GÖTTINGER ZENTRUM
FÜR BIODIVERSITÄTSFORSCHUNG UND ÖKOLOGIE
– GÖTTINGEN CENTRE FOR BIODIVERSITY AND ECOLOGY –

Soil greenhouse gas fluxes under elevated nutrient input along an elevation gradient of tropical montane forests in southern Ecuador

Dissertation zur Erlangung des Doktorgrades der
Mathematisch-Naturwissenschaftlichen Fakultäten der
Georg-August-Universität Göttingen

vorgelegt von

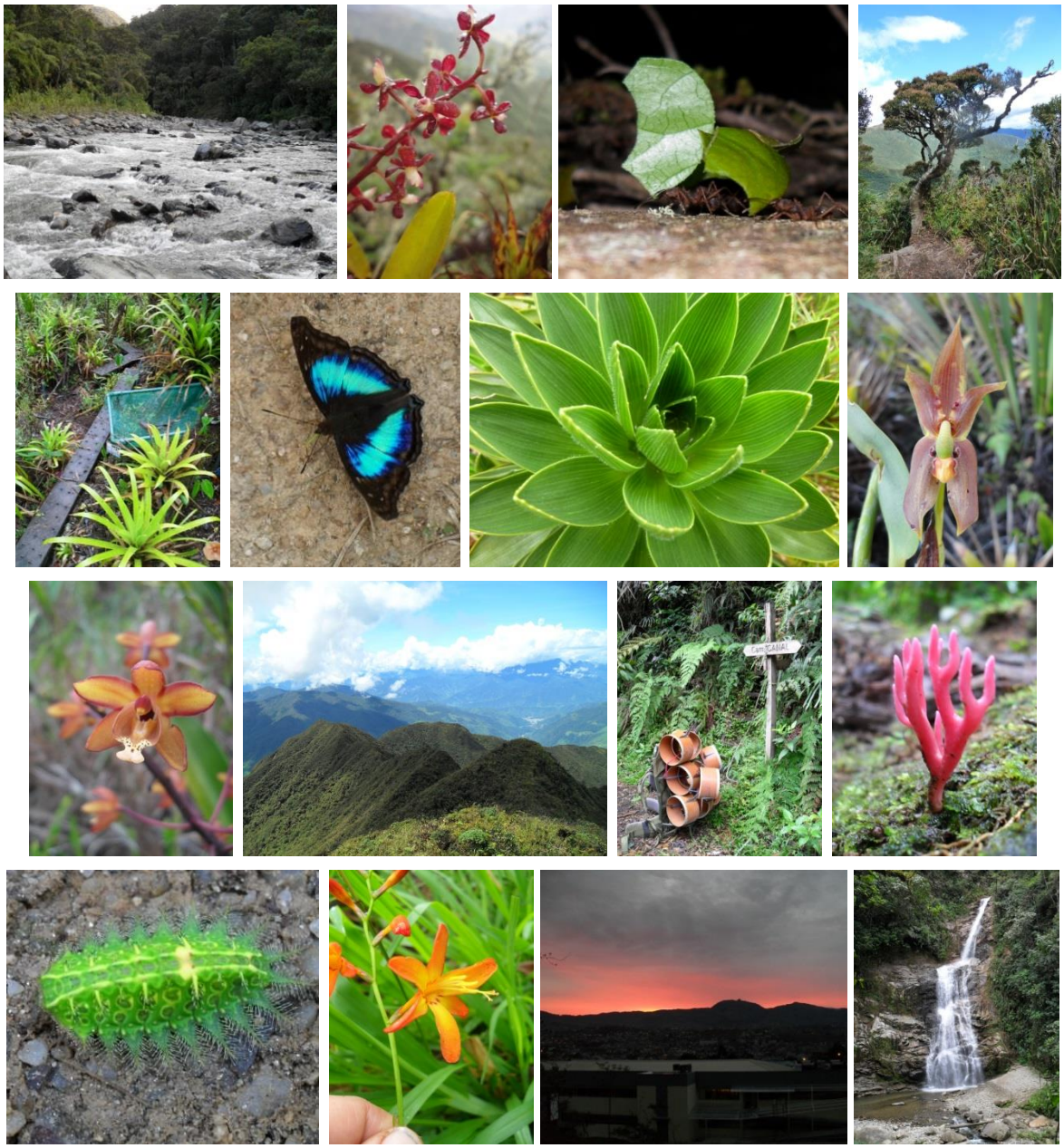
Diplom Agrarbiologin

Anke Katrin Müller

aus
Kirchheim/Teck

Göttingen, August 2014

Referentin/Referent:	Prof. Dr. Edzo Veldkamp
Korreferentin/Korreferent:	Dr. Marife D. Corre
Tag der mündlichen Prüfung:	30.09.2014



"Es gibt eine Theorie, die besagt, wenn jemals irgendwer genau herausfindet, wozu das Universum da ist und warum es da ist, dann verschwindet es auf der Stelle und wird durch noch etwas Bizarreres und Unbegreiflicheres ersetzt. - Es gibt eine andere Theorie, nach der das schon passiert ist."

Douglas Adams, Das Restaurant am Ende des Universums, 1980

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ZUSAMMENFASSUNG

Tropische Waldböden spielen für das Klima der Erde eine wichtige Rolle, da sie große Mengen an Treibhausgasen (THGs) mit der Atmosphäre austauschen. Diese wichtige Funktion könnte jedoch durch menschliche Aktivitäten verändert werden, da sie atmosphärische Nährstoffeinträge vor allem in tropischen Regionen erhöhen. Wie ansteigende Nährstoffeinträge THG Flüsse von tropischen Waldböden beeinflussen wurde jedoch bisher kaum untersucht und Nährstoff-Kontrollmechanismen in tropischen Bergregenwäldern (TBRWs) sind noch viel weniger verstanden. Nachdem TBRW-Böden ungefähr 11-21% der tropischen Waldfläche ausmachen, ist es unerlässlich Veränderungen in THG Flüssen unter Nährstoffeinträgen zu quantifizieren und vorherzusagen, da diese weitere globale Veränderung zur Folge haben könnten. Ziel dieser Doktorarbeit ist es, den Einfluss, den moderater Gaben von Stickstoff (N) und/oder Phosphor (P) auf Flüsse der drei THGs Kohlenstoffdioxid (CO_2), Distickstoffoxid (N_2O) und Methan (CH_4) haben, entlang eines Höhengradienten (1000 m, 2000 m, 3000 m) primärer TBRWs Südecuadors zu quantifizieren.

