Leuschner C (2007) Altitudinal change in LAI and stand leaf biomass in tropical montane forests: a transect shady in Ecuador and a pan-tropical meta-analysis. Ecosystems 10:924-935

Nadkarni NM (1994) Diversity of species and interactions in the upper tree canopy of forest ecosystems. American Zoologist 34:321-330

Nadkarni NM, Schaefer DA, Matelson TJ, Solano R (2002) Comparison of arboreal and terrestrial soil characteristics in a lower montane forest, Monteverde, Costa Rica. Pedobiologia 46: 23-33

Nadkarni NM, Schaefer DA, Matelson TJ, Solano R (2004) Biomass and nutrient pools of canopy and terrestrial components in a primary and a secondary montane cloud forest, Costa Rica. For Ecol Manag 198:223-236

Nohrstedt HO (1983) Conversion factor between acetylene reduction and nitrogen fixation in soil: effect of water content and nitrogenase activity. Soil Biol Biochem 15:275-279

Ozanne CM, Anhuf PD, Boulter SL, Keller M, Kitching RL, Körner C, Meinzer FC, Mitchell AW, Nakashizuka T, Dias PLS, Stork NE, Wright SJ, Yoshimura M (2003) Biodiversity meets the atmosphere: a global view of forest canopies. Science 301:183-186

Phoenix GK, Hicks WK, Cinderby S, Kuylenstierna JCI, Stock WD, Dentener FJ, Giller KE, Austin AT, Lefroy RDB, Gimeno BS, Ashmore MR, Ineson P (2006) Atmospheric nitrogen deposition in world biodiversity hotspots: the need for a greater global perspective in assessing N deposition impacts. Glob Change Biol 12:470-476

Prescott CE (2002) The influence of the forest canopy on nutrient cycling. Tree Physiology 22:1193-1200

Rai AN, Rowell P, Stewart WDP (1981) Nitrogenase activity and dark CO2 fixation in the lichen *Peltigera aphthosa* Willd. Planta 151:256-264

Reed SC, Cleveland CC, Townsend AR (2007) Controls over leaf litter and soil nitrogen fixation in two lowland tropical rain forests. Biotropica 39:585-592

Reed SC, Cleveland CC, Townsend AR (2008) Tree species control rates of free-living nitrogen fixation in a tropical rain forest. Ecology 89:2924-34

Reed SC, Townsend AR, Cleveland CC, Nemergut DR (2010) Microbial community shifts influence patterns in tropical forest nitrogen fixation. Oecologia 164:521-531

Reed SC, Cleveland CC, Townsend AR (2011) Functional ecology of free-living nitrogen fixation: a contemporary perspective. Annu Rev Evol System 42:489-512

Silver WL, Lugo AE, Keller M (1999) Soil oxygen availability and biogeochemistry along rainfall and topographic gradients in upland wet tropical forest soils. Biogeochemistry 44:301-328

Smit E, Leeflang P, Gommans S, van den Broek J, van Mil S, Wernars K (2001) Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. Appl Environ Microbiol 67:2284-2291

Stewart GR, Schmidt S, Handley LL, Turnbull MH, Erskine PD, Joly CA (1995). N-15 Natural abundance of vascular rainforest epiphytes – implications for nitrogen source and acquisition. Plant Cell Environ 18:85–90

Tozer WC, Hackell D, Miers DB, Silvester WB (2005) Extreme isotopic depletion of nitrogen in New Zealand lithophytes and epiphytes; the result of diffusive uptake of atmospheric ammonia? Oecologia 144:628-635

Vance E, Nadarni NM (1990) Microbial biomass and activity in canopy organic matter and the forest floor of a tropical cloud forest. Soil Biol Biochem 22:677-684

Vitousek PM, Hobbie, SE (2000) The control of heterotrophic nitrogen fixation in decomposing litter. Ecology 81:2366-2376

Vitousek PM, Porder S, Houlton BZ, Chadwick OA (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. Ecol Appl 20:5-15

Vitousek PM, Menge DNL, Reed SC, Cleveland CC (2013) Biological nitrogen fixation: rates, patterns and ecological controls in terrestrial ecosystems. Phil Trans R Soc B 368:20130119

Wania R, Hietz P, Wanek W (2002) Natural 15N abundance of epiphytes depends on the position within the forest canopy: source signals and isotope fractionation. Plant, Cell and Environment 25:581-589

Werner FA, Homeier J, Oesker M, Boy J (2012) Epiphytic biomass of a tropical Andean forest varies with topography. J Trop Ecol 28:23-31

Wullaert H, Homeier J, Valarezo C, Wilcke W (2010) Response of the N and P cycles of an old-growth montane forest in Ecuador to experimental low-level N and P amendments. For Ecol Manag 260:1434-1445

Zhang, Y (2012) Likelihood-based and Bayesian methods for Tweedie compound Poisson linear mixed models. Statistics and Computing doi:10.1007/s11222-012-9343-7

Chapter 3

NITROGEN CYCLING IN CANOPY SOILS OF TROPICAL MONTANE FORESTS RESPONDS RAPIDLY TO INDIRECT N AND P FERTILIZATION

3.1 - Abstract

Although the canopy can play an important role in forest nutrient cycles, canopy-based processes are often overlooked in studies on atmospheric deposition. In areas of nitrogen (N) and phosphorus (P) deposition, canopy soils may retain a significant proportion of direct atmospheric inputs, and also receive indirect enrichment through root uptake followed by throughfall or recycling of plant litter. We measured net and gross rates of N cycling in canopy soils of tropical montane forests along an elevation gradient and assessed indirect effects of elevated nutrient inputs to the forest floor. Net N cycling rates were measured using the buried bag method. Gross N cycling rates were measured using ^{15}N pool dilution techniques. Measurements took place in the field, in the wet and dry season, using intact cores of canopy soil from three elevations (1000, 2000 and 3000 m). The forest floor had been fertilized biannually with moderate amounts of N and P for 4 years; treatments included control, N, P and N+P. In control plots, gross rates of NH_4^+ transformations decreased with increasing elevation; gross rates of NO_3^- transformations did not exhibit a clear elevation trend but were significantly affected by season. Nutrient-addition effects were different at each elevation, but combined N+P generally increased N cycling rates at all elevations. When compared with a parallel study from the forest floor, canopy soils contributed up to 23% of total (canopy + forest floor) mineral N production in our soils. In contrast to theories that canopy soil is decoupled from nutrient cycling in forest floor soil, N cycling in the canopy soils of our forests was remarkably sensitive to even slight changes in forest floor N and P availability. Long-term atmospheric N and P deposition may lead to increased N cycling but also increased mineral N losses from the canopy soil system.

3.2 - Introduction

Although they have the potential to play an important role in forest nutrient cycles, canopy soils are rarely included in studies of nutrient cycling. In forests with large stores of canopy soil, such as those found in tropical montane regions, this could contribute to an incomplete understanding of total forest nutrient cycling.

Ecologists were the first to recognize the importance of the nutrient capital found in "crown humus" (Jeník, 1973) or "dead organic matter" (Nadkarni, 1984) and eventually this material began being referred to as a soil (i.e. as "arboreal soil" by Nadkarni, 2002 or "epiphytic soil" by Perez *et al.,* 2005). However, the first analysis of this material from a pedological perspective did not appear in literature until Enloe et al. (2006) identified soils found in Californian redwood trees as Typic Udifolists (soil order: Histosol - acidic, low-density soils primarily made up of organic material and developed in areas of restricted drainage [IUSS, 2006]). Canopy soils are a unique type of Histosol; although they are most commonly found in forests with high annual humidity and rainfall, they are not formed in areas with restricted drainage like most Histosols. Instead, being more subject to climatic variability (less protected from wind, precipitation, sun, etc.) than forest floor Histosols, they go through frequent dry/wet cycles. Studies have shown that Histosols from temperate or boreal regions that have been drained or have experienced changing water tables can exhibit higher rates of nitrogen (N) cycling, especially nitrification (Regina *et al.,* 1996; Venterink *et al.,* 2002; Yu & Ehrenfeld 2009), than those with relatively constant moisture conditions. Canopy Histosols have the potential, therefore, to have high N cycling rates, but there is still a paucity of data about N cycling in these soils.

Compared on a mass-based scale to forest floor soils, canopy soils are remarkably alike in many respects. They can have similar or higher C:N ratios and cation exchange capacity (Nadkarni *et al.,* 2002; Cardelus *et al.,* 2009), similar (Vance & Nadkarni, 1990) or higher (Cardelus *et al.,* 2009) microbial biomass C and N, similar N_2 fixation (Matson *et al.,* unpublished data), similar (Perez *et al.,* 2005) or both higher and lower (Cardelus *et al.,* 2009) net N cycling, and similar gross N cycling (Wanek *et al.,* 2002). However, canopy soils are usually more acidic than forest floor soils (Cardelus *et al.,* 2009), with significantly higher amounts of extractable aluminum (Nadkarni *et al.,* 2002). The relevance of canopy soil N cycling to the total forest nutrient cycle depends not only on N cycling rates, however, but also on the amount of canopy soil present in any given forest. Canopy soil biomass can range from 1000 kg ha^{-1} to 33,000 kg ha^{-1} (Vance & Nadkarni, 1990; Nadkarni *et al.,* 2004; Chen *et al.,* 2010; Werner *et al.,* 2012). In some forests, canopy soil can account for up to 80% of non-woody aboveground biomass (Nadkarni *et al.,* 2004). However, there are still only a handful of studies that have looked at N cycling in canopy soils and none have tried to assess the actual field rates (i.e. *in situ* with intact cores of soil) or investigated how these rates could change with nutrient deposition.

Due to disturbances such as forest clearing, industrialization and biomass burning, tropical regions are experiencing increasing amounts of atmospheric N and phosphorus (P) deposition (Galloway *et al.,* 2004; Mahowald *et al.,* 2005, 2008; Hietz *et al.,* 2011). Some of this occurs in agricultural or urban areas where the additional nutrients may not have a significant impact on an already-altered landscape, but deposition also occurs in neighboring forests that are otherwise undisturbed (Galloway *et al.,* 2003; Mahowald *et al.,* 2008). Many of these forests are expected to be N and/or P limited (Elser *et al.,* 2007; Vitousek *et al.,* 2010)

and could therefore be strongly affected by the deposition of these nutrients. This could be especially true for canopy soils. Canopy budget studies have shown that from 50% (Clark *et al.,* 1998, 2005) up to 80% (Gaige *et al.,* 2007) of N deposition to tropical forests may be retained by the canopy. Therefore, canopy soils may first receive the bulk of direct N deposition in a forest stand and then be indirectly enriched through root uptake and nutrient-enriched throughfall or recycling of nutrient-enriched plant litter. However, it is still uncertain how strongly or quickly internal N cycling in canopy soil would be altered by nutrient deposition. Decomposition is much slower in the canopy than on the forest floor (Nadkarni & Matelson 1991; Cardelus, 2010). Furthermore, in montane canopies, wind often removes the majority of litter from branches, resulting in the formation of canopy soil being largely dependent on epiphytes, which are thought to be disconnected from forest soil nutrient pools (Nadkarni & Matelson 1991; Hietz *et al.,* 2002). Hedin et al. (2009) use this disconnection to postulate that areas such as the canopy may remain N-limited and continue N_2 fixation even as N accumulates elsewhere in the forest. However, the canopy and forest floor were not entirely decoupled in a long-term fertilization study in Hawaii, where addition of P to the forest floor caused an increase in epiphyte abundance and richness - in particular N_2-fixing lichens - although addition of N and other nutrients did not (Benner *et al.,* 2007; Benner & Vitousek 2007). It has also been shown that when N and P start to accumulate on the forest floor, net canopy retention of both these nutrients decreases (Wullaert *et al.,* 2010). However, it is not clear whether N and P accumulation and retention processes are mainly controlled by and affecting epiphytes, or if canopy soil also plays a significant role in N and P dynamics.

In this study, we measured net and gross rates of N cycling in intact cores of canopy soil along an elevation gradient of tropical montane forests. Cores were taken from trees in plots

with N, P and combined N+P additions to the forest floor and in the control plots (without fertilization). Our objectives were to: (1) determine rates of N cycling in canopy soil (in comparison with forest floor rates which were measured in a separate, parallel study by another member of our working group) and (2) assess whether these rates were affected by indirect fertilization (through nutrient inputs to the forest floor). We hypothesized that in control plots, N cycling rates in canopy soils would be similar to forest floor soils, and would decrease with increasing elevation. We expected to see slightly (given the low amount of nutrients added and that our study was conducted in only the fourth year of treatment) higher N cycling rates in N- and P-fertilized plots as compared to control.

3.3 - Materials and Methods

3.3.1 Study sites

This study took place along an elevation gradient in and adjoining Podocarpus National Park, a tropical montane forest in the Andes of southern Ecuador. The gradient included three sites: 1000 m (ranging from 990-1100 m), 2000 m (ranging from 1950-2100 m) and 3000 m (ranging from 2900-3050 m) (Martinson *et al.,* 2013). With increasing elevation, annual temperature decreased from 19.4 °C to 9.4 °C and precipitation varied from 2230 mm (1000 m), to 1950 mm (2000 m), to 4500 mm (3000 m) (Moser *et al.,* 2007). Vegetation at 1000 m was classified as premontane, shifting to lower montane and then upper montane with increasing elevation (Homeier *et al.,* 2010). As elevation increased, stand height decreased (from 20 - 25 m at 1000 m to 6 - 8 m at 3000 m) and tree density increased (from 747.5 trees ha^{-1} at 1000 m to 1305.0 m trees ha^{-1} at 3000 m) (Martinson *et al.,* 2013). We assume that differences between the three study areas observed in our results are caused by elevation-

related climatic, soil and vegetation differences, but we do note that the elevations themselves were not replicated, so these results cannot be said to represent these elevations in tropical montane forests as a whole. Canopy soil parameters for each elevation are summarized in Table 3.1.

Table 3.1 Canopy soil characteristics from three study sites located along a 1000- to 3000-m elevation gradient in a tropical montane forest of southern Ecuador. Soil characteristics (mean ± SE; n = 4) were measured from the top 5 cm of soil in the upper canopy

	1000 m	**2000 m**	**3000 m**
Total carbon (%)	48.9 ± 0.9	48.0 ± 0.6	48.9 ± 1.5
Total nitrogen (%)	2.4 ± 0.3	1.7 ± 0.1	1.5 ± 0.2
C/N ratio	20.8 ± 2.2	28.7 ± 1.6	34.4 ± 2.3
$\delta^{15}N$	1.2 ± 0.4	-0.03 ± 0.7	0.1 ± 0.5
pH (1:4 soil-to-H_2O)	4.2 ± 0.2	3.7 ± 0.3	3.8 ± 0.2
Total aluminum (mg Al g^{-1})	2.8 ± 1.4	2.3 ± 0.4	1.6 ± 0.2
Total calcium (mg Ca g^{-1})	3.8 ± 0.7	0.7 ± 0.4	1.3 ± 0.5
Total iron (mg Fe g^{-1})	0.4 ± 0.1	0.8 ± 0.1	0.6 ± 0.1
Total magnesium (mg Mg g^{-1})	0.9 ± 0.2	0.6 ± 0.2	1.3 ± 0.2
Total manganese (mg Mn g^{-1})	0.23 ± 0.07	0.09 ± 0.05	0.16 ± 0.07
Total phosphorus (mg P g^{-1})	0.9 ± 0.2	0.5 ± 0.1	0.5 ± 0.0
Total potassium (mg K g^{-1})	2.2 ± 0.3	1.9 ± 0.4	1.4 ± 0.1
Total sodium (mg Na g^{-1})	0.03 ± 0.01	0.03 ± 0.00	0.06 ± 0.01
Total sulphur (mg S g^{-1})	2.2 ± 0.3	1.7 ± 0.2	1.4 ± 0.1

3.3.2 Nutrient addition

We focused on the possible effects of indirect fertilization by using canopy soil from trees within a pre-existing experimental setup. Samples were taken from trees within plots of the nutrient manipulation experiment (NUMEX) project (fully described by Martinson *et al.*, 2013), in which the forest floor of 20x20 m plots had been fertilized biannually with moderate

amounts of N (urea at 50 kg N ha^{-1} yr^{-1}) and P (analytical grade monosodium phosphate at 10 kg P ha^{-1} yr^{-1}) since 2008. Between 1998 and 2012, ambient deposition near our 2000 m site ranged from 14 to 45 kg N ha^{-1} yr^{-1} and 0.4 to 4.9 kg P ha^{-1} yr^{-1} (Homeier *et al.*, 2012), so fertilization rates were quite realistic in terms of projected deposition rates (Galloway *et al.*, 2004; Phoenix *et al.*, 2006), as opposed to other nutrient manipulation experiments that have used much higher rates (e.g. Hall & Matson 2003; Corre *et al.*, 2010). The solid fertilizer was distributed by hand; in 2011 and 2012, all elevations were fertilized once between February and April and once in August/September. Treatments plots (control, N, P and N+P) were separated from each other by a minimum of 10 m. Each elevation had four replicate blocks containing each treatment, making a total of 16 plots per elevation.

Although we originally intended to limit the study to specific tree species, there were no species – even within each elevation – that appeared in all of the treatment plots, contained an adequate volume of canopy soil, and had soil in a location that was accessible. Since we wanted to work in the NUMEX plots where the forest floor measurements were underway, and knowing that there is evidence that canopy soil in montane forests is largely derived from epiphytes rather than host tree material (Nadkarni & Matelson 1991; Hietz *et al.*, 2002), we decided to see whether we could observe treatment effects regardless of any variability caused by tree species. We chose sample trees by first looking for a high volume of canopy soil (≥ 5 cm in depth) and then eliminating any trees where soil was in an area that could not be reached using rope techniques or a ladder. Of the remaining trees, we chose the individual that was furthest from the edge of the plot in order to avoid edge effects.

3.3.3 N cycling measurements

N cycling was measured twice: once in the dry season (July/August 2011) and once in the wet season (Jan/Feb 2012), using ^{15}N pool dilution techniques (Davidson *et al.,* 1991). Climate and soil moisture data for these specific dates are shown in Table 3.2. Six intact soil cores were taken from the upper canopy (near the top of a tree in an area relatively open to sun/wind/rain) and lower canopy (mid to lower area of a tree, with less exposure to sun/wind/rain) in each treatment plot (except at 1000 m, where lower canopy was excluded because there was little to no soil present at mid-stem positions); cores were 4.7 cm high with a volume of 78.1 cm^3. In all cores, twigs and leaves were removed before incubation, but moss, lichens and decomposing litter were left, when removing them would have disturbed the soil within the core.

In each set of six cores, two were injected with $^{15}NH_4^+$ (in the form of $(^{15}NH_4)_2SO_4$) to measure gross N mineralization rates and two were injected with $^{15}NO_3^-$ (in the form of $K^{15}NO_3$) to measure gross nitrification rates. Of the remaining two cores, one was used to measure background N concentrations and the other was incubated in the field for 7-9 days to measure net N mineralization and nitrification rates, using the buried bag method (Hart *et al.,* 1994). The ^{15}N cores were labeled using side-port needles, with five, 1-mL injections; the solutions were added to maximize the homogeneity of the label in the core without increasing the gravimetric soil moisture more than 30% (based on previous field measurements). The solutions added 50 µg of $^{15}NH_4^+$ and 25 µg $^{15}NO_3^-$ (both 99% ^{15}N enriched) to their respective cores, corresponding to 7 µg ^{15}N g^{-1} dry soil in the N mineralization cores and 3.5 µg ^{15}N g^{-1} dry soil in the nitrification cores. Once they were labeled, one of each set of cores was immediately mixed and extracted with 0.5 M K_2SO_4, and the soil and extract bottle stored

in a container with ice for transport back to the field station. The other core from each pair was incubated in the field for 24 hours (N mineralization) or 48 hours (nitrification). Since the NO_3^- pool was quite small, we incubated nitrification cores for longer in order to allow time for dilution to be seen in the labeled pool. The 1- and 2-day incubated cores were also extracted with 0.5 M K_2SO_4 in the field and transported back to the field station on ice.

Table 3.2 Soil and climatic[a] parameters in montane forests along a 1000- to 3000-m elevation[b] gradient, during the dry season (July/August 2011) and wet season (Jan/Feb 2012), on days when N cycling was measured

	1000 m	**2000 m**	**3000 m**
Dry Season[c]			
Air temperature (°C)	22.9^a	14.9^b	6.25^c
Wind speed (m s^{-1})	1.28^b	1.12^b	11.2^a
Relative humidity (%)	62.4^c	76.5^b	96.5^a
Solar radiation (W m^{-2})	761.5^a	293.1^b	226.6^c
WFPS (%)	31.2 ± 1.7^a	31.7 ± 3.0^a	30.0 ± 6.8^a
Wet Season[c]			
Air temperature (°C)	21.3^a	15.1^b	7.10^c
Wind speed (m s^{-1})	0.38^b	0.76^b	5.85^a
Relative humidity (%)	87.4^b	82.8^b	97.9^a
Solar radiation (W m^{-2})	235.2^a	281.4^a	230.8^a
WFPS (%)	42.0 ± 2.0^b	33.5 ± 2.4^b	56.0 ± 6.0^a

[a] Unpublished data used with the permission of T. Peters

[b] Values with different letters indicate significant differences between elevations (linear mixed effects models with Tukey HSD test at $P < 0.05$).

[c] Differences between seasons were not significant, except the difference in solar radiation at 1000 m (Paired T test at $P = 0.09$).

At the field station, a subsample of soil from each core was dried to a constant weight at 105°C, to measure gravimetric water content. In 2012, in order to determine microbial biomass N, a subsample of the soil from the 1- and 2-day incubated cores was fumigated with $CHCl_3$ (Brookes *et al.,* 1985). Fumigation was initiated as soon as the cores returned from the

field and lasted 5 days, after which soil was extracted with 0.5 M K_2SO_4. All extracts were double-filtered using pre-washed filter paper and then frozen before transport to Germany for further analysis.

3.3.4 Laboratory Analyses

In Germany, the concentrations of NH_4^+ and NO_3^- in the extracts were analyzed using continuous flow injection colorimetry (Cenco/Skalar Instruments, Breda, Netherlands). NH_4^+ was determined using the Berthelot reaction method (Skalar Method 155-000) and NO_3^- was determined using the copper-cadmium reduction method (Skalar Method 461-000). Organic N was determined by persulfate digestion of the extract followed by analysis of the NO_3^- concentration as described above.

Extracts were analysed for $^{15}NH_4^+$, $^{15}NO_3^-$ and ^{15}N in organic pools by NH_3 diffusion (which for NO_3^- and organic N digested to NO_3^- include a reduction step to NH_4^+) onto polytetrafluoroethylene-encased acid traps (Stark & Hart, 1996), which were then analysed using isotope ratio mass spectrometry (IRMS) (Delta C, Finnigan MAT, Bremen, Germany). The full procedure used by our working group has been outlined in detail in previous studies (e.g. Corre *et al.,* 2007, 2010). Rates of gross N mineralization and were calculated according to Davidson *et al.* (1991). Note that we define N both net and gross mineralization to be the production of NH_4^+ alone, *not* NH_4^+ and NO_3^- combined. Microbial biomass N was calculated as the difference between extractable N in the original and fumigated soils, divided by k_N = 0.68 (Brookes *et al.,* 1985).

3.3.5 Additional soil analyses

In 2011, soil samples were taken from the upper, mid and lower canopy positions in each plot and used to measure additional soil parameters. Natural abundance (δ) ^{15}N signatures of the soils were measured using IRMS (Delta Plus, Finnigan MAT, Bremen, Germany). Total C and N were measured by dry combustion in a CN analyzer (Elementar Vario EL; Elementar Analysis Systems GmbH, Hanau, Germany). Total elemental concentration of P, Fe, Mg, Mn, Na, S, Fe, K and Al were determined using pressure digestion of the sample in concentrated nitric acid followed by analysis with an inductively coupled plasma-atomic emission spectrometer (Spectroflame, Spectro Analytical Instruments, Kleve, Germany). Soil pH (H_2O) was analyzed in a 1:4 soil-to-water ratio. Soil moisture was expressed as water-filled pore space (WFPS), calculated using an organic soil particle density of 1.40 g cm^{-3} (Breuer *et al.*, 2002).

3.3.6 Statistical analyses and calculations

Results were analyzed using the R (version 2.15.3) open source software. N cycling rates and climate data were all tested for normality and those exhibiting non-normal distributions were square root or log transformed. Positional (i.e. upper vs. lower canopy) differences in the control plots were tested for each elevation using an Independent T test; as differences between positions were only sporadically significant, they are not discussed further except to mention when they interact with seasonal effects. Control plots were tested for seasonal differences using Paired T tests for 1000 m (where only one canopy position was sampled) and linear mixed effect models (LME) for 2000 m and 3000 m with plot (replicate) and canopy position (nested in plot) as random effects and season as the fixed effect; possible

interaction effects between season and canopy position were also tested. Elevation differences between control plots were also tested using LME with season, plot (replicate) and canopy position as random effects and elevation as the fixed effect. If significant seasonal differences in the control plots were detected, separate LME for each season were used. Nutrient-addition effects were also tested using LME and conducted for each elevation. For this, models included season, plot (replicate) and canopy position as random effects and treatment as the fixed effect, and were tested for interactions between treatment and season. Where significant interactions existed, separate LME for each season were used to determine treatment effects. The climate data in Table 3.2 was also tested for differences between seasons and elevations using a LME. We used a Tukey HSD test for multiple comparisons. For correlations between soil properties and N cycling rates, we tested the upper canopy values from control plots across all three elevations using Pearson correlations. We accepted P values of $P < 0.10$ as significant.

In order to understand the relevance of canopy rates in the forest as a whole, we converted our rates to kg ha^{-1} yr^{-1} using data from Werner et al. (2012). They found an average of 3877 kg ha^{-1} canopy soil on trees from mid-slope positions at 2000 m, in the same forest area where we worked. We used this number as the mass of canopy soil in our site at 2000 m and then (based on our field observations) made a rough approximation of the amount of soil at 1000 m and 3000 m, using half that amount for 1000 m and twice that amount for 3000 m. To upscale to a per year rate, we assumed that the wet and dry season each lasted for six months.

3.4 - Results

3.4.1 Control plots: seasonal pattern

Comparing between the two sampling dates, relative humidity and WFPS increased, and wind speed decreased, in the wet season at all elevations (although only WFPS at 1000 m and 3000 m was significant) (Table 3.2). Seasonal differences in N cycling were also seen at each elevation (Table 3.3). At 1000 m, all of the following were significantly higher in the wet season than the dry season: NO_3^- concentrations ($P < 0.01$), gross nitrification ($P = 0.02$), gross consumption of NO_3^- ($P = 0.05$) and the mean residence time (MRT) of NO_3^- ($P = 0.01$). At 2000 m, NO_3^- concentrations were also higher in the wet season ($P = 0.01$), while net nitrification and MRT of NO_3^- had significant interactions with season and canopy position ($P = 0.01$ for net nitrification and $P = 0.02$ for MRT of NO_3^-), with upper canopy values decreasing and lower canopy values increasing in the wet season (data per canopy position are not shown). At 3000 m, NH_4^+ concentrations were higher in the wet season ($P = 0.01$) and gross nitrification showed an interaction with season and canopy position ($P = 0.06$), with rates in the upper canopy decreasing and in the lower canopy increasing from dry season to wet season (data per canopy position are not shown). Note that microbial biomass N (MBN) was only measured in the wet season, so it could not be tested for seasonal differences.

Table 3.3 Nitrogen pools and cycling rates in the canopy soils of the control plots in tropical montane forests along a 1000- to 3000-m elevation gradient. Values shown (mean ± SE; n = 4) were measured in intact cores from the top 5 cm of organic material found on branches in the canopy. Measurements were taken in the dry season (Jul./Aug. 2011) and wet season (Jan./Feb. 2012)

Elevation[a]	Season[b]	Mineral N (mg N kg^{-1})	Mean residence time (d)	Net cycling (mg N $kg^{-1}d^{-1}$)	Gross cycling (mg N $kg^{-1}d^{-1}$)	Consumption (mg N $kg^{-1}d^{-1}$)	DNRA[c] (mg N $kg^{-1}d^{-1}$)	Microbial biomass N (mg N kg^{-1})
		NH_4^+	*NH_4^+*	*Mineralization*	*Mineralization*	*NH_4^+*		
1000 m	Dry	30.7 ± 7.4^{AB}	0.65 ± 0.09	0.80 ± 0.37^{AB}	53.0 ± 21.2^{A}	66.9 ± 27.8^{A}	NA	-
	Wet	27.1 ± 4.6	0.89 ± 0.24	0.59 ± 0.73^{AB}	38.6 ± 13.2^{A}	41.4 ± 12.5^{A}	NA	347 ± 63
2000 m	Dry	31.5 ± 6.4^{A}	1.50 ± 0.71	-0.77 ± 0.75^{B}	41.2 ± 16.3^{AB}	40.7 ± 18.0^{AB}	NA	-
	Wet	24.6 ± 4.1	1.61 ± 0.87	-0.11 ± 0.68^{B}	20.4 ± 7.5^{AB}	25.7 ± 7.1^{AB}	NA	357 ± 67
3000 m	Dry	17.3 ± 3.7^{Bb}	4.31 ± 4.12	0.11 ± 0.63^{A}	20.6 ± 7.3^{B}	23.2 ± 8.0^{B}	NA	-
	Wet	24.8 ± 3.4^{a}	3.26 ± 2.38	2.58 ± 2.10^{A}	24.8 ± 14.1^{B}	28.5 ± 15.0^{B}	NA	444 ± 69
		NO_3^-	*NO_3^-*	*Nitrification*	*Nitrification*	*NO_3^-*		
1000 m	Dry	0.80 ± 0.47^{b}	0.52± 0.37^{b}	2.84 ± 1.60	1.19 ± 0.41^{b}	1.05 ± 0.39^{b}	0.03 ± 0.02	-
	Wet	3.42 ± 1.27^{Aa}	1.19 ± 0.42^{a}	0.29 ± 0.32	2.77 ± 0.32^{a}	4.06 ± 0.79^{a}	0.71 ± 0.62	347 ± 63
2000 m	Dry	0.73 ± 0.11^{b}	0.98 ± 0.17*	0.01 ± 0.02*	0.86 ± 0.26	1.79 ± 0.39	0.08 ± 0.02	-
	Wet	2.38 ± 1.37^{ABa}	0.81 ± 0.48*	0.26 ± 0.26*	2.24 ± 1.05	2.14 ± 1.30	0.36 ± 0.29	357 ± 67
3000 m	Dry	0.62 ± 0.12	0.73 ± 0.24	-0.02 ± 0.03	1.14 ± 0.36*	2.17 ± 0.54	0.14 ± 0.03	-
	Wet	0.85 ± 0.21^{B}	0.70 ± 0.20	-0.03 ± 0.04	1.26 ± 0.43*	2.35 ± 0.66	0.58 ± 0.32	444 ± 69

[a] Values with different uppercase letters indicate significant differences between elevations (linear mixed effects model with Tukey HSD test at $P \leq 0.04$).

[b] Values with different lowercase letters indicate significant differences between seasons for each elevation (Paired T test at $P \leq 0.06$).

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

[*] Significant interaction between season and canopy position (linear mixed effects model at $P \leq 0.05$).

3.4.2 Control plots: elevation differences

In our canopy soils, total N and total P concentrations decreased with elevation, the C/N ratio increased, and other soil nutrients had varying trends (Table 3.1). Similarly, mineral N concentrations and gross rates of N mineralization, nitrification, and NH_4^+ or NO_3^- consumption tended to decrease with increasing elevation, while MRT and MBN tended to increase and net rates of N cycling had no consistent trend (Table 3.3). However, not all of the trends were statistically significant. NH_4^+ concentrations in the dry season were lower at 3000 m than at 2000 m ($P = 0.01$), but there was no difference in NH_4^+ at 1000 m and either 2000 m ($P = 0.80$) or 3000 m ($P = 0.15$). NO_3^- concentrations in the wet season were lower at 3000 m than at 1000 m ($P = 0.01$), but there was no difference in NO_3^- at 2000 m and either 1000 m ($P = 0.34$) or 3000 m ($P = 0.20$).