Hierfür haben wir fünf Jahre lang THG Flüsse von Böden in einem Nährstoffmanipulationsexperiment (‘NUMEX‘, Abkürzung vom Englischen herrührend) mit unbehandelte Kontrollflächen und N (50 kg N ha^{-1} yr^{-1}), P (10 kg P ha^{-1} yr^{-1}) sowie N+P gedüngten Flächen gemessen. Messungen erfolgten monatlich *in situ* mit belüfteten statischen Hauben und darauffolgender gaschromatographischer Analyse. Um einen detaillierten Einblick in Prozesse zu erhalten, welche an dem Austausch von THGs zwischen Boden und Atmosphäre beteiligt sind, wurden weitere Untersuchungen durchgeführt. Unter anderem untersuchten wir grundlegenden Faktoren die die THG Flüsse von Böden beeinflussen (Bodentemperatur, -feuchte und mineralischer Boden-N Gehalt), verschiedener Komponenten von CO_2 Flüssen, netto N-Umsatzraten in Böden und

Komponenten der N_2O Flüsse. Hierfür wurden folgende Techniken *in situ* angewandt: Entfernen frischen Laubstreus, Ausschluss von Wurzeln (*trenching*), Bodeninkubation (*buried bag method*) und temporäre Markierung von Böden mit ^{15}N.

THG Flüsse von Waldböden in unserem Untersuchungsgebiet waren vergleichbar mit Flüssen von anderen TBRWs entsprechender Höhenstufen, mit Ausnahme von N_2O. N_2O Flüsse, welche sich hauptsächlich aus Denitrifikationsprozessen ableiten, waren für einen TBRW relative klein, was wir auf einen konservativen Boden N-Kreislauf in unserer Flächen zurückführen. Böden waren CO_2 und N_2O Quellen (wobei die Stärke mit zunehmender Höhe abnahm) und über alle Höhenstufen hinweg CH_4 Senken.

Unsere Ergebnisse zeigen, dass sich die Auswirkungen der Nährstoffgaben auf gemessenen THG Flüsse mit der Höhenstufe unterscheiden. Die Reaktionen der CO_2 Flüsse von Böden veränderten sich zudem mit der Dauer der Nährstoffgabe und der Art zugegebener Nährstoffe. Auf 1000 m Höhe veränderten sich CO_2 Flüsse von Böden unter Zugabe von N nicht, wohingegen sie unter Zugabe von P und N+P in dem ersten und vierten bis fünften Jahr abnahmen. Auf 2000 m Höhe stiegen CO_2 Flüsse unter Zugabe von N und N+P in dem ersten Jahr an; danach nahmen sie mit Zugabe von N ab, wohingegen die Zugabe von N+P keine Auswirkungen mehr hatte; Zugabe von P hatte keine Folge. Auf 3000 m Höhe stiegen CO_2 Flüsse unter Zugabe von N durchgehend; wobei sie unter Zugabe von P und N+P nur in dem ersten Jahr anstiegen, ohne weitere Auswirkungen in den folgenden Jahren. Differentielle Auswirkungen der Nährstoffgaben hingen mit dem ursprünglichen N und P Status der Böden sowie unterschiedlichen Reaktionen von Komponenten der Bodenrespiration zusammen.

Reaktionen von N_2O und CH_4 Flüssen zeigten große Schwankungen zwischen den Jahren. Die Zugabe von N in den Jahr drei bis fünf veränderte N_2O Flüsse nicht, obwohl während der ersten zwei Jahre desselben Experiments signifikante Effekte beobachteter

werden konnten. Wir führen das Ausbleiben einer Reaktion auf relativ geringe Bodenfeuchtegehalte während unseres Messzeitraumes in den Jahren 2010-2012 zurück. Entlang des gesamten Höhengradienten nahmen N_2O-Flüsse und mineralische Boden-N Gehalte durch Zugabe von P ab, vermutlich da dies die P Limitierung der Nettoprimärproduktion abschwächte, wodurch Pflanzen mehr N aufnahmen. Die Zugabe von N+P zeigte ähnliche Trends wie die Zugabe von N, wobei die Ausprägung durch die gegenläufige Wirkung der P Zugabe geringerer ausfiel.

Während der ersten zwei Jahre hatten Nährstoffgaben auf keiner Höhenstufe einen Einfluss auf die CH_4 Flüsse. Wir führen dies auf die Kombination moderater Nährstoffgaben, starker Immobilisierung zugegebener Nährstoffe und die räumliche Trennung des Ortes höchster CH_4 Aufnahmekapazität im Unterboden von dem Ort der Nährstoffgabe auf der Bodenoberfläche zurück. Drei bis fünfjährige Nährstoffgaben erhöhten die CH_4 Aufnahme von Böden, jedoch variierten die Effekte unter Zugabe von N und P entlang des Höhengradienten: auf 1000 m Höhe stieg die jährliche CH_4 Aufnahme unter Zugabe von N und N+P um 20-60% an. Auf 2000 m Höhe stieg sie unter Zugabe von P und N+P um 21-50% an; und auf 3000 m Höhe stieg sie unter Zugabe von N um 34-40% an. Diese unterschiedlichen Effekte der Nährstoffgaben könnten mit dem anfänglichen Nährstoffstatus der Böden sowie unterschiedlichen Auswirkungen von Nährstoffgaben auf Ökosystemkomponenten je Höhenstufe zusammenhängen.