Comparing across seasons, net N mineralization was higher at 3000 m than at 2000 m ($P = 0.04$), but 1000 m was not different from 2000 m ($P = 0.13$) or 3000 m ($P = 0.98$). Gross N mineralization and consumption of NH_4^+ were lower at 3000 m than at 1000 m ($P = 0.03$ for N mineralization and $P = 0.04$ for NH_4^+ consumption), but 2000 m was not different from 1000 m ($P = 0.35$ for N mineralization and $P = 0.20$ for NH_4^+ consumption) or 3000 m ($P = 0.34$ for a N mineralization and $P = 0.63$ for NH_4^+ consumption).

3.4.3 Control plots: correlations between N cycling and soil properties

In both seasons, gross N mineralization and gross NH_4^+ consumption had a strong negative correlation with the C/N ratio ($P < 0.02$) (Table S3.1), which was also evident within each elevation, suggesting it was not simply a correlation with elevation. In the dry season (Table S3.1a), net nitrification and MRT of NO_3^- were positively correlated with total P ($P <$

0.01 for nitrification and *P = 0.03* for MRT) and net nitrification was negatively correlated with gross NO_3^- consumption (*P = 0.03*). There were no significant correlations between NH_4^+-related rates and NO_3^--related processes. In the wet season (Table S3.1b), gross N mineralization, gross NH_4^+ consumption and net nitrification were all positively correlated with total P (*P = 0.02* for N mineralization, *P = 0.03* for consumption and *P = 0.04* for nitrification). Gross nitrification and gross NO_3^- consumption were negatively correlated with the C/N ratio (*P = 0.01* for both). Relating NH_4^+-related rates and NO_3^--related rates, net N mineralization was positively correlated with MRT of NO_3^- (*P = 0.02*), and gross N mineralization and gross NH_4^+ consumption were positively correlated with gross nitrification and gross NO_3^- consumption (all *P < 0.01*).

3.4.4 Effects of nutrient addition to the forest floor on canopy N cycling

At 1000 m, net nitrification was lower in P plots than in N+P plots (*P = 0.01*), and gross nitrification was lower in N plots than in control (*P = 0.01*) and N+P plots (*P < 0.01*), and marginally lower in P plots than in N+P plots (*P = 0.06;* Table 3.4). Of the measured soil properties, $\delta^{15}N$ at 1000 m was higher in N+P plots as compared to all other treatments (*P < 0.01* for control and P plots, and *P = 0.06* for N plots), and also higher in N plots than P plots (*P = 0.03*). Both total P (*P < 0.01* for control, *P = 0.03* for P plots, *P = 0.01* for N+P plots) and total Mn concentrations (*P < 0.01* for control, *P = 0.03* for P plots, *P = 0.09* for N+P plots) were lower in N plots than in all other treatments.

Table 3.4 Nitrogen (N) pools and cycling rates in the canopy soils of a nutrient manipulation experiment in tropical montane forests along a 1000- to 3000-m elevation gradient. Low levels of N, phosphorus (P) and combined N+P were added to the forest floor biannually starting in 2008. Values shown (mean ± SE; n = 4) were measured in the top 5 cm of organic material found on branches in the canopy, in the dry season (Jul./Aug. 2011) and wet season (Jan./Feb. 2012)

Elevation	Nitrogen measurement	Season	Treatment[a]			
			Control	Added N	Added P	Added N+P
1000 m	Net nitrification (mg N $kg^{-1}d^{-1}$)	Wet/dry	1.56 ± 1.27^{ab}	1.78 ± 1.76^{ab}	0.47 ± 0.48^{b}	2.86 ± 1.04^{a}
	Gross nitrification (mg N $kg^{-1}d^{-1}$)	Wet/dry	1.98 ± 0.54^{ab}	0.56 ± 0.21^{c}	1.32 ± 0.78^{bc}	2.56 ± 0.98^{a}
2000 m	Gross N mineralization (mg N $kg^{-1}d^{-1}$)	Wet/dry	30.8 ± 13.4^{b}	36.9 ± 12.2^{ab}	48.5 ± 15.3^{a}	34.6 ± 9.9^{ab}
	NO_3^- concentration (mg N kg^{-1})	Wet/dry	1.55 ± 1.06^{ab}	1.31 ± 0.37^{ab}	1.40 ± 0.89^{b}	2.80 ± 1.68^{a}
	Net nitrification (mg N $kg^{-1}d^{-1}$)	Wet/dry	0.11 ± 0.17^{ab}	0.16 ± 0.23^{ab}	0.03 ± 0.05^{b}	0.34 ± 0.26^{a}
	Gross nitrification (mg N $kg^{-1}d^{-1}$)	Dry	0.86 ± 0.26^{b}	0.62 ± 0.16^{b}	0.76 ± 0.19^{b}	1.61 ± 0.31^{a}
3000 m	Gross N mineralization (mg N $kg^{-1}d^{-1}$)	Wet/dry	22.7 ± 10.9^{b}	36.1 ± 13.0^{ab}	25.9 ± 11.2^{ab}	39.2 ± 11.6^{a}
	Gross NH_4^+ consumption (mg N $kg^{-1}d^{-1}$)	Wet/dry	25.9 ± 11.7^{b}	38.8 ± 14.5^{ab}	33.9 ± 14.8^{ab}	46.3 ± 14.3^{a}

[a] For each elevation, values with different lowercase letters indicate significant differences between treatments (linear mixed effects models with Tukey HSD test at $P < 0.09$).

At 2000 m, gross N mineralization was higher in P plots as compared to control (*P = 0.05*), and NO_3^- concentration and net nitrification were higher in N+P plots than in P plots (*P = 0.08* for NO_3^- and *P = 0.00* for nitrification). Gross nitrification displayed a significant interaction between season and treatment; in the dry season, gross nitrification was higher in N +P plots than in all other treatments (*P = 0.02* for control and *P < 0.01* for both N plots and P plots). Of the measured soil properties, $\delta^{15}N$ at 2000 m was higher in N+P plots as compared to P plots (*P = 0.01*). In the mid-canopy, total K was lower in N plots than in all other treatments (all *P < 0.01*).

At 3000 m, gross N mineralization and gross NH_4^+ consumption were higher in N+P plots as compared to control (*P < 0.01*). There were no treatment effects on canopy soil properties at 3000 m.

3.5 - Discussion

3.5.1 Canopy vs. forest floor

Gross N mineralization rates in our canopy soils were larger on a mass basis than those measured on the forest floor, in a parallel study conducted by another member of our working group (Table S3.2; Baldos *et al.*, unpublished data), whereas gross nitrification rates were comparable or slightly lower in the canopy soils. However, N cycling rates in the canopy soils were considerably lower on an area basis, since canopy soil mass is considerably lower than that of the forest floor. In the canopy, gross N mineralization ranged from 32 to 64 kg N ha^{-1} yr^{-1} across the elevations gradient and gross nitrification ranged from 1.4 to 3.4 kg N ha^{-1} yr^{-1}. In comparison, the rates in the top 5-cm depth of forest floor soils ranged from 219 to 858 kg N ha^{-1} yr^{-1} for gross N mineralization and 91 to 321 kg N ha^{-1} yr^{-1} for gross nitrification. The contribution of canopy soil at 3000 m was larger (23% of the sum of gross N mineralization

from forest floor and canopy soils), where N cycling on the forest floor was low, than at the lower elevations (4-6 %) where N cycling on the forest floor was high. In a similar trend across the elevation gradient, gross nitrification in the canopy soil contributed 4% to the total gross nitrification (forest floor and canopy soils) at 3000 m and only 0.4% at 1000 m.

The only other measurements of gross N cycling in canopy soils that we are aware of, come from canopy soils in a lowland tropical forest in Costa Rica (Wanek *et al.,* 2002). They measured mass-based gross rates of N mineralization and nitrification similar to those found in our study (27.5 mg N kg^{-1} d^{-1} for gross N mineralization and 2.1 mg N kg^{-1} d^{-1} for gross nitrification), and found no significant difference in N cycling between canopy and forest floor soils. Similarity in NH_4^+ concentrations and MBN between canopy and forest floor soils was also reported for a lower montane forest in Costa Rica by Vance and Nadkarni (1990) and in wet-season net N cycling for lowland canopy soils in Costa Rica by Cardelus et al. (2009).

Since the available studies did not detect differences in N cycling rates between canopy and forest floor soils, and in the absence of other studies on gross N cycling in canopy soils, we compare our results to tropical forest floor soils. Published rates of gross N cycling vary considerably, but our values were well within the range of other tropical montane forest studies that used field incubation of intact cores. When expressed on an area-based scale, the significance of N cycling rates in canopy soils of most forests will likely be small (Table S3.2). However, when compared on a mass-based scale, gross N cycling rates in a lower montane forest in Puerto Rico (Silver *et al*., 2001; Templer *et al*., 2008) were lower than those which we measured in canopy soils, whereas gross N mineralization rates were similar with those from a lower montane forest soil in Ecuador (Arnold *et al*., 2009) and a lower montane forest soil in Panama (Corre *et al*., 2010). Similar to what we observed in our study area, our

gross nitrification rates were lower than these published values, possibly because canopy soils tend to be more acidic than forest floor soils and therefore have less potential for nitrification (Vance & Nadkarni, 1990).

3.5.2 Environmental effects (seasonality, elevation and soil properties)

Seasonality appeared to have a stronger effect on NO_3^--related processes than on NH_4^+-related processes (Table 3.3). A simple reason for this could be that the NO_3^- pool is much smaller than the NH_4^+ pool and therefore even small fluctuations are likely to be significant. However, there are also mechanistic explanations for this observance. Where seasonal differences were significant, we saw increased pool sizes/rates in the wet season as compared to the dry season. Although nitrification is an aerobic process that is normally expected to decrease in soils during the wet season (Breuer *et al.,* 2002), nitrification may increase in organic soils such as ours (also seen by Ingwersen *et al.,* (1999) in a forest floor organic layer) since high porosity allows them to remain aerobic as moisture increases. Gross N mineralization did not increase during the wet season; however, gross N mineralization rates were at least a factor of 10 higher than nitrification rates throughout the study at all elevations and the higher nitrification rates may be related to lower NH_4^+ immobilization during the wet season. Alternately, the changes may not have been related to increased N cycling rates due to higher moisture, but instead due to increased inputs of mineral N through precipitation during the wet season. A study in our 2000 m forest site consistently observed net loss of dissolved organic N from the canopy during rainfall events, but during some rainfall events, net retention of NH_4^+ and NO_3^- was detected (Zimmerman *et al.,* 2007). They also measured higher concentrations of NO_3^- as compared to NH_4^+ in rainfall. Mineral N addition to our soils

with concurrent decreases in organic N could help to explain why NO_3^--related processes increased while NH_4^+-related processes remained relatively unchanged. A contribution of external nutrient sources to N cycling in our soils is supported by the correlations that we observed. Whereas only gross N mineralization and gross NH_4^+ consumption were correlated in the dry season (Table S3.1a), gross N mineralization, gross NH_4^+ consumption, gross nitrification and gross NO_3^- consumption were all correlated with one another in the wet season (Table S3.1b), perhaps because increased nutrient availability through precipitation removed local limitations on N cycling in some of the soils. Cardelus (2009) saw a similar effect in the wet season; discriminant function analyses of canopy soil properties showed that tree species were more similar to each other in the wet season whereas they were all significantly different in the dry season. Dry-season differences may then be a result of microclimate and/or host tree chemistry, which become more important when there are fewer nutrient inputs from external sources.

Along our elevation gradient, we expected N cycling in the canopy soil to decrease with elevation, as temperature decreased (inhibiting microbial activity), wind speeds increased (removing litter and/or speeding up evaporation) and C/N ratios increased (indicating decreasing organic matter quality; Booth *et al.*, 2005). Although not always significant, we did often observe this pattern in N cycling rates (Table 3.3). The importance of organic matter quality, in particular, was highlighted by the negative correlations that we saw with N cycling rates and C/N ratios in both seasons (Table S3.1). However, N cycling rates measured at 2000 m occasionally did not fall between the other two elevations - as was the case with net N mineralization (Table 3.3). In addition, the $\delta^{15}N$ in the canopy soil (Table 3.1) shows that although there were definitely higher rates of N cycling at 1000 m as compared to the other

elevations, there seemed to be no difference in $\delta^{15}N$ between 2000 m and 3000 m. This is likely related to specific differences in our site characteristics; our 2000 m elevation actually received less precipitation than the 1000 m or 3000 m sites, and in our study, the WFPS of the canopy soil at 2000 m was not different between the two seasons (Table 3.2). Additionally, several characteristics in the canopy soil at 2000 m (namely Ca, Fe, Mg and Mn; Table 3.1) were either higher or lower than both of the other elevations, rather than exhibiting intermediate values. Such specific site differences are not uncommon in elevation gradient studies, and simply need to be taken into account when making sweeping statements about elevation effects (Körner 2007).

3.5.3 Response to four years of indirect fertilization

The response of canopy soils to indirect fertilization showed a clear tendency for N+P plots at all three elevations to have significantly higher N concentrations and cycling rates than the other treatments (Table 3.4). Increases in N cycling rates in response to N and N+P additions were also seen in the forest floor soil of our site, and were attributed to increased substrate quality and quantity (Baldos *et al.*, unpublished data). We are likely seeing a similar effect in the canopy, as there was no evidence of treatment effects on MBN, but we did observe significant negative correlations with soil C:N ratio and net nitrification, gross N mineralization, gross nitrification and gross consumption of NH_4^+ and NO_3^- (Table S3.1).

Homeier et al. (2012) proposed that the combination of N and P was important for N cycling in our forest floor soils because P limits the involved microorganisms, whereas N is limiting as a substrate. Our results indicate that canopy soils were also limited by both elements as shown by significant increases in net nitrification and gross nitrification (at1000

m and 2000 m) and in gross N mineralization and gross NH_4^+ consumption (at 3000 m) with N+P addition (Table 3.4). Furthermore, at both 1000 m and 2000 m, the significantly higher $\delta^{15}N$ in N+P plots as compared to control (1000 m) or P plots (2000 m) emphasizes that the *combination* of N and P increased N cycling rates at these elevations. This was also shown by the fact that net nitrification was lowest in the P plots at both 1000 m and 2000 m, suggesting with additional available P, more N was immobilized. At 2000 m, the significant increase in gross N mineralization in P plots (Table 3.4) could indicate that the N required as a substrate was relatively sufficient or that additional P increased another source of N. For example, as was also reported by Benner et al. (2007) for Hawaiian montane forests, P fertilization to the forest floor at 2000 m had increased N_2 fixation in the canopy soils (Matson *et al.,* unpublished data). Finally, it is notable that N+P addition at 1000 m and 2000 m increased mineral N production rates but not mineral N consumption rates. This could be an early indication that N cycling in these canopy soils may become decoupled (immobilization not keeping pace with production). Strong decoupling of the N cycle was observed by Baldos *et al.* (unpublished data) in our forest floor soils in both the N and N+P treatments.

In contrast to the increases observed in N+P plots, the decrease in gross nitrification in N plots (observed at both 1000 m and 2000 m, although only significant at 1000 m) may be a response to increased acidity. The significant decrease in total Mn concentrations in the canopy soil of N plots at 1000 m and the decrease of total K concentrations in the canopy soil at mid-canopy position of N plots at 2000 m could be a result of NO_3^- leaching. Gaige et al. (2007) observed that the relative amount of throughfall NO_3^- (as compared to dissolved organic N and NH_4^+) increased in N plots compared to control plots. NO_3^- leaching can, in turn, lead to cation leaching and soil acidity (Matson *et al.,* 1999) and acidity may inhibit

nitrification in the soil (Vance & Nadkarni, 1990). We also observed lower total P concentrations at 1000 in the N plots as compared to the control, which would be consistent with higher P mobilization through N mineralization, followed by leaching; although we do not have data for the 1000 m site, an increase in throughfall P concentration in N-amended plots compared to control was observed in our 2000 m site (Homeier *et al.*, 2012).