Wir zeigen hiermit, dass sich in TBRWs die THG Flüsse von Böden und demnach das Netto-Treibhauspotential von Böden entlang eines Höhengradienten stark verändern kann, wobei es mit zunehmender Höhe tendenziell zu einer Abnahme kommt. Unsere Ergebnisse deuten ferner an, dass in TBRW der Anden, erhöhte N und P Depositionen die THG Flüsse von Böden stark beeinflussen können. Auswirkungen von Nährstoffgaben auf THG Flüssen von Böden hängen allerdings stark von dem anfänglichen Nährstoffstatus der

Böden, der Dauer der Nährstoffgabe und jährlichen klimatischen Schwankungen ab. Da sich Nährstoffeffekte nicht linear mit der Dauer der Nährstoffgabe veränderten und komplexe Interaktionen mit anderen Ökosystemkomponenten existieren, gibt es einige Unsicherheit was die Prognose der Auswirkungen von Nährstoffdepositionen auf THG Flüsse von Böden betrifft. Dennoch liefern wir hiermit die ersten Daten über mittelfristige Auswirkungen der Nährstoffzugabe von N, P und N+P, auf die drei wichtigsten THG Flüsse von Böden entlang eines Höhengradienten in TBRWs der Anden. Unsere Ergebnisse deuten an, dass das Netto-Treibhauspotential von Böden entlang des Höhengradienten unter zunehmenden N Einträgen leicht zunehmen könnte, wohingegen es unter zunehmenden P und N+P Einträgen abnehmen könnte.

SUMMARY

Tropical forest soils play an important role in Earth's climate, by exchanging large amounts of greenhouse gases (GHGs) with the atmosphere. This important function might however be altered by human activities, which increase nutrient deposition to terrestrial ecosystems - especially in tropical regions. How increasing nutrient inputs affect soil GHG fluxes from tropical forests is relatively understudied, though, and nutrient controls in tropical montane forests (TMFs) are even less understood. Since TMFs represent about 11-21% of tropical forest area, it is vital to be able to predict and quantify changes in soil GHG fluxes with nutrient input, as they might further feedback to other global changes. This dissertation aims to quantify the impact of moderate nitrogen (N) and/or phosphorus (P) addition on fluxes of three soil GHGs: carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4), along an elevation gradient (1000 m, 2000 m, 3000 m) of old-growth TMFs in southern Ecuador.

Over five years, we measured soil GHG fluxes in a nutrient manipulation experiment (NUMEX), with control, N (50 kg N ha^{-1} yr^{-1}), P (10 kg P ha^{-1} yr^{-1}) and N+P addition. In situ measurements were done monthly using static vented chambers, followed by gas chromatographic analysis. To achieve an in-depth look into the processes involved in soil-atmosphere GHG exchange, further investigations included monitoring of basic controlling factors (soil temperature, moisture and mineral N concentrations), different components of soil CO_2 fluxes, net soil N cycling rates and sources of soil N_2O fluxes. To do so, we used litter removal and trenching techniques, the buried bag method and a short-term ^{15}N labeling approach.

Soil GHG fluxes in our study forests were within the range of fluxes reported from other TMFs at comparable elevations, except for N_2O. N_2O fluxes, which were mainly derived from denitrification, were low for a TMF, which we attribute to the conservative

soil N cycling in our sites. Soils were sources of CO_2 and N_2O (source strength decreases with increasing elevation) and across all elevations, they were CH_4 sinks.

We found differential nutrient effects on all measured soil GHG fluxes with elevation. Responses of soil CO_2 fluxes changed with the duration and type of nutrient addition. At 1000 m, N addition did not affect soil CO_2 fluxes, whereas P and N+P additions decreased fluxes in the first and fourth-fifth years. At 2000 m, N and N+P additions increased CO_2 fluxes in the first year; thereafter, N addition decreased fluxes whereas N+P addition no longer showed any effect; P addition showed no effect. At 3000 m, N addition increased CO_2 fluxes consistently; P and N+P additions increased fluxes only in the first year showing no effect thereafter. Differential nutrient effects were related to initial soil N and P status and varied responses of soil respiration components.

Responses of N_2O and CH_4 fluxes to nutrient addition showed large inter-annual variability. N_2O fluxes were not affected by three to five years of N addition, despite the significant effects observed during the first two years of the same experiment. We attribute the lack of response in later years to the relatively low soil moisture contents during our 2010-2012 measurement period. Across the elevation gradient, P addition decreased N_2O fluxes and mineral N concentrations, presumably because it alleviated P limitations to net primary production, which increased plant N uptake. N+P addition showed similar trends to N addition, but less pronounced because of the counteracting effects of P addition.

During the first two years of nutrient addition, CH_4 fluxes were not affected at any elevation, which we attribute to the combination of moderate amounts of added nutrients, strong immobilization of added nutrients, and the separation of the highest CH_4 uptake capacity in the subsoil from the surface of the soil, where fertilizers were added. In years three to five, nutrient additions increased soil CH_4 uptake. However, effects of N and P varied along the elevation gradient: at 1000 m, N and N+P addition increased annual CH_4

uptake by 20-60%; at 2000 m, P and N+P addition increase uptake by 21-50%; and at 3000 m, N addition increased CH_4 uptake by 34-40%. These differential effects of nutrient addition may be related to initial soil nutrient status and differential responses of ecosystem components to nutrient addition at each elevation.

We show that soil GHG fluxes and consequently net soil global warming potential of TMFs can change considerably along an elevation gradient, following a general descending trend with increasing elevation. Results indicated further, that elevated N and P deposition can strongly affect soil GHG fluxes in Andean TMFs, but responses of soil GHG fluxes to nutrient addition depend largely on initial soil nutrient status, duration of nutrient addition and inter-annual variability in climatic conditions. Since nutrient addition effects were not linear with time of exposure, and complex interactions with other ecosystem components exist, there are some uncertainties in predicting effects of nutrient depositions on soil GHG fluxes. However, we provide the first data on mid-term nutrient effects of N, P and N+P on fluxes of the three main soil GHGs along an elevation gradient of Andean TMFs. Our results suggest that the net soil global warming potential across the elevation gradient might slightly increase with increasing N input, whereas it might decrease with increasing P and N+P inputs.