It is important to stress that all observed fertilization effects were a result of *indirect* fertilization. This means that we may not have seen treatment effects in some of the treatment plots because the canopy soils in that treatment were simply not *yet* enriched (as seen by Benner and Vitousek (2007) with N addition), considering that our study was conducted in only the fourth year since this nutrient manipulation experiment started. From other studies done in our site at 2000 m, we know that several indices of relevance to canopy nutrient enrichment increased as a result of forest floor N and/or P addition: N or P return with throughfall, N or P concentration in litterfall, N or P return with litter, total leaf litter production, foliar N or P concentration and litter decomposition (Homeier *et al.*, 2012). However, we also know that in plots with just addition of N, litter may be less quickly decomposed in the canopy, as P has been shown to limit decomposition in some canopies (Cardelus *et al.*, 2010). In addition, Nadkarni and Matelson (1991) showed that due to the prevalence of wind, very little tree litter actually remains and decomposes in montane forest canopies, and of the litter that remained, decomposition was significantly slower than on the forest floor. Therefore, although we cannot exclude that some nutrient-rich litter may be contributing to canopy soil, it is more likely that canopy soils were largely dependent on throughfall and stemflow for nutrient enrichment (Wullaert *et al.*, 2010).

3.5.4 Implications

This study had three key implications for N cycling in canopy soils. First, N cycling in canopy soil can be an important component of total forest N cycling. This is most likely to occur in humid, high altitude tropical montane forests, where N cycling rates in forest floor soil are relatively low and the amount of canopy soil is relatively high. Second, in contrast to theories that canopy soil is decoupled from soil on the forest floor, the canopy in our forests was remarkably sensitive to changes in nutrient availability in the forest floor soil. Our canopy soils responded very rapidly (within 4 years from the onset of nutrient addition) to relatively low amounts of added N and P. However, N cycling processes in our canopy soils were clearly limited by both N and P, so a final key implication of this study is that the canopy soil response to nutrient deposition will depend on local nutrient limitations. In canopy soils like ours, chronic N and P deposition may stimulate mineral N production and consumption processes, but over time a decoupling of the N cycle could occur, with the decreased immobilization of mineral N providing more N for plant uptake and/or causing increased leaching of N to the forest floor. In canopy soils where N cycling processes are limited by both N and P, the response of N cycling to a single limiting nutrient is more complex. Based on our observations, an increase in P without a concurrent increase in N may cause increased N cycling through a more efficient use of extant N stores and/or increased rates of N_2 fixation in the canopy soil. However, an increase in N without a concurrent increase in P may lead to nutrient mining of the canopy soil, with stores of P used up first, followed by a loss of soil cations through increased NO_3^- leaching.

3.6 - References

Arnold J, Corre MC, Veldkamp E (2009) Soil N cycling in old-growth forests across an Andosol toposequence in Ecuador. *Forest Ecology and Management,* **257**, 2079–2087.

Benner JW, Conroy S, Lunch CK, Toyoda N, Vitousek PM (2007) Phosphorus fertilization increases the abundance and nitrogenase activity of the cyanolichen *Pseudocyphellaria crocata* in Hawaiian montane forests. *Biotropica,* **39**, 400–405.

Benner JW, Vitousek PM (2007) Development of a diverse epiphyte community in response to phosphorus fertilization. *Ecology Letters,* **10**, 628–636.

Booth MS, Stark JM, Rastetter E (2005) Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. *Ecological Monographs,* **75**, 139–157.

Breuer L, Kiese R, Butterbach-Bahl K (2002) Temperature and moisture effects on nitrification rates in tropical rain-forest soils. *Soil Science Society of America Journal*, **66**, 834–844.

Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology and Biochemistry*, **17**, 837–842.

Cardelús CL, Mack MC, Woods CL, DeMarco J. Treseder, K (2009) Nutrient cycling in canopy and forest floor soils in a lowland tropical wet forest, Costa Rica. *Plant and Soil*, **318**, 47–61.

Cardelús CL (2010) Litter decomposition within the canopy and forest floor of three tree species in a tropical lowland rain forest, Costa Rica. *Biotropica*, **42**, 300–308.

Chen L, Liu WY, Wang GS (2010) Estimation of epiphytic biomass and nutrient pools in the subtropical montane cloud forest in the Ailao Mountains, southwestern China. *Ecological Research*, **25**, 315–325.

Clark KL, Nadkarni NM, Gholz HL (1998) Growth, net production, litter decomposition, and net nitrogen accumulation by epiphytic bryophytes in a tropical montane forest. *Biotropica,* **30**, 12–23.

Clark KL, Nadkarni NM, Gholz HL (2005) Retention of inorganic nitrogen by epiphytic bryophytes in a tropical montane forest. *Biotropica,* **37**, 328–336.

Corre MD, Brumme R, Veldkamp E, Beese FO (2007) Changes in nitrogen cycling and retention processes in soils under spruce forests along a nitrogen enrichment gradient in Germany. *Global Change Biology*, **13**, 1509–1527.

Corre MD, Veldkamp E, Arnold J, Wright SJ (2010) Impact of elevated N input on soil N cycling and losses in old-growth lowland and montane forests in Panama. *Ecology*, **91**, 1715–1729.

Davidson EA, Hart SC, Shanks CA, Firestone MK (1991) Measuring gross nitrogen mineralization, immobilization, and nitrification by ^{15}N isotopic pool dilution in intact soil cores. *Journal of Soil Science*, **42**, 335–349.

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters*, **10**:1135–1142.

Enloe HA, Graham RC, Sillett SC (2006) Arboreal Histosols in old growth redwood forest canopies, northern California. *Soil Science Society of America Journal*, **70**, 408–418.

Gaige E, Dail DB, Hollinger DY, Davidson EA, Fernandez IJ, Sievering H, White A, Halteman W (2007) Changes in canopy processes following whole-forest canopy nitrogen fertilization of a mature spruce-hemlock forest. *Ecosystems*, **10**, 1133–47.

Galloway JN, Aber JD, Erisman JW, Seitzinger SP, Howarth RW, Cowling EB, Cosby BJ (2003) The Nitrogen Cascade *BioScience*, **53**, 341–356.

Galloway JN, Dentener FJ, Capone DG *et al.* (2004) Nitrogen cycles: past, present, and future. *Biogeochemistry*, **70**, 153–226.

Hall SJ, Matson PA (2003) Nutrient status of tropical rain forests influences soil N dynamics after N additions. *Ecological Monographs*, **73**, 107–129.

Hart SC, Stark JM, Davidson EA, Firestone MK (1994) Nitrogen mineralization, immobilization, and nitrification. In: Weaver R (ed.), Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties. SSSA Book Series No. 5, American Society of Agronomy, Madison, WI, pp. 985–1019.

Hedin LO, Brookshire ENJ, Menge DNL, Barron AR (2009) The nitrogen paradox in tropical forest ecosystems. *Annual Review of Ecology, Evolution and Systematics,* **40**, 613-635.

Hietz P, Wanek W, Wania R, Nadkarni NM (2002) Nitrogen-15 natural abundance in a montane cloud forest canopy as an indicator of nitrogen cycling and epiphyte nutrition. *Oecologia*, **131, 350**–355.

Hietz P, Turner BL, Wanek W, Richter A, Nock CA, Wright SJ (2011) Long-term change in the nitrogen cycle of tropical forests. *Science,* **334,** 664–666.

Homeier J, Breckle SW, Gunter S, Rollenbeck RT, Leuschner C (2010) Tree diversity, forest structure and productivity along altitudinal and topographical gradients in a species-rich Ecuadorian montane rain forest. *Biotropica*, **42**, 140–148.

Homeier J, Hertel D, Camenzind T *et al.* (2012) Tropical Andean forests are highly susceptible to nutrient inputs—rapid effects of experimental N and P addition to an Ecuadorian montane forest. *PLoSONE* **7**, doi:10.1371/journal.pone.0047128.

IUSS Working Group WRB. 2006. World Reference Base for Soil Resources 2006. World Soil Resources Reports No. 103. FAO, Rome.

Jeník J (1973) Root systems of tropical trees. 8:Stilt-roots and allied adaptations. *Preslia*, **45**, 250–264.

Koerner C (2007) The use of 'altitude' in ecological research. *Trends in Ecology and Evolution,* **22**, 569–574.

Mahowald, NM, Artaxo P, Baker AR, Jickells TD, Okin GS, Randerson JT, Townsend AR (2005) Impacts of biomass burning emissions and land use change on Amazonian atmospheric phosphorus cycling and deposition. *Global Biogeochemical Cycles*, **19**, GB4030.

Mahowald N, Jickells TD, Baker AR *et al.* (2008) Global distribution of atmospheric phosphorus sources, concentrations and deposition rates, and anthropogenic impacts. *Global Biogeochemical Cycles*, **22**, GB4026.

Martinson G, Corre MD, Veldkamp E (2013) Responses of nitrous oxide fluxes and soil nitrogen cycling to nutrient additions in montane forests along an elevation gradient in southern Ecuador. *Biogeochemistry*, **112**, 625–636.

Matson PA, McDowell WH, Townsend AR, Vitousek PM (1999) The globalization of N deposition: ecosystem consequences in tropical environments. *Biogeochemistry*, **46**, 67–83.

Moser G, Hertel D, Leuschner C (2007) Altitudinal change in LAI and stand leaf biomass in tropical montane forests: a transect study in Ecuador and a pan-tropical meta-analysis. *Ecosystems*, **10**, 924–935.

Nadkarni, NM (1984) Epiphytic biomass of a neotropical elfin forest. *Biotropica* **16**, 249-256

Nadkarni NM, Matelson TJ (1991) Fine litter dynamics within the tree canopy of a tropical cloud forest. *Ecology*, **72**, 2071–2082.

Nadkarni NM, Schaefer DA, Matelson TJ, Solano R (2002) Comparison of arboreal and terrestrial soil characteristics in a lower montane forest, Monteverde, Costa Rica**.** *Pedobiologia***, 46**, 24**–**33.

Nadkarni NM, Schaefer DA, Matelson TJ, Solano R (2004) Biomass and nutrient pools of canopy and terrestrial components in a primary and a secondary montane cloud forest, Costa Rica**.** *Forest Ecology and Management*, **198**, 223**–**236.

Perez CA, Guevara R, Carmona MR, Armesto JJ (2005) Nitrogen mineralization in epiphytic soils of an old-growth *Fitzroya cupressoides* forest, southern Chile. *Ecoscience*, **12,** 210-215.

Phoenix GK, Hicks WK, Cinderby S *et al.* (2006) Atmospheric nitrogen deposition in world biodiversity hotspots: the need for a greater global perspective in assessing N deposition impacts. *Global Change Biology*, **12**, 470–476.

Regina K, Silvola J, Martikainen PJ (1999) Short-term effects of changing water table on N_2O fluxes from peat monoliths from natural and drained boreal peatlands. *Global Change Biology*, **5**, 183–189.

Silver WL, Herman DJ, Firestone MK (2001) Dissimilatory nitrate reduction to ammonium in upland tropical forest soils. *Ecology*, **82**, 2410–2416.

Stark JM, Hart SC (1996) Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitrogen-15 analysis. *Soil Science Society of America Journal*, **60**, 1846–1855.

Templer PH, Silver WL, Pett-Ridge J. DeAngelisand KM, Firestone MK (2008) Plant and microbial controls on nitrogen retention and loss in a humid tropical forest. *Ecology,* **89**, 3030–3040.

Vance E, Nadkarni NM **(1990**) Microbial biomass and activity in canopy organic matter and the forest floor of a tropical cloud forest. Soil Biology and Biochemistry, **22**, 677–684.

Venterink HO, Davidsson TE, Kiehl K, Leonardson L. (2002) The impact of drying and re-wetting on N, P and K dynamics in a wetland soil. *Plant and Soil*, **243,** 119**–**130.

Vitousek PM, Porder S, Houlton BZ, Chadwick OA (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen – phosphorus interactions. Ecological Applications, **20**, 5–15.

Wanek W, Arndt SK, Huber W, Popp M (2002) Nitrogen nutrition during ontogeny of hemiepiphytic *Clusia* species. *Functional Plant Biology*, **29**, 733–740.

Werner FA, Homeier J, Oesker M, Boy J (2012) Epiphytic biomass of a tropical Andean forest varies with topography. *Journal of Tropical Ecology*, **28**, 23–31.

Wullaert H, Homeier J, Valarezo C, Wilcke W (2010) Response of the N and P cycles of an old-growth montane forest in Ecuador to experimental low-level N and P amendments. *Forest Ecology and Management*, **260**, 1434–1445.

Yu S, Ehrenfeld, JG (2009) The effects of changes in soil moisture on nitrogen cycling in acid wetland types of the New Jersey Pinelands (USA). *Soil Biology and Biochemistry*, **41**, 2394–2405.

Zimmermann A, Wilcke W, Elsenbeer H (2007) Spatial and temporal patterns of throughfall quantity and quality in a tropical montane forest in Ecuador. *Journal of Hydrology*, **343**, 80–96.

Table S3.1 Pearson correlation coefficients between N cycling rates and nutrient concentrations measured in the dry season (a) and wet season (b), in upper canopy soils of control plots in tropical montane forests along a 1000- to 3000-m elevation gradient (n = 12)

(a)	C:N	Net N mineralization	Net nitrification	Gross N mineralization	Gross NH_4^+ consumption	Gross nitrification	Gross NO_3^- consumption	MRT NH_4^+	MRT NO_3^-
N	**-0.99****	0.17	**0.89****	**0.73****	**0.77****	0.24	-0.49	**-0.62***	0.41
C:N		0.13	**-0.86****	**-0.77****	**-0.80****	-0.15	0.49	**0.66***	-0.41
Net N mineralization			0.27	-0.01	0.00	0.32	-0.31	-0.08	-0.23
Net nitrification				0.54	0.56	0.33	**-0.63***	-0.44	0.52
Gross N mineralization					**0.99****	0.39	0.05	**-0.91****	-0.10
Gross NH_4^+ consumption						0.39	0.01	**-0.87****	-0.04
Gross nitrification							0.22	-0.32	-0.33
Gross NO_3^- consumption								-0.01	**-0.72****
MRT NH_4^+									0.27

(b)	C:N	Net N mineralization	Net nitrification	Gross N mineralization	Gross NH_4^+ consumption	Gross nitrification	Gross NO_3^- consumption	MRT NH_4^+	MRT NO_3^-
N	**-0.99****	-0.11	0.48	0.56	**0.60***	**0.62***	**0.68***	-0.10	0.00
C:N		0.17	-0.53	**-0.66***	**-0.69***	**-0.70***	**-0.74****	0.22	0.10
Net N mineralization			-0.02	-0.27	-0.19	-0.08	0.09	0.38	**0.75***
Net nitrification				0.48	0.52	0.20	0.18	-0.17	-0.22
Gross N mineralization					**0.98****	**0.79****	**0.78****	**-0.92****	-0.32
Gross NH_4^+ consumption						**0.80****	**0.82****	**-0.82****	-0.35
Gross nitrification							**0.94****	-0.61	-0.31
Gross NO_3^- consumption								-0.57	0.00
MRT NH_4^+									0.12

$^{*, **}$ Significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table S3.2 Nitrogen (N) cycling rates in canopy and forest floor[a] soils of tropical montane forests along a 1000- to 3000-m elevation gradient. Values shown (mean ± SE; *n* = 4) were measured in the top 5 cm of organic material found on branches in the canopy or from the top 5 cm of forest floor soil (corresponding to a mineral soil at 1000 m and an organic soil at 2000 m and 3000 m)

Elevation	Nitrogen cycling measure	Mass-based rates (mg N kg^{-1} d^{-1})		Area-based rates (kg N ha^{-1} yr^{-1})		
		Canopy	*Forest floor*	*Canopy*[b]	*Forest floor*	*Canopy contribution*
1000 m	Gross N mineralization	45.8 ± 16.8	5.60 ± 0.71	32.4 ± 11.9	858 ± 110	3.6%
	Gross nitrification	1.98 ± 0.54	2.10 ± 0.29	1.40 ± 0.38	321 ± 44	0.4%
2000 m	Gross N mineralization	30.8 ± 13.4	21.2 ± 5.9	43.6 ± 18.9	697 ± 193	5.9%
	Gross nitrification	1.55 ± 0.82	3.89 ± 0.22	2.20 ± 1.17	128 ± 7	1.7%
3000 m	Gross N mineralization	22.7 ± 10.9	10.9 ± 1.8	64.2 ± 30.8	219 ± 37	22.7%
	Gross nitrification	1.20 ± 0.39	4.55 ± 1.09	3.40 ± 1.10	91.3 ± 22	3.6%

[a] Forest floor data adapted from Baldos et al. (unpublished data) .

[b] For details on how area-based rates were calculated for canopy soils, see *Materials and methods: statistical analyses and calculations.*

Chapter 4

CANOPY SOILS ARE NOT SIGNIFICANT SOURCES OR SINKS OF CARBON DIOXIDE, METHANE OR NITROUS OXIDE IN TROPICAL MONTANE FORESTS

4.1 - Abstract

Canopy soils can contribute significantly to aboveground labile biomass, especially in tropical montane forests. Whether this means that they also contribute to the exchange of greenhouse gases is unknown. We quantified fluxes of CO_2, CH_4 and N_2O in canopy soils along a 1000- to 3000-m elevation gradient of tropical montane forests and assessed the indirect effects of nutrient addition to the forest floor on canopy soil gas exchange. Gas fluxes were measured using both static chambers with permanent bases, and intact soil cores sealed in jars. The forest floor had been fertilized biannually with moderate amounts of N and P for 4 years; treatments included control, N, P and N+P. Canopy soil CO_2 emissions based on chamber area (10.5 to 109.5 mg CO_2-C m^{-2} h^{-1}) were similar to those from the forest floor, but emissions based on forest area made up only 5-11% of total (canopy + forest floor) soil CO_2 emissions. Canopy soil CH_4 fluxes (-0.07 to 0.02 kg CH_4-C ha^{-1} yr^{-1}) and N_2O fluxes (0.00 to 0.01 kg N_2O-N ha^{-1} yr^{-1}) made up less than 5% of total soil fluxes. At all elevations, canopy soils in P plots were a slightly stronger CH_4 sink than in other treatments. At 2000 m only, canopy soils in N plots became a slight N_2O source, whereas P addition decreased CO_2 emissions by approximately 50%. Results suggest that GHG-related processes in canopy soils will respond to long-term atmospheric N and/or P deposition. However, fluxes in canopy soils are unlikely to significantly contribute to total forest greenhouse gas budgets.

4.2 - Introduction

Canopy soils are the collection of non-living organic material commonly found on the branches of trees from humid forests (Coxson and Nadkarni 1995); they are primarily made up of decomposed epiphytic material from epiphytes but also include intercepted litter, dust, invertebrates, fungi and microorganisms (Freiberg and Freiberg 2000; Hietz et al. 2002; Nadkarni et al. 2002). The relevance of canopy soil nutrient cycling to the forest as a whole depends, in part, on the amount of canopy soil present in a given stand. This can range from 1000 kg ha^{-1} to 33,000 kg ha^{-1} (Chen et al. 2010; Freiberg and Freiberg 2000; Nadkarni et al. 2004; Vance and Nadkarni, 1990; Werner et al. 2012). In some forests, canopy soil can account for up to 80% of aboveground labile (non-woody) biomass (Nadkarni et al. 2004). Therefore, despite their relatively low biomass, canopy soils can be an important part of the overall nutrient cycle.

Canopy soils also have the potential to be sources or sinks of the greenhouse gases (GHGs) carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O); as compared to the forest floor, canopy soils can have similar or higher microbial biomass carbon (C) and nitrogen (N) (Cardelus et al. 2009; Vance and Nadkarni 1990), as well as slightly higher microbial respiration (Vance and Nadkarni 1990). However, canopies are unique environments that are home to many 'canopy specialists' not found on the forest floor (Beaulieu et al. 2010; Nadkarni et al. 1994, 2002; Pittl et al. 2010), so it cannot be assumed that canopy soil activity will always mimic that of forest floor soils. Regarding CO_2 emissions, it is known that canopy soils emit CO_2 through soil respiratory activity (Vance and Nadkarni 1990; Wardle et al. 2003), but data is still lacking on estimates of CO_2 emissions from canopy soils on a forest-area basis. As for CH_4, although significant emissions have been

measured from canopy bromeliads (Martinson et al. 2010), substantial emissions from canopy soils are unlikely. Methanogenesis is a strictly anaerobic process (Le Mer and Roger 2001); since the structure of trees is not conducive to standing water and canopy soils are highly porous, they are unlikely to ever be completely anaerobic. Canopy soils are, however, a potential CH_4 sink. Although studies from boreal and temperate forest soils report that organic layers have little ability to oxidize CH_4 (Butterbach-Bahl et al. 2002; Maurer et al. 2008; Saari et al. 1998; Steinkamp et al. 2001) studies in both temperate and tropical montane forests have found that organic layers can act as a strong CH_4 sink (Chan et al. 2005; Wolf et al. 2012). Finally, concerning N_2O, the contribution of canopy soils is uncertain. Although canopies are generally understood to be N limited (Hedin et al. 2009), making them an unlikely source of N_2O, they otherwise have many of the right environmental conditions. Denitrifiers are heterotrophic (Knowles 1982), suggesting that they could thrive in canopy soils, which may have less recalcitrant C than on the forest floor (Vance and Nadkarni 1990). In addition, canopy soils are acidic (Cardelus et al. 2009), which inhibits the final reaction of N_2O to N_2 (Knowles 1982), and although they are unlikely to ever be completely anaerobic, canopy soil moisture could reach 70-80% WFPS, which is the accepted threshold for N_2O production (Davidson et al. 2000).

Not only is it important to quantify current fluxes, however, it is also necessary to understand how GHG fluxes might change under future global change scenarios. Due to anthropogenic disturbances such as forest clearing, industrialization and biomass burning, tropical regions are experiencing increasing amounts of atmospheric N and phosphorus (P) deposition (Galloway et al. 2004; Hietz et al. 2011; Mahowald et al. 2005, 2008). Studies have shown that GHG fluxes in tropical montane forest soils can exhibit contrasting responses to

these nutrients. For example, a recent study from Ecuador initially showed increased N_2O emissions in response to low levels of N addition (Martinson et al. 2013), but the effect of N addition on N_2O disappeared as the study continued (Mueller et al. unpublished data). In the same study, N and P additions both increased and decreased CO_2 emissions, depending on elevation and the duration since onset of nutrient addition, and increased CH_4 uptake, but with shifting limiting nutrients along the elevation gradient (Mueller et al. unpublished data). Other studies have also shown mixed responses of CO_2, CH_4 and N_2O to nutrient addition, depending on the forest type, elevation and type/amount/duration of nutrient addition (Cleveland and Townsend 2006; Corre et al. 2010, 2014; Cusack et al. 2011; Hall and Matson 2003; Koehler et al. 2009; Veldkamp et al. 2013; Zhang et al. 2011).

In this study, we measured greenhouse gas (CO_2, CH_4 and N_2O) fluxes in canopy soils along an elevation gradient of tropical montane forests. Canopy soils were located in trees of plots with and without N and P addition *to the forest floor*. Our objectives were to: (1) quantify the magnitude of GHG fluxes in canopy soils and asses their contribution to total soil (forest floor and canopy) fluxes and (2) assess whether these rates were affected by indirect fertilization through nutrient inputs to the forest floor. We hypothesized that canopy soil fluxes of CH_4 and N_2O would be low, but that CO_2 emissions would be similar to those measured on the forest floor. Since canopies are assumed to be N limited, we did not expect to see treatment effects on N_2O emissions, but postulated that both N and P could stimulate CH_4 uptake and improve litter quality, increasing CO_2 emissions.

4.3 - Materials and Methods

4.3.1 Study sites

This study took place along an elevation gradient from 1000- to 3000-m asl, in and adjoining Podocarpus National Park, a tropical montane forest in the Andes of southern Ecuador. The sites have been thoroughly described by Richter et al. (2013), but basic climate details, soil parameters and vegetative cover are summarized in Table 4.1.

Table 4.1 Site and canopy soil characteristics from three study sites located along an elevation gradient in a tropical montane forest of southern Ecuador. Soil characteristics (mean (SE); n=4) are measured from the top 5 cm of soil in the upper canopy.

	1000 m	**2000 m**	**3000 m**
Annual temperature (°C)[1]	19.4	15.7	9.4
Annual rainfall (mm)[1]	2230	1950	4500
Vegetation[2]	Premontane	Lower montane	Upper montane
Stand height (m)[3]	20-25	10-14	6-8
Tree density (trees ha^{-1})[3]	747.5	1142.5	1305.0
Total carbon (%)	48.9 (0.9)	48.0 (0.6)	48.9 (1.5)
Total nitrogen (%)	2.4 (0.3)	1.7 (0.1)	1.5 (0.2)
C/N ratio	20.8 (2.2)	28.7 (1.6)	34.4 (2.3)
$\delta^{15}N$	1.2 (0.4)	-0.03 (0.7)	0.1 (0.5)
pH (1:4 soil-to-H_2O)	4.2 (0.2)	3.7 (0.3)	3.8 (0.2)

[1]Moser *et al.,* (2007)

[2]Homeier *et al.,* (2010)

[3]Martinson *et al.,* (2013)

4.3.2 Nutrient addition

We focused on the effects of indirect fertilization by studying canopy soil from trees in a pre-existing fertilization experiment. The trees were in plots of the nutrient manipulation experiment (NUMEX) project (fully described in Martinson et al. 2013), in which the forest floor had been fertilized biannually with moderate amounts of N (urea at 50 kg N ha^{-1} yr^{-1}) and P (analytical grade monosodium phosphate at 10 kg P ha^{-1} yr^{-1}) since 2008; treatments

included control, N, P and N+P. Between 1998 and 2012, ambient deposition near our 2000 m site ranged from 14 to 45 kg N ha^{-1} yr^{-1} and 0.4 to 4.9 kg P ha^{-1} yr^{-1} (Homeier et al. 2012), so fertilization rates were quite realistic in terms of projected deposition rates (Galloway et al. 2004; Phoenix et al. 2006). The solid fertilizer was broadcast by hand; in 2011 and 2012 fertilization occurred between February and April, and in August or September.

Although we had intended to limit the study to specific tree species, there were no species – even within each elevation – that appeared in all of the plots, contained an adequate volume of canopy soil, and had soil in a location that was accessible. Therefore, we decided to look for general treatment effects, which were visible across tree species. Sample trees were chosen by identifying the individual in each plot that was furthest from the edge (to avoid edge effects), but still had a high volume of canopy soil (≥ 5 cm in depth) in a location accessible using rope techniques or a ladder. Both chambers and rings (described in detail in the next section) were installed in the canopy soil for use in measuring gas fluxes. Chambers were installed in the upper canopy (near the top of a tree in an area relatively open to sun/wind/rain) of trees within three blocks of the NUMEX plots at 2000 m and 3000 m in January/February 2011. Lower canopy chambers (mid to lower area of a tree, with less exposure to sun/wind/rain) were added after the first two sampling dates (June 2011). Rings (for intact soil cores) were installed at 1000 m in August, 2011 and for comparison they were also installed in the other two elevations in September, 2011.

4.3.3 Gas flux field sampling

Soil CO_2, CH_4 and N_2O fluxes were measured five times between June 2011 and April 2012 (June, September, November, January and April). Gas flux was measured in canopy soil

at 2000 m and 3000 m using chambers (all five dates) and intact cores (last four dates). At 1000 m, only intact cores were used (last four dates). The two-piece, closed, vented chambers were made of telescopic PVC fitted with caps; the cap of each chamber had a septum for gas sampling. The bases were inserted 2-3 cm into the canopy soil, fixed to the branch with nylon cable ties, and remained in the field throughout the study. Two sizes of chambers were used; the smaller chambers had a 1.09 L headspace and 0.008 m^{-2} surface area and the larger chambers had a 1.64 L headspace and a 0.012 m^{-2} surface area. The intact cores were 5 cm high and covered a surface area of 0.002 m^{-2}; bottoms of the intact cores were fitted with a piece of plastic mesh, which hindered soil loss when removing the cores for sampling, but allowed for water flow and nutrient exchange when the core was in the tree. Cores remained in the tree throughout the study, except during gas sampling.

When sampling, chambers were fitted onto the permanent bases installed in the trees, whereas intact cores were carefully removed from the tree and sealed in 500 mL glass jars fitted with septa for gas sampling. The jars were then partially buried, so they were still exposed to light while keeping the cores at a realistic field temperature. Gas was sampled over a period of 30 to 60 minutes, during which gas samples were taken five times, using a 20-mL syringe, and injected into 12-mL Labco Exetainer® (Labco Limited, Lampeter, UK) evacuated tubes. Since jars were not vented, each time an air sample was removed from a jar, an equal volume of ambient air was injected in order to prevent an under-pressure in the jar. This dilution was accounted for during calculations. During or immediately following gas sampling on each date, air pressure, air temperature and soil temperature were measured for use in flux calculations.

4.3.4 Gas and soil analyses

Gas samples were analyzed using a using a gas chromatograph (Shimadzu GC-14B, Duisburg, Germany) equipped with a flame ionization detector (FID) and an electron capture detector (ECD); this is the same system described in Martinson *et al.* (2013) and Wolf *et al.* (2012), but adjusted to measure the smaller 12-mL tubes. Gas concentrations were calculated by comparing integration peaks with three standard gases containing CO_2, CH_4 and N_2O (Deuste Steiniger GmbH, Mühlhausen, Germany). Standard gases were included during each analytical run to check for drift and calculate the minimum detectable concentration difference (MDCD; explained in detail in Yates et al. 2006). The MDCD was then used to determine if gas flux was significantly different from zero. If fluxes were significant, they were calculated as the linear (CH_4) or both linear and quadratic (CO_2 and N_2O) change in concentration over time. All fluxes - including zero fluxes - were used in calculations.

In 2011, soil samples were taken from the upper, mid and lower canopy soil in each plot and used to measure additional soil parameters. Natural abundance ^{15}N signatures of the soils were measured using IRMS (Delta Plus, Finnigan MAT, Bremen, Germany). Total C and N were measured by dry combustion in a CN analyzer (Elementar Vario EL; Elementar Analysis Systems GmbH, Hanau, Germany). Soil pH (H_2O) was analyzed in a 1:4 soil-to-water ratio.

4.3.5 Statistics and calculations

Results were analyzed using the R (version 2.15.3) open source software. Control blocks were tested for elevation effects using nested linear mixed-effect models (LME), with date, block (replicate) and position as random effects, and elevation as the fixed effect. Positional (i.e. upper vs. lower canopy) differences were tested for each elevation using an Independent

T-test, but as there were only infrequent differences, position is not discussed further. Treatment effects were also tested using LME, with separate models at each elevation. For all LME, we used a Tukey-HSD test for multiple comparisons and used the AIC to determine whether the quadratic or linear fluxes (for CO_2 and N_2O) were the best fit for our data (Wolf et al. 2012). We accepted *P*-values of $P < 0.10$ as significant.

In order to upscale fluxes to ha^{-1} yr^{-1}, we converted the fluxes from a chamber-area basis to one based on canopy soil mass, by using the soil core depth (5 cm) and average soil bulk densities at each elevation (1000 m: 0.07 g cm^{-3}, 2000 m: 0.10 - 0.11 g cm^{-3}, 3000 m: 0.09 g cm^{-3}), and then adjusted for the total mass of canopy soil per hectare at each elevation. Werner *et al.* (2012) found an average of 3877 kg ha^{-1} canopy soil on trees from mid-slope positions near our 2000 m site. We used this number as the mass of canopy soil in our 2000 m plots and then (based on our field observations) made a rough approximation for the other elevations, using half that amount for 1000 m and twice that amount for 3000 m. To upscale to per year fluxes at each elevation, we used the average fluxes that we measured over the entire study period and assumed that these were representative of the whole year.