RESUMEN

Los suelos de los bosques tropicales desempeñan un papel importante en el clima de la Tierra mediante el intercambio con la atmosfera de grandes cantidades de gases de efecto invernadero (GEI). Sin embargo, esta importante función podría ser alterada por las actividades humanas causando el aumento en la deposición de nutrientes en los ecosistemas terrestres, especialmente en las regiones tropicales. Las causas de cómo el incremento de las cantidades de nutrientes está afectando los flujos de suelo de los GEI de los bosques tropicales es relativamente poco conocida, por ello los monitoreos de nutrientes *in situ* de los bosques montanos tropicales (BHT) son aún menos comprendidos. Ya que los BHT representan alrededor del 11-21% de la superficie forestal tropical, es de vital importancia predecir y cuantificar los cambios en los flujos de GEI del suelo en respuesta a la adición de nutrientes ya que podrían favorecer la retroalimentación a otros cambios globales. Esta tesis tiene como objetivo cuantificar el impacto de adición moderada de nitrógeno (N) y/o fósforo (P) en los flujos de tres GEI en suelo: dióxido de carbono (CO_2), óxido nitroso (N_2O) y el metano (CH_4), a lo largo de un gradiente altitudinal (1000 m, 2000 m, 3000 m) de los BHT primarios en el sur de Ecuador.

Desde hace más de cinco años, se ha medido los flujos de GEI del suelo en un experimento de manipulación de nutrientes ('NUMEX', por sus siglas en inglés), con replicas para control, y la adición de N (50 kg N ha^{-1} $año^{-1}$), P (10 kg P ha^{-1} $año^{-1}$) y N+P. Las mediciones *in situ* se realizaron mensualmente utilizando cámaras ventiladas estáticas, seguido por un análisis de cromatografía de gases para conseguir una perspectiva más profunda sobre los procesos implicados en el intercambio suelo-atmósfera de GEI. Se realizaron nuevas investigaciones incluyendo el monitoreo de factores básicos de control (i.e. temperatura del suelo, humedad y las concentraciones del N mineral), los diferentes componentes de los flujos de CO_2 del suelo, tasas de reciclaje netos de N y fuentes de los

flujos de N_2O del suelo. Con este propósito, se utilizó la extracción de hojarasca y técnicas de excavación de zanjas (*trenching technique*), incubación de las muestras *in situ* (*buried bag method*) y el etiquetaje de ^{15}N de corto plazo.

Los flujos de GEI del suelo en los bosques que estudiados se mostraron en el rango de aceptado de los flujos de gases de otras BHT en elevaciones comparables, excepto para el N_2O. Los flujos de N_2O, que se derivan principalmente de la des nitrificación, fueron bajos para un TMF lo que se puede atribuir a los ciclos conservativos de N del suelo en nuestros sitios de estudios. Los suelos fueron fuentes de CO_2 y N_2O (la intensidad del recurso disminuye al aumentar la altitud) y en todas las elevaciones el CH_4 es bajo.

Encontramos efectos de los nutrientes en todos los flujos de GEI medidos en cada elevación. Las respuestas de los flujos de CO_2 del suelo cambian con la duración y el tipo de nutrientes adicionado. En 1000 m, la adición del N no afecta los flujos de CO_2 del suelo, mientras que las adiciones de P y N+P disminuyeron los flujos en el primer y cuarto a quinto año. En 2000 m., la adición de N y N+P incrementa los flujos de CO_2 en el primer año; a partir de entonces, la adición del N disminuye los flujos mientras que la adición de N + P no mostro ningún efecto la adición de P carece de efectos. En 3000 m, la adición de N además incrementó los flujos de CO_2 constantemente; la adición de P y N+P aumentaron los flujos sólo en el primer año a partir de entonces no existió ningún efecto. Los efectos diferenciales de los nutrientes estuvieron relacionados a un estatus del N y P y respuestas variadas de los componentes de la respiración del suelo.

Las respuestas de los flujos de N_2O y CH_4 a la adición de nutrientes mostraron gran variabilidad entre años. Los flujos de N_2O no se vieron afectados por la adición de tres a cinco años de N a pesar de las diferencias significativas observadas durante los dos primeros años del mismo experimento. Atribuimos la ausencia de las respuestas en años mas tardíos debido a los contenidos bajos de humedad del suelo en nuestro periodo de

monitoreo 2010-2012. En todo el gradiente altitudinal, la adición de P disminuyó los flujos de N_2O y las concentraciones de N mineral, presumiblemente debido a que alivió de la limitación del P en la producción primaria neta, lo que aumentó la captación de N a través de las plantas. La adición de N+P además mostró tendencias similares las respuestas a la adición de N solamente, pero con efectos menos fuertes debido a los efectos contrapuestos de la adición de P.

Durante los dos primeros años de la adición de nutrientes, los flujos de CH_4 no se vieron afectados en ninguna elevación, lo cual atribuimos a la combinación de cantidades moderadas de nutrientes añadidos, la fuerte inmovilización de nutrientes, y la separación de la más alta capacidad de absorción de CH_4 en el subsuelo de la superficie del suelo donde se añaden fertilizantes. En el tercer a quinto año, la adición de nutrientes del suelo aumentaron la captación de CH_4, aunque los efectos de N y P variaron a lo largo del gradiente altitudinal: en 1000 m, la adición de N y N+P aumentó la captación anual de CH_4 a 20-60%; en 2000 m P y N+P incrementaron la captación a 21-50%; y en 3000 m la adición de P y N+P incrementó la captación de CH_4 a 34-40%. Estos efectos diferenciales de la adición de nutrientes pueden estar relacionados con el estatus inicial de del suelo y respuesta diferenciales de otros componentes del ecosistema a la adición de nutrientes en cada elevación.