4.4 - Results

4.4.1 CO_2 fluxes

Using both the chamber and jar methods, positive CO_2 fluxes were consistently measured at all elevations (Table 4.2). Compared on a forest-area-based scale, canopy soil CO_2 fluxes made up 5-11% of forest floor and canopy fluxes combined. Using the chamber method (2000 m and 3000 m only), CO_2 emissions from control plots were higher at 2000 m than 3000 m (*P = 0.05*) whereas using the jar method (all elevations), CO_2 emissions were higher at 1000 m

than at 2000 m and 3000 m ($P = 0.01$ for 2000 m and $P < 0.01$ for 3000 m) but 2000 m and 3000 m were not different from one another ($P = 0.78$).

At 2000 m, chamber CO_2 emissions were 45-56% lower in P plots as compared to control ($P = 0.02$) and N+P ($P < 0.01$) plots. In the jars at 2000 m, CO_2 emissions were again lower in P plots (51-54%) as compared to control plots and N+P plots ($P < 0.01$ for both). There were no treatment effects on CO_2 fluxes at 1000 m ($P = 0.44$) or 3000 m ($P > 0.39$).

4.4.2 CH_4 and N_2O fluxes

Compared on a forest-area-based scale, CH_4 and N_2O fluxes in canopy soils contributed only marginally (up to 5%) to combined canopy and forest floor fluxes (Tables 4.2 and S4.1); standard errors indicated that fluxes of both gases could be slightly positive or negative.

At 1000 m, net CH_4 emissions were lower in P plots than N plots ($P = 0.06$); canopy soils in P plots were a slight CH_4 sink (-2.94 ± 4.15 μg CH_4-C m^{-2} h^{-1}), while in N plots they were a slight source (9.03 ± 11.2 μg CH_4-C m^{-2} h^{-1}). At 2000 m, using the jar method, canopy soils in P plots were also a slight CH_4 sink (-8.93 ± 9.62 μg CH_4-C m^{-2} h^{-1}), so that net CH_4 emissions in P plots were lower than the slight CH_4 source from control (2.10 ± 3.85 μg CH_4-C m^{-2} h^{-1}; $P = 0.05$) and N+P plots (1.71 ± 10.6 μg CH_4-C m^{-2} h^{-1}; $P = 0.06$). At 3000 m, using the chamber method, soils in P plots were again a CH_4 sink (-10.8 ± 13.5 μg CH_4-C m^{-2} h^{-1}), with net emissions lower than control plots (1.24 ± 5.32 μg CH_4-C m^{-2} h^{-1}; $P = 0.01$), N plots (-0.13 ± 5.16 μg CH_4-C m^{-2} h^{-1}; $P < 0.01$) and N+P plots (0.23 ± 2.87 μg CH_4-C m^{-2} h^{-1}; $P < 0.02$).

N_2O fluxes exhibited one significant treatment effect; at 2000 m, using the jar method, soils in N plots were a slight N_2O source (2.43 ± 3.72 μg N_2O-N m^{-2} h^{-1}), which was higher ($P < 0.01$) than soils in control plots, which were a slight sink (-2.03 ± 2.97 μg N_2O-N m^{-2} h^{-1}).

Table 4.2 Average CO_2 fluxes, CH_4 fluxes and N_2O fluxes of tropical canopy soils along an elevation gradient, averaged from measurements on Sept. 2011, Nov. 2011, Jan. 2012 and April 2012. Gas was measured in three replicate blocks (n=3, SE shown in brackets), using two methods: static, vented chambers and soil cores sealed in jars.

Greenhouse gas	Elevation	Rates based on chamber/jar area		Rates based on forest area[1]		*Canopy contribution (jar values) to total forest soil emissions (CO_2/N_2O) or uptake (CH_4)*
		Chamber	*Jar*	*Chamber*	*Jar*	
CO_2		(mg CO_2-C m^{-2} h^{-1})		(Mg CO_2-C ha^{-1} yr^{-1})		
	1000 m	-	109.5 (43.4)	-	0.51 (0.20)	5%
	2000 m	17.5 (6.1)	47.3 (24.3)	0.11 (0.04)	0.30 (0.16)	5%
	3000 m	10.5 (5.4)	26.5 (10.9)	0.15 (0.08)	0.39 (0.16)	11%
CH_4		(μg CH_4-C m^{-2} h^{-1})		(kg CH_4-C ha^{-1} yr^{-1})		
	1000 m	-	0.93 (3.91)	-	0.00 (0.02)	0%
	2000 m	-6.04 (6.76)	2.10 (3.85)	-0.04 (0.04)	0.01 (0.02)	0%
	3000 m	1.24 (5.32)	-4.62 (6.61)	0.02 (0.08)	-0.07 (0.10)	4%
N_2O		(μg N_2O-N m^{-2} h^{-1})		(kg N_2O-N ha^{-1} yr^{-1})		
	1000 m	-	0.99 (4.44)	-	0.00 (0.00)	0%
	2000 m	-0.37 (0.46)	-2.03 (2.97)	0.00 (0.00)	0.00 (0.00)	0%
	3000 m	2.33 (3.52)	3.43 (5.15)	0.00 (0.00)	0.01 (0.01)	undefined[2]

[1] For details on how area-based rates were calculated for canopy soils, see *Materials and methods: statistics and calculations*

[2] The sum of the canopy and forest floor rates was zero (Table S4.1), so a contribution for the canopy could not be calculated

4.5 - Discussion

4.5.1 Canopy vs. forest floor

Although CO_2 emissions based on chamber area were similar between canopy and forest floor soil, when compared using forest area, canopy soils had much lower GHG fluxes – less CO_2 and N_2O production and less CH_4 consumption (Table 4.2; Table S4.1). Therefore, we can conclude that the canopy soils in our study forests were not making a significant contribution to total (canopy and forest floor) soil GHG flux, with the possible exception of CO_2 emissions from canopy soils at higher altitudes (i.e. at 3000 m canopy soils comprised 11% of total emissions).

4.5.2 GHG fluxes in canopy soil - CO_2 (C turnover)

Combining the chamber and jar relationships that we observed, there was a significant decrease in CO_2 fluxes with increasing elevation, as observed in other studies from tropical montane forest elevation gradients (Purbopuspito et al. 2006; Wolf et al. 2012; Zimmermann et al. 2010), including the forest floor of our plots (Mueller et al. unpublished data; Table S4.1). Although the percentage of total C in the canopy soil at all three elevations was similar (Table 4.1), there was significantly more canopy soil biomass with increasing elevation (personal observation). This indicates that C turnover likely has a strong elevation trend. Using our total C and CO_2 flux measurements (Tables 4.1 and 4.2) to make a rough comparison (assuming all CO_2 is heterotrophic respiration and all C is equally available), the C turnover time along our elevation gradient would increase from approximately 2 years at 1000 m up to 16 years at 3000 m. This has implications when considering climate change, suggesting that the proposed future increase in temperature and decrease in moisture (Foster 2001; Loope et al. 1998; Nadkarni and Solano 2002) could cause faster turnover and less retention of C in higher elevation canopies.

Studying the simulated effect of climate change on the canopy community in a tropical cloud forest in Costa Rica, Nadkarni and Solano (2002) suggest that these forests may experience significant losses in epiphytes followed by loss of canopy soil altogether. Loss of canopy soil would, in turn, have effects on forest diversity. The forests in our study area are considered diversity hot-spots (Richter et al. 2013), but in Ecuador, over 25% of vascular plant diversity in montane forests has been shown to be a result of epiphytes (Jørgensen and León-Yánez 1999). Although all epiphytes are not necessarily dependent on the presence of canopy soil, diversity is higher in trees where organic matter is present (Barthlott et al. 2001; Cardelus and Mack 2010).

At 2000 m, the decrease in CO_2 with added P at first seemed counterintuitive, as there were few live roots left in the cores used for the jar method (ruling out changes as a result of differences in root biomass and/or exudates) and decomposition in the canopy has been found to be P limited (Cardelus 2010). However, we can offer two possible explanations. First, P addition decreased litter production in our study sites at all elevations (Homeier et al. 2012), so there would have been less fresh litter as a substrate for canopy decomposition. Second, decreased CO_2 may have been caused by decreases in microbial C. Keller et al. (2006) observed decreases in CO_2 with added P in an ombrotrophic bog (comparable to our soils in the sense that they are also ombrotrophic organic soils) and attributed it to direct inhibition of the microbial community by P. This result may be stemming specifically from an inhibition of enzymatic activity through the addition of P and possibly N. We measured a general increase in N_2 fixation in P-amended plots across all elevations, as well as an increase in gross mineralization in these same P plots at 2000 m (Matson et al., unpublished data). Increases in N, in turn, have been documented to inhibit enzymatic activity and result in decreased microbial biomass C in soils (Baldos et al. unpublished data; Carreiro et al. 2000; Sinsabaugh et al. 2002).

4.5.3 GHG fluxes in canopy soil - CH_4

The low fluxes of CH_4 at all elevations were within the same magnitude as the standard error of the forest floor soils (Table S4.1). Production of CH_4 requires anaerobic conditions (Le Mer and Roger 2001), so although some CH_4 was produced in our soils - probably in anaerobic microsites - the high porosity of the canopy soils means they are unlikely to ever be a significant CH_4 source. The high porosity also means that, in terms of CH_4 oxidation, only high-affinity oxidation should be possible in our soils, as low-affinity requires high CH_4 concentrations (Le Mer and Roger 2001). Furthermore, most CH_4 oxidizers are neutrophiles and mesophiles, which may have been inhibited in our acidic, montane canopy soils (Whittenbury et al. 1970). Although species of methanotrophs have been found in more extreme ecosystems (Op den Camp et al. 2009; Trotsenko and Khmelenina 2002), they do not appear to be active in these soils.

The general decrease of CH_4 flux with P addition suggests that additional P in the canopy either inhibits CH_4 production or promotes CH_4 uptake. Higher rates of CH_4 uptake with P and N+P addition were seen in our forest floor soils at 2000 m (Mueller et al., unpublished data) and were attributed to an increase in methanotrophic activity, since consumption of CH_4 was the dominant CH_4 flux, and a previous study had shown that the forest floor soils in our study area produced very little CH_4 (Wolf et al. 2012). However, decreased methanogenic activity from anaerobic microsites is also possible. If, as suggested above, P was inhibiting enzyme activity, causing a decrease in microbial C, this would have likely resulted in decreased methanogenic activity in addition to decreases in total respiration. It is notable, for example, that in the ombrotrophic bog where CO_2 was inhibited by P, CH_4 emissions also decreased with P addition (Keller et al. 2006). Therefore, we cannot rule out either explanation given our available data.

4.5.4 GHG fluxes in canopy soil - N_2O

Similar to CH_4, the low N_2O fluxes at all elevations were within the same magnitude as the standard error of the forest floor fluxes (Table S4.1). Unlike CH_4, there was a good possibility that our soils could have exhibited significant N_2O fluxes. On the forest floor, the majority of N_2O emissions comes from denitrification (Mueller et al. unpublished data), and although canopy soils are unlikely to ever be completely anaerobic, the moisture threshold for N_2O emissions via denitrification is generally considered to be 70-80% WFPS (Butterbach-Bahl et al. 2013). However, even in the wet season, we rarely observed moistures higher than 60% WFPS in our soils (Matson et al. unpublished data), which is probably why N_2O emissions were so trivial.

Indirect fertilization of our soils showed that, in addition to a lack of moisture, N_2O emissions may have been limited by available N (significant at 2000 m). Increases in N_2O emissions were also seen with N addition to our forest floor soils during the first two years of the nutrient manipulation experiment (Martinson et al., 2012). Given that we saw an N effect in this study even when our fluxes were so low, and we know that in the wet season, soil NO_3^- concentrations and nitrification increase in these soils (Matson et al. unpublished data), in very wet years these canopy soils could be a slight N_2O source - especially under conditions of N deposition. However, given that future climate scenarios predict that montane forests will become warmer and drier (Foster 2001; Loope et al. 1998; Nadkarni and Solano 2002), canopy soils are unlikely to ever become a significant source of N_2O.

4.5.5 Measuring gas fluxes in canopy soil

Gas exchange measurements in canopy soil presented a unique challenge due to the high porosity of the soil. Although static chamber bases were fixed firmly to a branch, in at least 3 cm

of soil, we suspect that as gas concentrations started to increase in the chamber headspace, gas could still diffuse out of the chamber through the porous soil. In contrast, although the jar method precluded diffusion of gas from the sampling headspace, they necessitated a slight disturbance of the soil each time they were measured (the act of removing the core from the tree and dislodging any new roots that had formed). On hot days, the soil in jars may also have warmed slightly, which may have also affected gas flux. Therefore, the two methods likely represent a slight under-estimation (chambers) and a slight over-estimation (jars), yet fluxes from both methods were within the same order of magnitude. Future measurements of canopy gas flux should be done by enclosing whole branch areas in a sampling chamber that allows an acceptable volume of soil to be completely contained, and fluxes measured, without requiring any soil disturbance. However, we also draw attention to the results of this study, which strongly indicate that GHG fluxes are unlikely to be significant in canopy soils.

4.6 - References

Barthlott W, Schmit-Neuerburg V, Nieder J, Engwald S (2001) Diversity and abundance of vascular epiphytes: a comparison of secondary vegetation and primary montane rain forest in the Venezuelan Andes. Plant Ecology 152:145–156

Beaulieu F, Walter DE, Proctor HC, Kitching RL (2010) The canopy starts at 0.5 m: predatory mites (Acari: Mesostigmata) differ between rain forest floor soil and suspended soil at any height. Biotropica 42:704–709

Butterbach-Bahl K, Baggs EM, Dannenmann M, Kiese R, Zechmeister-Boltenstern S (2013) Nitrous oxide emissions from soils: how well do we understand the processes and their controls? Phil Trans R Soc 368:20130122

Butterbach-Bahl K, Papen H (2002) Four years continuous record of CH4-exchange between the atmosphere and untreated and limed soil of a N-saturated spruce and beech forest ecosystem in Germany. Plant Soil 240:77–90

Cardelús CL (2010) Litter decomposition within the canopy and forest floor of three tree species in a tropical lowland rain forest, Costa Rica. Biotropica 42:300–308

Cardelus CL, Mack MC (2010) The nutrient status of epiphytes and their host trees along an elevational gradient in Costa Rica. Plant Ecol 207:25–37

Cardelús CL, Mack MC, Woods CL, DeMarco J. Treseder, K (2009) Nutrient cycling in canopy and forest floor soils in a lowland tropical wet forest, Costa Rica. Plant Soil 318:47–61

Carreiro MM, Sinsabaugh RL, Repert DA, Parkhurst DF (2000) Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81:2359–2365

Chan AS, Steudler PA, Bowden, RD et al. (2005) Consequences of nitrogen fertilization on soil methane consumption in a productive temperate deciduous forest. Biol Fert Soils 41:182–189

Chen L, Liu WY, Wang GS (2010) Estimation of epiphytic biomass and nutrient pools in the subtropical montane cloud forest in the Ailao Mountains, southwestern China. Ecol Res 25:315–325

Cleveland CC, Townsend AR (2006) Nutrient additions to a tropical rain forest drive substantial soil carbon dioxide losses to the atmosphere. PNAS 103:10316–10321

Corre MD, Veldkamp E, Arnold J, Wright SJ (2010) Impact of elevated N input on soil N cycling and losses in old-growth lowland and montane forests in Panama. Ecology 91:1715–1729

Corre MD, Sueta JP, Veldkamp E (2014) Nitrogen-oxide emissions from tropical forest soils exposed to elevated nitrogen input strongly interact with rainfall quantity and seasonality. Biogeochemistry 118:103–120

Coxson DS, Nadkarni NM (1995) Ecological Role of Epiphytes in Nutrient Cycles. *in* Lowman MD, Nadkarni NM (eds.), Forest Canopies. Academic Press, New York, pp. 495–543

Cusack DF, Silver WL, Torn MS, McDowell WH (2011) Effects of nitrogen additions on above- and belowground carbon dynamics in two tropical forests. Biogeochemistry 104:203–225

Davidson EA, Keller M, Erickson HE, Verchot LV, Veldkamp E (2000) Testing a conceptual model of soil emissions of nitrous and nitric oxides. BioScience 50:667–680

Foster P (2001) The potential negative impacts of global climate change on tropical montane cloud forests. Earth-Science Reviews 55:73–106

Freiberg M, Freiberg E (2000) Epiphyte diversity and biomass in the canopy of lowland and montane forests in Ecuador. Journal of Tropical Ecology 16:673–688

Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, Karl DM, Michaels AF, Porter JH, Townsend AR, Vörösmarty CJ (2004) Nitrogen cycles: past, present, and future. Biogeochemistry 70:153–226

Hall SJ, Matson PA (2003) Nutrient status of tropical rain forests influences soil N dynamics after N additions. Ecological Monographs 73:107–129

Hedin LO, Brookshire ENJ, Menge DNL, Barron AR (2009) The nitrogen paradox in tropical forest ecosystems. Annu Rev Ecol Evol S 40:613–635

Hietz P, Wanek W, Wania R, Nadkarni NM (2002) Nitrogen-15 natural abundance in a montane cloud forest canopy as an indicator of nitrogen cycling and epiphyte nutrition. Oecologia 131:350–355

Hietz P, Turner BL, Wanek W, Richter A, Nock CA, Wright SJ (2011) Long-term change in the nitrogen cycle of tropical forests. Science 334:664–666

Homeier J, Breckle SW, Gunter S, Rollenbeck RT, Leuschner C (2010) Tree diversity, forest structure and productivity along altitudinal and topographical gradients in a species-rich Ecuadorian montane rain forest. Biotropica 42:140–148

Homeier J, Hertel D, Camenzind T et al. (2012) Tropical Andean forests are highly susceptible to nutrient inputs—rapid effects of experimental N and P addition to an Ecuadorian montane forest. PLoSONE doi:10.1371/journal.pone.0047128

Jørgensen PM, Leon-Yanez S (eds) (1999) Catalogue of the vascular plants of Ecuador. Monographs in systematic botany of the Missouri Botanical Garden, 75. Missouri Botanical Garden, St Louis.