Demostramos que los flujos de GEI del suelo y consecuentemente la red potencial de calentamiento global del suelo pueden cambiar considerablemente a lo largo de un gradiente de elevación, siguiendo una tendencia general de disminución con el aumento de la elevación. Los resultados indican además que la elevada deposición de N y P puede afectar los flujos de GEI del suelo en los BHT Andinos, pero las respuestas a los flujos de GEI a la adición de nutrientes depende del estatus inicial de los nutrientes del suelo, la duración de la adición de nutrientes y la variabilidad inter-anual de las condiciones

climáticas. Puesto que los efectos de la adición de nutrientes fueron no lineares con el tiempo de exposición y a la par existen complejas interacciones con otros componentes del ecosistema, aún quedan muchas incertidumbres en la predicción exacta de los efectos de la deposición de nutrientes en los flujos de GEI. Sin embargo, ofrecemos los primeros datos sobre los efectos de nutrientes a medio plazo de N, P y N+P en los flujos de los tres principales gases de efecto invernadero del suelo a lo largo de un gradiente altitudinal de los BHT Andina. Nuestros resultados sugieren que la red potencial de calentamiento global de los suelos en todo el gradiente altitudinal podría aumentar ligeramente con la entrada contribución de N, mientras que podría disminuir con el aumento de la contribución de P y N+P.

CHAPTER 1

General introduction

1.1 Global change - significance and complexity

Human activities are changing global environmental processes in a largely unregulated way, with limited knowledge as to the consequences; however, these changes could affect the basic functioning of the Earth system and thus human life (Steffen et al. 2004).

Major global changes currently impacting the earth include the alteration of biogeochemical cycles (e.g. nitrogen [N], carbon [C]) and rising atmospheric greenhouse gas (GHG) concentrations, the latter directly changing the earth's climate (IPCC 2013). Atmospheric concentrations of the three major GHGs: carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) have increased between 20 and 150 % since pre-industrial times (Table 1.1). Increasing concentrations drive global warming by increasing global surface temperatures, which are likely to exceed 2°C in 2100 relative to 1850-1900 (IPCC 2013). This would move temperatures well outside the range of natural variability that has been exhibited for thousands of years. This temperature shift could potentially exceed tipping points, causing the Earth system to switch quickly from its current state to another state, which may prove much less amenable to human life (Steffen et al. 2004).

Although increases in atmospheric GHG concentrations are primarily driven by fossil-fuel emissions, they are also linked to other global changes, and cascade through the Earth system in complex ways. Cultivation of N-fixing plants, fossil fuel and fertilizer use, biomass burning, and industrialization, for example, more than double the amount of reactive N cycling globally, with consequent increases in atmospheric N deposition (Galloway et al. 2008). Increasing atmospheric N deposition, in turn, can affects soil GHG fluxes, and is therefore indirectly responsible for the 0.4-1.3 Tg N yr^{-1} of anthropogenic N_2O land emissions. This range of emissions is similar in magnitude to direct emissions due to fossil-fuel use and industrial processes (0.2-1.0 Tg N yr^{-1}; IPCC 2013). This

comparison, which does not include potential feedbacks of changing temperature on land emissions, illustrates the complexity of human impacts on the Earth system, and the need to understand and quantify global changes, in order to predict, manage and possibly prevent potential negative impacts.

Table 1.1 An overview of climate-relevant characteristics of the three greenhouse gases carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) (IPCC 2013)

	CO_2	N_2O	CH_4
Pre-industrial atmospheric concentrations in 1750	278 ± 2 ppm	270 ± 7 ppb	722 ± 25 ppb
Atmospheric concentrations in 2011	391 ± 0.2 ppm	324 ± 0.1 ppb	1803 ± 2.0 ppb
Change in atmospheric concentrations (%) between 1750-2011	41 (113 ppm)	20 (54 ppb)	150 (1081 ppb)
Absolute change in radiative forcing (W/m^2)	1.82 ± 0.19	0.17 ± 0.03	0.48 ± 0.05
Atmospheric lifetime (yrs)	50-200*	131	9
Global warming potential (100 yrs)**	1	298	34

*No single lifetime can be given; range reported by Batjes and Bridges 1992

**including climate-carbon feedbacks

1.2 Greenhouse gas fluxes from tropical forest soils

Although the current atmospheric GHG concentrations of CO_2, N_2O and CH_4 are dominated by human activities, soils - especially tropical forest soils - are an important natural controller of these GHGs and thus important for the earth's climate.

Soil CO_2 emissions are the second-largest flux in the global C cycle (Schlesinger and Andrews 2000). Tropical forest soils have higher annual CO_2 emission rates than any other forest biome (Luyssaert et al. 2007), which is significant in terms of climate change, since, after water vapor, CO_2 is the most abundant GHG in the atmosphere (Table 1.1). However, due to the ability of plants to fix CO_2 via photosynthesis, intact forest ecosystems appear to

be CO_2 sinks (Dalal and Allen 2008; Luyssaert et al. 2007). In soils, CO_2 is produced via root and heterotrophic respiration (Figure 1.1) and the relative contributions of these sources, although critical to the understanding of total soil CO_2 emissions, have only rarely been quantified (Kuzyakov 2006); soil CO_2 emissions are still one of the least understood fluxes in the C cycle (Houghton 2007; Malhi et al. 1999). The two main controlling factors for CO_2 emission are soil temperature and moisture (Schwendenmann et al. 2003). However, several indirect factors such as soil type, vegetation, landscape position and nutrient availability can also affect soil CO_2 fluxes (Luo and Zhou 2006; Raich 1998).

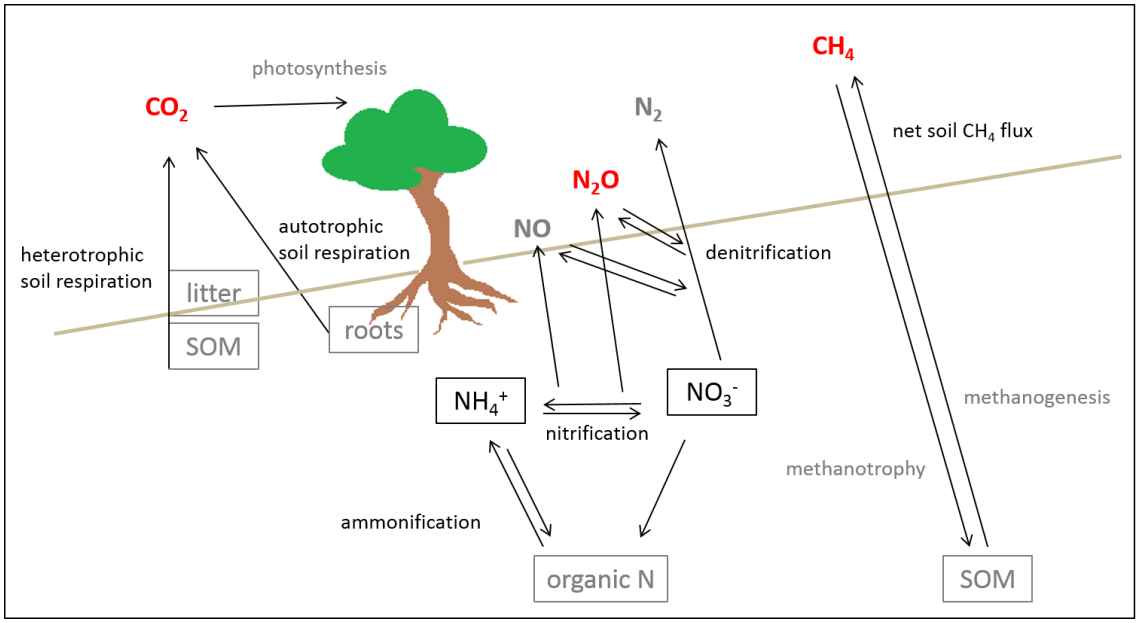


Figure 1.1 Flow diagram of processes involving the production and consumption of the three main soil greenhouse gases (red) in forest soils; processes and stocks that were measured as part of this study (see Chapters 2-4) are indicated in black.

The biggest natural source of atmospheric N_2O are soils (Denman et al. 2007), and tropical forest soils contribute most to these emissions, accounting for 30% (Dentener et al. 2001). Although N_2O fluxes and concentrations in the atmosphere are low, even small changes in atmospheric N_2O concentrations can largely affect the global climate, due to the 298 times higher global warming potential (GWP) of N_2O compared to CO_2 (Table 1.1). N_2O is produced in soils mainly during the microbial processes of nitrification and

denitrification, although N_2O can also be consumed during the anoxic process of denitrification (Figure 1.1; Chapuis-Lardy et al. 2007). How the two main controlling factors, N cycling and soil water content, affect N_2O fluxes has been described in the conceptual 'hole-in-the-pipe' model (Firestone and Davidson 1989). However, several other factors such as soil temperature, organic C contents (Weier et al. 1993) and soil pH have also been found to be important controls of soil N_2O fluxes (Weslien et al. 2009).

Finally, soils are important natural biogenic sinks and sources of CH_4; forest soils are generally strong net CH_4 sinks (Le Mer and Roger 2001), although in tropical forests, canopy wetlands have been found to be CH_4 sources (Martinson et al. 2010). Tropical forest soils contribute about 28% to the global annual CH_4 uptake by soils (Dutaur and Verchot 2007). Consequently, they represent important sinks of atmospheric CH_4 concentrations, which have increased dramatically since levels before the industrial revolution (Table 1.1; Etheridge et al. 1998). In combination with its relatively higher GWP compared to CO_2, this makes CH_4 the second most important GHG causing global warming (Denman et al. 2007). In soils, CH_4 is produced via anaerobic oxidation of C, mainly by methanogenic archae, and consumed via oxidation by methanotrophic bacteria (Figure 1.1; Le Mer and Roger 2001). The dominance of one process over the other determines if soils are sinks or sources of CH_4; generally wetland soils are net sources of CH_4 and aerated upland forest soils are net sinks for atmospheric CH_4 (Le Mer and Roger 2001). The strength and direction of CH_4 fluxes in aerated soils are mainly controlled by soil moisture (Bowden et al. 1998), soil texture (Dörr et al. 1993) and the presence of organic layers (Saari et al. 1998). However, soil temperature (Le Mer and Roger 2001) and N availability (Bodelier and Laanbroek 2004) have also been shown to be important controlling factors.

1.3 Nutrient deposition in tropical regions, tropical montane forests and nutrient effects on soil greenhouse gas fluxes

Increasing N deposition due to human impacts have been shown to affect many ecosystem functions, causing acidification (Matson et al. 1999), aquatic eutrophication (Smith et al. 1999), biodiversity loss (Phoenix et al. 2006) and changes in soil GHG fluxes (Corre et al. 2014). Currently, dramatic increases in atmospheric N deposition are occurring in tropical areas (Galloway et al. 2004; Hietz et al. 2011) and further increases are predicted within the next decades, with predicted rates exceeding 25 kg N ha^{-1} yr^{-1} (Figure 1.2; Phoenix et al. 2006). Additionally, in tropical forests of South America, atmospheric phosphorus (P) depositions are expected to increase due to biomass burning and dust inputs (Mahowald et al. 2005; Okin et al. 2004). Changes in P deposition will be relatively small as compared to N deposition, but since P and N are both major nutrients limiting net primary productivity (NPP), not only their single but also their combined impact is of interest in tropical forests. Studying forest response to nutrient additions is especially important in tropical regions, since these highly diverse forests have recently been recognized to contradict Liebig's law (which posits a single limiting factor for plant growth), instead having complex and multiple nutrient limitations (Homeier et al. 2012; Kaspari et al. 2008; Wright et al. 2011). Tropical montane forests (TMFs), which seem to be co-limited by N and P (Homeier et al. 2012; Tanner et al. 1998), might particularly be affected by increasing N and P depositions, due to the importance of cloud water deposition in this ecosystem (Carillo et al. 2002). Not only are ion concentrations higher in fog water compared to rain water (Rollenbeck et al. 2008), but this form of water input reduces the risk of immediate nutrient losses via leaching or overland flow, which often occur with heavy rainfall events.