Keller JK, Bauers AK, Bridgham SD, Kellogg LE, Iversen CM (2006) Nutrient control of microbial carbon cycling along an ombrotrophic-minerotrophic peatland gradient. J Geophys Res 111:G03006

Knowles R (1982) Denitrification. Microbiol Rev 46:43–70

Koehler B, Corre MD, Veldkamp E, Wullaert H, Wright SJ (2009) Immediate and long-term nitrogen oxide emissions from tropical forest soils exposed to elevated nitrogen input. Glob Change Biol 15:2049–2066

Le Mer J, Roger P (2001) Production, oxidation, emission and consumption of methane by soils: a review. Eur J Soil Biol 37:25–50

Loope LL, Giambelluca TW (1998) Vulnerability of island tropical montane forest to climate change, with special reference to East Maui, Hawaii. Clim Change 39:503–517

Mahowald, NM, Artaxo P, Baker AR, Jickells TD, Okin GS, Randerson JT, Townsend AR (2005) Impacts of biomass burning emissions and land use change on Amazonian atmospheric phosphorus cycling and deposition. Global Biogeochem Cycles 19:GB4030

Mahowald N, Jickells TD, Baker AR, Artaxo P, Benitez-Nelson CR, Bergametti G, Bond TC, Chen Y, Cohen DD, Herut B, Kubilay N, Losno R, Luo C, Maenhaut W, McGee KA, Okin GS, Siefert RL, Tsukuda S (2008) Global distribution of atmospheric phosphorus sources, concentrations and deposition rates, and anthropogenic impacts. Global Biogeochem Cycles 22:GB4026

Martinson GO, Werner FA, Scherber C, Conrad R, Corre MD, Flessa H, Wolf K, Klose M, Gradstein SR, Veldkamp E (2010) Methane emissions from tank bromeliads in neotropical forests. Nat Geosci 3:766–769

Martinson G, Corre MD, Veldkamp E (2013) Responses of nitrous oxide fluxes and soil nitrogen cycling to nutrient additions in montane forests along an elevation gradient in southern Ecuador. Biogeochemistry 112:625–636

Maurer D, Kolb S, Haumaier L, Borken W (2008) Inhibition of atmospheric methane oxidation by monoterpenes in Norway spruce and European beech soils. Soil Biol Biochem 40:3014–3020

Moser G, Hertel D, Leuschner C (2007) Altitudinal change in LAI and stand leaf biomass in tropical montane forests: a transect shady in Ecuador and a pan-tropical meta-analysis. Ecosystems 10:924–935

Nadkarni NM (1994) Diversity of species and interactions in the upper tree canopy of forest ecosystems. American Zoologist 34:321–330

Nadkarni NM, Schaefer DA, Matelson TJ, Solano R (2002) Comparison of arboreal and terrestrial soil characteristics in a lower montane forest, Monteverde, Costa Rica. Pedobiologia 46:24–33

Nadkarni NM, Schaefer DA, Matelson TJ, Solano R (2004) Biomass and nutrient pools of canopy and terrestrial components in a primary and a secondary montane cloud forest, Costa Rica. For Ecol Manag 198:223–236

Nadkarni NM, Solano R (2002) Potential effects of climate change on canopy communitiesin a tropical cloud forest: an experimental approach. Oecologia 131:580–586

Op den Camp HJM, Islam T, Stott MB, Harhangi HR, Hynes A, Schouten S, *et al*. (2009) Environmental, genomic and taxonomic perspectives on methanotrophic *Verrucomicrobia*. Environ Microbiol Rep 1: 293–306

Purbopuspito J, Veldkamp E, Brumme R, Murdiyarso D (2006) Trace gas fluxes and nitrogen cycling along an elevation sequence of tropical montane forests in Central Sulawesi, Indonesia. Globacl Biogeochemical Cycles 20:GB3010

Pittl E, Innerebner G, Wanek W, Insam H (2010) Microbial communities of arboreal and ground soils in the Esquinas rainforest, Costa Rica. Plant Soil 329:65–74

Richter M, Beck E, Rollenbeck R, Bendix J (2013) The study area. *in* Bendix J, Beck E, Bräuning A, Makeschin F, Mosandl R, Scheu S, Wilcke W. (eds) Ecosystem Services, Biodiversity and Environmental Change in a Tropical Mountain Ecosystem of South Ecuador. Ecological Studies 221:3–18

Saari A, Heiskanen J, Martikainen PJ (1998) Effect of the organic horizon on methane oxidation and uptake in soil of a boreal Scots pine forest. Fems Microbiology Ecology 26:245–255

Sinsabaugh RL, Carreiro MM, Repert DA (2002) Allocation of extracellular enzyme activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60:1–24

Steinkamp R, Butterbach-Bahl K, Papen H (2001) Methane oxidation by soils of an N limited and N fertilized spruce forest in the Black Forest, Germany. Soil Biol Biochem 33:145–153

Trotsenko YA, Khmelenina VN (2002) Biology of extremophilic and extremotolerant methanotrophs. Arch Microbiol 177:123–131

Vance E, Nadkarni NM (1990) Microbial biomass and activity in canopy organic matter and the forest floor of a tropical cloud forest. Soil Biol Biochem 22:677–84

Veldkamp E, Koehler B, Corre MD (2013) Indications of nitrogen-limited methane uptake in tropical forest soils. Biogeosciences 10:5367–5379

Wardle DA, Yeates GW, Barker GM, Bellingham PJ, Bonner KI, Williamson WM (2003) Island biology and ecosystem functioning in epiphytic soil communities. Science 301:1717–1720

Werner FA, Homeier J, Oesker M, Boy J (2012) Epiphytic biomass of a tropical Andean forest varies with topography. J Trop Ecol 28:23–31

Whittenbury R, Phillips KC, Wilkinson JF (1970) Enrichment, isolation and some properties of methane utilizing bacteria. J Gen Microbiol 61:205–218

Wolf K, Flessa H, Veldkamp E (2012) Atmospheric methane uptake by tropical montane forest soils and the contribution of organic layers. Biogeochemistry 11:469–483

Yates, T.T., Si, B.C., Farrell, R.E. and Pennock, D.J. 2006. Probability distribution and spatial dependence of nitrous oxide emission: temporal change in hummocky terrain. Soil Sci Soc Am J 70:753–762

Zhang T, Zhu W, Mo J, Liu L, Dong S, Wang X (2011) Increased phosphorus availability mitigates the inhibition of nitrogen deposition on CH4 uptake in an old-growth tropical forest, southern China. Biogeosciences 8:2805–2813

Zimmermann M, Meir P, Bird MI, Malhi Y, Ccahuana AJQ (2010) Temporal variation and climate dependence of soil respiration and its components along a 3000 m altitudinal tropical forest gradient. Global Biogeochem Cycles 24:GB4012

Table S4.1 Average CO_2 fluxes, CH_4 fluxes and N_2O fluxes of tropical forest floor soils along an elevation gradient, from measurements in Sept. 2011, Nov. 2011, Jan. 2012 and April 2012 (Mueller et al. unpublished data). On each date, gas was measured in three replicate blocks (n=3, SE shown in brackets).

Greenhouse gas units	Elevation	Rates based on chamber area	Rates based on forest area
		(mg CO_2-C m^{-2} h^{-1})	(Mg CO_2-C ha^{-1} yr^{-1})
CO_2-C	1000 m	123 (12)	10.8 (1.0)
	2000 m	71.3 (19.2)	6.25 (1.68)
	3000 m	33.6 (11.8)	2.94 (1.03)
		(µg CH_4-C m^{-2} h^{-1})	(kg CH_4-C ha^{-1} yr^{-1})
CH_4-C	1000 m	-19.5 (8.2)	-1.71 (0.71)
	2000 m	-33.7 (7.1)	-2.95 (0.62)
	3000 m	-19.7 (3.7)	-1.73 (0.32)
		(µg N_2O-N m^{-2} h^{-1})	(kg N_2O-N ha^{-1} yr^{-1})
N_2O-N	1000 m	3.11 (5.57)	0.27 (0.49)
	2000 m	1.44 (1.60)	0.13 (0.14)
	3000 m	-0.09 (0.94)	-0.01 (0.08)

Chapter 5

SYNTHESIS

5.1 - Cracking open the canopy 'black box'

Key Finding: Soil N cycling processes were active in canopy soils along the whole elevation gradient, with mass-based rates similar to those found on the forest floor.

Canopy science is still a relatively young branch of forest biology. It has only been in the past three decades that safe and effective methods were established to access canopies, before which the canopy was mostly studied from below (Lowman and Schowalter 2012). Early canopy research, which relied on ground observations and measurements to gather data about the canopy (i.e. Ford 1976), was not able to address specific questions about within-canopy diversity and processes. Once it was possible to work in the canopy, the number of canopy-based research studies increased rapidly (Nadkarni and Parker 1994). However, in a recent review about canopy science, Lowman and Schowalter (2012) outlined many 'black boxes' that still remain, including the need to understand pathways of nutrient transport and to predict how future disturbances might affect the canopy.

Previous research has customarily focused on canopy soils in their role as a bulk nutrient source for plants, rather than looking specifically at internal nutrient cycling rates. Yet in this study, we found that N cycling was quite active in canopy soils, with many parallels to what was seen in the corresponding forest floor soils. Figure 5.1 shows a possible N budget for the canopy at 2000 m, using the data from Chapters 2, 3 and 4, additional data (gross NH_4^+ and NO_3^- immobilization) not included in Chapter 3, and other measurements from research done within our study area, namely: canopy soil biomass (Werner et al. 2012), total N in rainfall and dry deposition (Wullaert et al. 2010), total N in throughfall (Homeier et al. 2012), and relative abundance of different N forms in throughfall (Zimmerman et al. 2007).

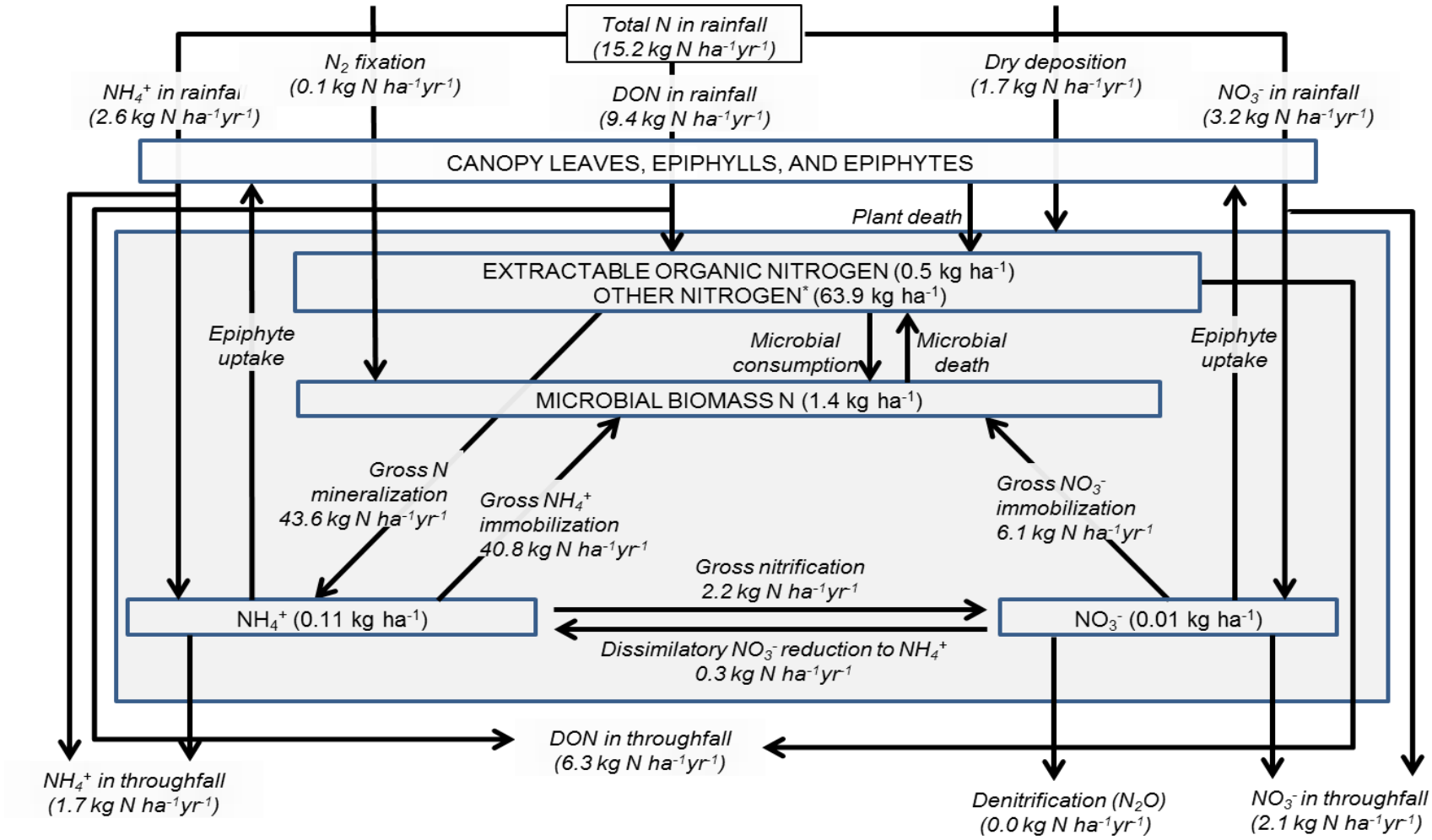

Figure 5.1 Nitrogen (N) inputs and losses from canopy soil (the shaded region of the figure) of a tropical montane forest at 2000 m. Values were taken from this study, except total N in rainfall and dry deposition (Wullaert et al. 2010) and total N in throughfall (Homeier et al. 2012). Forms of N in rainfall and throughfall were calculated using the proportion of each N form given by Zimmerman et al. (2007). Other N* is the difference between total canopy soil N (based on the canopy soil biomass from Werner et al. (2012) and the % N that we measured in canopy soil) and all other measured N pools.

This study provided a considerable amount of new information regarding N cycling in canopy soils, but there are still some points that remain unclear. In the soil NH_4^+ pool shown in Fig. 5.1, the sum of total inputs was 46.5 kg N ha^{-1} yr^{-1} and the sum of total outputs was 44.7 kg N ha^{-1} yr^{-1}. Discounting other possible fates of NH_4^+ (i.e. retention through abiotic processes; Johnson et al. 2000), this suggests that 1.8 kg NH_4-N ha^{-1} yr^{-1} was taken up by epiphytes. Werner et al. (2012) found the combined epiphytic biomass of lichens, bryophytes and vascular plants at this elevation to be, on average, 1898 kg ha^{-1}. Combining this with the average foliar N concentration (1.5 %) that Stewart et al. (1995) found in epiphytes, this amount of NH_4^+ uptake would account for 6% of epiphyte biomass N. However, uptake could be considerably larger, given the lack of stemflow data for this area and the uncertainty in the rainfall and throughfall estimates (see below). In the NO_3^- pool, the sum of total inputs was 5.4 kg N ha^{-1} yr^{-1} and the sum of total outputs was 8.5 kg N ha^{-1} yr^{-1}. This suggests that there was minimal uptake of NO_3^- by epiphytes, which is consistent with epiphyte preference for NH_4^+ over NO_3^- , as observed by Wanek et al. (2002). Since outputs exceeded inputs by 3.1 kg N ha^{-1} yr^{-1} (only 1.2 kg N ha^{-1} yr^{-1} of which can be explained by standard error), it is likely that stemflow (which was not measured in our study area) and throughfall from further up in the canopy make up much of this missing source.

The amount of N that epiphytes and canopy soil contribute to throughfall and/or receive from throughfall remains unclear. Normally, studies looking at throughfall only take two measurements: before any interaction with the canopy (incident rainfall) and what reaches the ground after interaction with the canopy (throughfall). But, from a canopy perspective, there are also intermediate values that would need to be calculated to understand N dynamics. Figure 5.1 shows rainfall interacting with live parts of the canopy and then possibly entering

the canopy soil or simply terminating on the ground as throughfall. So, when doing an N budget for canopy soil, which is the correct 'input' to the canopy soil: the rainfall or the throughfall? For simplicity, in Figure 5.1, rainfall is the input and throughfall is the output, but it should be noted that, in reality, those numbers could be very different. Studies have shown that nutrient concentrations in throughfall measured from collectors under large accumulations of epiphytes and/or canopy soil vary from other measured values of throughfall in a forest (Fleischbein et al. 2005; Zimmerman et al. 2007). Compiling a proper N budget of canopy soil would therefore require a much more detailed look at throughfall N concentrations along a vertical gradient in the canopy. Adriaenssens et al. (2012) used a vertical profile to study throughfall within beech and spruce canopies in a temperate forest, and they observed significant differences in the chemical composition of throughfall at different canopy heights.

5.2 - Moving from a 'top down' to a connected view of canopy and forest floor soil

Key Finding: The canopy and forest floor soil fertility in these forests is closely linked. Changes in canopy N cycling and GHG flux were seen after only a short period of moderate nutrient inputs to the forest floor.

Most research linking forest floor and canopy fertility uses a 'top down' approach: 'How do canopies affect nutrient cycling on the forest floor?' Canopies are considered to be collectors and reservoirs of available nutrients, which will eventually add to the nutrient capital of terrestrial soil. This generally occurs in one of two ways: detritus or precipitation. Detritus can move from the canopy to the forest floor through epiphytic litterfall, storm breakage of branches or growth of epiphytes beyond the capacity of the host tree (Diaz et al.

2010; Lowman and Schowalter 2012; Tejo 2013). Precipitation can, in the form of throughfall and stemflow, wash adsorbed minerals from different canopy components (leaves, epiphytes, bark) or leach nutrients from canopy soil (Coxson and Nadkarni 1995; Lowman and Schowalter 2012; Tukey 1970; Zimmerman et al. 2007). However, although the general mechanism for precipitation-related nutrient mobilization is clear, the specific processes are still not completely understood. It is assumed that epiphytes and canopy soil contribute to the high spatial variability of throughfall nutrient concentrations in some tropical forests (Fleischbein et al. 2005; Veneklaas and Van Ek 1990; Zimmerman et al. 2007), but differences in areas with and without epiphytes are not always significant (Fleischbein et al. 2005). In a lowland forest in Costa Rica, Umana and Wanek (2010) isolated single branches that contained epiphytes and canopy soil, and looked specifically at how epiphytic material affected N in throughfall; they found large differences between net and gross canopy retention and exchange processes. This suggests that more research is required to explore the complexity of throughfall in large areas.

But what about 'bottom up' processes? Although many studies look at how canopies affect the forest floor, studies reporting nutrient effects in the opposite direction are less common, possibly because this link in the cycle is poorly understood. In our study, four years of moderate fertilization (50 kg N ha^{-1} yr^{-1} and 10 kg P ha^{-1} yr^{-1}) had significant effects on N_2 fixation, N cycling and GHG flux, indicating that the fertility of canopy and forest floor soils in our study area was very closely linked. There is, however, scant literature with which to compare this. Benner et al. (2007) and Benner and Vitousek (2007) reported that after 14 years of high (100 kg ha^{-1} yr^{-1}) P addition to the forest floor, they observed significant increases in epiphyte abundance and diversity (in particular N_2-fixers) in a tropical forest.

However, they specifically mention that they did not expect epiphytic N_2-fixers to be affected by fertilization of the forest floor. The expectation for the canopy to be largely decoupled from forest floor nutrient status has been shown or postulated in many studies (Hedin et al. 2009; Hietz et al. 2002; Stewart et al. 1995; Tozer et al. 2005; Wania et al. 2002). Even in their study area where P had an effect on epiphytes, Benner and Vitousek (2007) observed that other nutrients (N and a combination of micronutrients) did not have significant effects. On the other hand, the mechanism for nutrient enrichment of the canopy is clear. There are studies from temperate forests, dating back almost 20 years, which have shown that nutrients leaching from host tree leaves and bark can affect epiphytic lichens (Gauslaa 1995; Goward and Arsenault 2000; Hauck 2003; Hauck and Runge 2002). Additionally, in a follow-up to the fertilization study, Benner (2011) showed that epiphytes in Hawaiian forests preferentially colonize unfertilized trees with naturally high P concentrations. Perhaps the link between all of these studies is the nutrient limitation in each specific area. In our study, we saw more increases in N cycling with N+P addition than with the addition of only one of the two nutrients, suggesting that our canopy soils were co-limited by N and P. So, the canopy soils may remain essentially ‘decoupled’ from forest floor nutrients if they are co-limited but only have access to a single limiting nutrient. Our study clearly indicates the need for more research into canopy-forest floor nutrient interactions.

5.3 - Atmospheric deposition and global change - how will they affect canopies?

Key Finding: Elevated levels of N and P significantly affected nutrient cycling in canopy soils and indicated that these canopies are co-limited by both nutrients.

We found that all of the processes that we measured in the canopy soil (N_2 fixation, N cycling and GHG flux) were in some way affected by moderate nutrient addition to the forest floor. So, chronic atmospheric N and P deposition has the potential to significantly change the dynamics of nutrient cycling in these canopies. As outlined in the summary at the beginning of this dissertation, N deposition may lead to inhibition of N_2 fixation, with hotspots still occurring where P is present. Internal N cycling in canopy soils will likely be stimulated by N and P deposition, but chronic nutrient deposition may also lead to increased mineral N losses from the canopy soil. GHG-related processes in canopy soils will probably also respond to N and P deposition, but with the exception of CO_2 emissions, fluxes in canopy soils are unlikely to significantly contribute to total forest GHG budgets. So what does that mean for the total canopy ecosystem? In terms of these specific processes, N_2 fixation is a relatively minor input compared to other sources (Fig. 5.1), so complete inhibition would likely have a negligible effect, while increases in N_2 fixation in areas of P deposition could become a useful N source (Benner et al. 2007). Increases in soil mineral N cycling should benefit the epiphytes that use canopy soil resources, but if leaching also increases, it might result in the loss of other important nutrients (i.e. cations) from the soil. Changes in GHG fluxes are unlikely to be significant in terms of GHG budgets, but may be significant in terms of changes in C cycling (see below).

More research would be required before we could formulate a more detailed theory as to how nutrient deposition might affect canopies. However, if deposition accompanies other global change processes, like climate change, the most significant driver of change in the canopy will likely be changes in the moisture regime. Water supply is considered the most powerful determinant of epiphyte distribution (Benzing 1998) and several studies have postulated that the dependence of epiphytes and canopy soil on atmospheric moisture will make them very sensitive to fluctuations in moisture levels with climate change (Benzing 1998; Foster 2001; Nadkarni and Solano 2002). In Chapter 4, we suggested that future increased temperature and decreased moisture could cause faster turnover and less retention of C in higher elevation canopies. This was linked to a study from a tropical cloud forest in Costa Rica (Nadkarni and Solano 2002), which simulated how climate change may effect epiphytes in the canopy; they concluded that canopies may experience significant losses in epiphytes, followed by the loss of canopy soil altogether. Such dramatic changes to the canopy would probably have cascading effects to the rest of the forest. Since epiphytes provide habitat, food and water for a vast number of invertebrates and vertebrates, a significant reduction in epiphytes would almost certainly lead to decreases in species abundance and perhaps even to some extinctions (Foster 2001).

5.4 - References

Adriaenssens S, Hansen K, Staelens J, Wuyts K, De Schrijver A, Baeten L, Boeckx P, Samson R, Verheyen K (2012) Throughfall deposition and canopy exchange processes along a vertical gradient within the canopy of beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* (L.) Karst). Science of the Total Environment 420:168-182

Benner JW (2011) Epiphytes preferentially colonize high-phosphorus host trees in unfertilized Hawaiian montane forests. The Bryologist 114:335-345

Benner JW, Conroy S, Lunch CK, Toyoda N, Vitousek PM (2007) Phosphorus fertilization increases the abundance and nitrogenase activity of the cyanolichen Pseudocyphellaria crocata in Hawaiian montane forests. Biotropica 39:400-405

Benner JW, Vitousek PM (2007) Development of a diverse epiphyte community in response to phosphorus fertilization. Ecology Letters 10:628–636

Benzing DH, (1998) Vulnerabilities of tropical forests to climate change: the significance of resident epiphytes. Clim Change 39:519–5440

Coxson DS and Nadkarni NM (1995) Ecological Role of Epiphytes in Nutrient Cycles. *in* Lowman MD, Nadkarni NM (eds.), Forest Canopies. Academic Press, New York, pp. 495–543

Díaz IA, Sieving KE, Pena-Foxon ME, Larraín J, Armesto JJ (2010) Epiphyte diversity and biomass loads of canopy emergent trees in Chilean temperate rain forests: A neglected functional component. Forest Ecology and Management 259:1490-1501

Fleischbein K, Wilcke W, Goller R, Boy J, Valarezo C, Zech W, Knoblich K (2005) Rainfall interception in a lower montane rain forest in Ecuador: effects of canopy properties. Hydrol Process 19:1355–1371

Ford E (1976) The canopy of a Scots pine forest description of a surface of complex roughness. Agnc Meterol 17:9-32

Foster P (2001) The potential negative impacts of global climate change on tropical montane cloud forests. Earth-Science Reviews 55:73–106

Gauslaa Y (1995) The Lobarion, an epiphytic community of ancient forests threatened by acid rain. Lichenologist 27:59–76

Goward T, Arsenault A (2000) Cyanolichen distribution in young unmanaged forests: a dripzone effect? Bryologist 10:28–37

Hauck M (2003) Epiphtic lichen diversity and forest dieback: the role of chemical site factors. Bryologist 106:257-269

Hauck M, Runge M (2002) Stemflow chemistry and epiphytic lichen diversity in dieback-affected spruce forest of the Harz Mountains, Germany. Flora 197:250–261

Hedin LO, Brookshire ENJ, Menge DNL, Barron AR (2009) The nitrogen paradox in tropical forest ecosystems. Annu Rev Ecol Evol S 40:613-635

Hietz P, Wanek W, Wania R, Nadkarni NM (2002) Nitrogen-15 natural abundance in a montane cloud forest canopy as an indicator of nitrogen cycling and epiphyte nutrition. Oecologia 131:350-355

Homeier J, Hertel D, Camenzind T et al. (2012) Tropical Andean forests are highly susceptible to nutrient inputs—rapid effects of experimental N and P addition to an Ecuadorian montane forest. PLoSONE doi:10.1371/journal.pone.0047128

Johnson DW, Cheng W, Burke IC (2000) Biotic and abiotic nitrogen retention in a variety of forest soils. Soil Sci Soc Am J 64:1503–1514

Lowman MD, Schowalter TD (2012) Plant science in forest canopies – the first 30 years of advances and challenges (1980–2010). New Phytologist 194:12–27

Nadkarni NM, Matelson TJ (1989) Bird use of epiphyte resources in neotropical trees. Condor 69:891–907

Nadkarni NM, Parker GG (1994) A profile of forest canopy science and scientists–who we are, what we want to know, and obstacles we face: results of an international survey. Selbyana 15:38-50

Nadkarni NM, Solano R (2002) Potential effects of climate change on canopy communitiesin a tropical cloud forest: an experimental approach. Oecologia 131:580–586

Stewart GR, Schmidt S, Handley LL, Turnbull MH, Erskine PD, Joly CA (1995). N-15 Natural abundance of vascular rainforest epiphytes – implications for nitrogen source and acquisition. Plant Cell Environ 18:85–90

Tejo C (2013) Canopy soils, litterfall and litter decomposition in a coastal old-growth temperate rainforest, Washington. Ph.D. Thesis. University of Washington, USA

Tozer WC, Hackell D, Miers DB, Silvester WB (2005) Extreme isotopic depletion of nitrogen in New Zealand lithophytes and epiphytes; the result of diffusive uptake of atmospheric ammonia? Oecologia 144:628-635

Tukey H (1970) Leaching of substances from plants. Ann Rev Plant Physio 21:305–324

Umana NH, Wanek W (2010) Large canopy exchange fluxes of inorganic and organic nitrogen and preferential retention of nitrogen by epiphytes in a tropical lowland rainforest. Ecosystems 13:367–381

Veneklaas EJ, Van Ek R (1990) Rainfall interception in two tropical montane rain forests, Colombia. Hydrol Process 4:311–326

Wanek W, Arndt SK, Huber W, Popp M (2002) Nitrogen nutrition during ontogeny of hemiepiphytic *Clusia* species. Functional Plant Biology 29**:**733–740

Wania R, Hietz P, Wanek W (2002) Natural 15N abundance of epiphytes depends on the position within the forest canopy: source signals and isotope fractionation. Plant, Cell and Environment 25:581–589

Werner FA, Homeier J, Oesker M, Boy J (2012) Epiphytic biomass of a tropical Andean forest varies with topography. J Trop Ecol 28:23–31

Wullaert H, Homeier J, Valarezo C, Wilcke W (2010) Response of the N and P cycles of an old-growth montane forest in Ecuador to experimental low-level N and P amendments. Forest Ecology and Management 260:1434–1445

Zimmermann A, Wilcke W, Elsenbeer H (2007) Spatial and temporal patterns of throughfall quantity and quality in a tropical montane forest in Ecuador. Journal of Hydrology 343:80–96

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DECLARATION OF ORIGINALITY AND CERTIFICATE OF AUTHORSHIP

I, Amanda Matson, hereby declare that I am the sole author of this dissertation entitled "Canopy soil nutrient cycling and response to elevated nutrient levels along an elevation gradient of tropical montane forests", and that all references and data sources have been appropriately acknowledged. I furthermore declare that this work has not been submitted elsewhere in any form as part of another dissertation procedure. I certify that the manuscripts presented in chapters 2, 3 and 4 have been written by me as first author.

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CONFERENCES

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