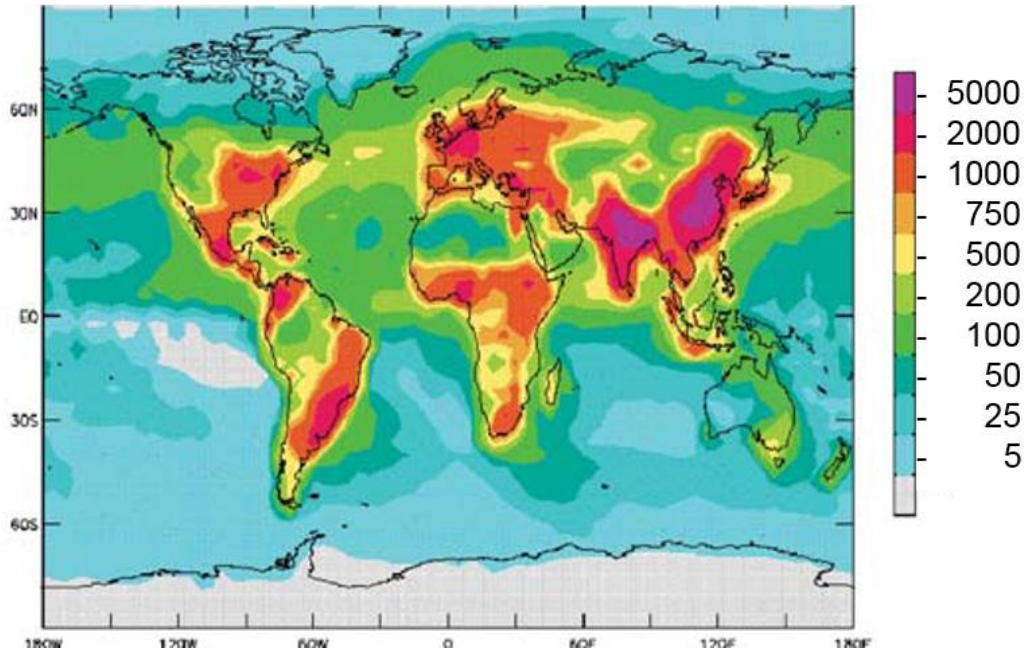


Figure 1.2 Projected total inorganic N deposition in 2050 (mg N m^{-2} yr^{-1}) (from Galloway et al. 2004)

TMFs occur within mountainous regions in tropical latitudes, spanning altitudinal gradients of 300 - 3,900 m above sea level (asl) (Stadtmüller 1987) and comprising a remarkable variety of climatic, floral and soil characteristics. However, moving upwards along elevation gradients, some general changes consistently occur (Figure 1.3); these include decreases in: temperature, tree height, complexity of forest strata and leaf size, and increases in: tree density, epiphytic density, the amount of gnarled trees, the tendency towards sclerophyll leaves and cloud incidence (Bruijnzeel and Hamilton 2000; Hamilton 1995; Richter 2008; Stadtmüller 1987). Although soil characteristics vary greatly, shallow soils with densely rooted organic layers of increasing thickness dominate at higher elevations (Wilcke et al. 2002).

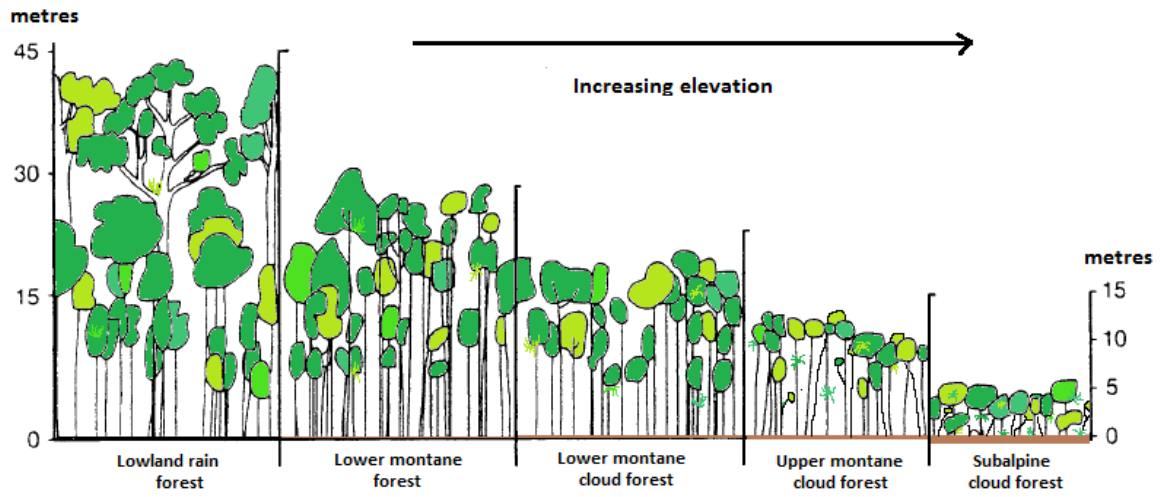


Figure 1.3 Generalized altitudinal forest formation series in the humid tropics (adapted from Bruijnzeel and Hamilton 2000)

Globally, TMFs represent an estimated 11 – 21% of global tropical forests (Bubb et al. 2004; FAO 1993; Spracklen and Righelato 2014), rank among the world's most important biodiversity hotspots (Myers et al. 2000) and fulfill important ecosystem services. They provide a reliable supply of high-quality water (Bruijnzeel 2005) and have important climate regulation functions, since they do not only cycle large amounts of CO_2 through plants, but their soils are also sinks and sources of the three major atmospheric GHGs (CO_2, N_2O and CH_4). Still, TMFs are one of the least-studied forest ecosystems in terms of ecosystem functioning (Bruijnzeel and Hamilton 2000; Bruijnzeel 2005) and their research history is rather short (Stadmüller 1987). A process-orientated understanding of TMFs is particularly lacking, and can be attributed to their high local and regional variability (Townsend et al. 2008) in combination with scarcity of long-term and ecosystem-integrated monitoring studies (Bruijnzeel and Hamilton 2000; Hamilton 1995). This is problematic, however, since the impact of human activities (locally and globally) is increasing rapidly in tropical regions and substantial changes in ecosystem processes and functioning are expected. For example, changes due to increasing nutrient deposition are still largely unquantified and poorly understood (Boehmer 2011).

Although nutrient deposition in tropical regions is increasing, most studies reporting effects of increasing nutrient inputs (mainly of N) on forest soil GHG fluxes stem from temperate regions (Wei et al. 2008). In these studies, N-addition does not always affect soil GHG fluxes, but studies reporting significant effects have generally found soil N_2O fluxes to increase and soil CH_4 uptake to decreases with N addition, while the effect on soil CO_2 fluxes varied from increasing to decreasing, depending, among others, on duration of nutrient addition. The effect of P addition on soil GHG fluxes is generally less studied in forest ecosystems, and plants seem to play a larger role in the response of GHG fluxes to P addition compared to N addition (Keith et al. 1997; Zhang et al. 2011). However, in tropical forests, nutrient effects might differ from temperate forests, due to their high diversity and thus heterogeneity (Townsend et al. 2008), year-round biological activity and NP-co-limitation of NPP (Hobbie and Vitousek 2000). In addition, many studies looking at GHG fluxes do not do so in an ecologically-relevant manner.

Of the studies looking at nutrient effects on GHG fluxes, several have been laboratory studies (e.g. Flessa et al. 1996; Saari et al. 1997; Teklay et al. 2006). Although such studies are helpful tools to investigate direct nutrient effects on soil GHG fluxes, by excluding ecosystem components their results are often different than in-situ manipulations and measurements (Cleveland and Townsend 2006). Since it is important to understand potential nutrient effects on soil GHG fluxes from TMFs on an ecosystem-scale, in-situ measurements are necessary, preferably using large-area and long-term measurements. Although some in-situ nutrient manipulation studies have been conducted in TMFs, studies often restrict measurements to one elevation (Hall and Matson 2003; Koehler et al. 2009a,b). Furthermore, in many studies, applied nutrient amounts are unrealistically high compared to expected nutrient depositions, with plot sizes that are too small to represent the highly diverse tropical forest ecosystem (Cleveland and Townsend 2006; Fisher et al.

2013). Finally, studies frequently concentrate on only one GHG for a short period of time (measurements rarely exceed 1 year) (Fisher et al. 2012; Hall and Matson 2003). Although these studies certainly contribute to the general understanding of nutrient input on soil GHG fluxes, they do not provide reliable data on the *long-term* impact of increasing nutrient deposition in TMFs and their resulting contribution to climate change.

1.4 Objectives

The aim of this study was to investigate the effect of moderate nutrient input of N and/or P (up to five years) on greenhouse gas fluxes (CO_2, N_2O, CH_4) from tropical montane forest soils along an elevation gradient in southern Ecuador.

We expected nutrient addition to affect soil GHG fluxes, in the same way as observed in other studies from tropical forests and previous results from our study area. We tested the following hypotheses (a detailed justification for each is given in the introductory sections of Chapters 2 to 4):

(1) Soil CO_2 fluxes will decrease with increasing elevation and response to nutrient addition will change over time, since different components of soil CO_2 fluxes will react with different magnitudes and directions. The combined addition of N and P will lead to stronger effects than the addition of single nutrients.

(2) Net soil-N cycling and soil N_2O fluxes, which increased within the first two years of N and N+P addition in our experiment (Martinson et al. 2013) will continue to increase, while P addition will have a minimal effect or might even decrease soil N_2O emissions. Soil N_2O fluxes will be dominated by denitrification processes in these moist tropical forest soils.

(3) N, P and N+P addition will increase soil CH_4 uptake, since forests showed evidence of N and P co-limitation (Homeier et al. 2012, 2013) and there are indications of N-limited CH_4 uptake in our study area (Wolf et al. 2012).

1.5 Material and methods

1.5.1 Study area and experimental design

Our study was conducted in three TMF sites, located along an elevation gradient (1000 m, 2000 m and 3000 m asl) in the Cordillera Real, a mountain chain in the eastern range of the South Ecuadorian Andes. While tropical forests formerly dominated the landscape of the Ecuadorian Andes, their extent has been significantly decreased through anthropogenic influences and deforestation rates are still high (Beck et al. 2008). However, protected old-growth forests remain in the Podocarpus National Park (~1460 km²; Naughton-Treves et al. 2006) and parts of the adjacent 'Reserva Biológica San Francisco' (~11.2 km²; 1600-3140 m as; Beck et al. 2008), which lay within the Ecuadorian provinces of Loja and Zamora Chinchipe. This area of forests, which served as our study area for this research (Figure 1.4), has been identified as a center of endemism and diversity for major groups of organisms including birds, various insects (e.g. moths) and vascular plants (e.g. Beck and Richter 2008; Beck et al. 2008; Brehm et al. 2005; Brummitt and Lughadha 2003; Jørgensen et al. 2011). A detailed description of the study area is given by Richter et al. (2008) and several ecosystem aspects within this area have already been investigated (Beck et al. 2008; Bendix et al. 2013).

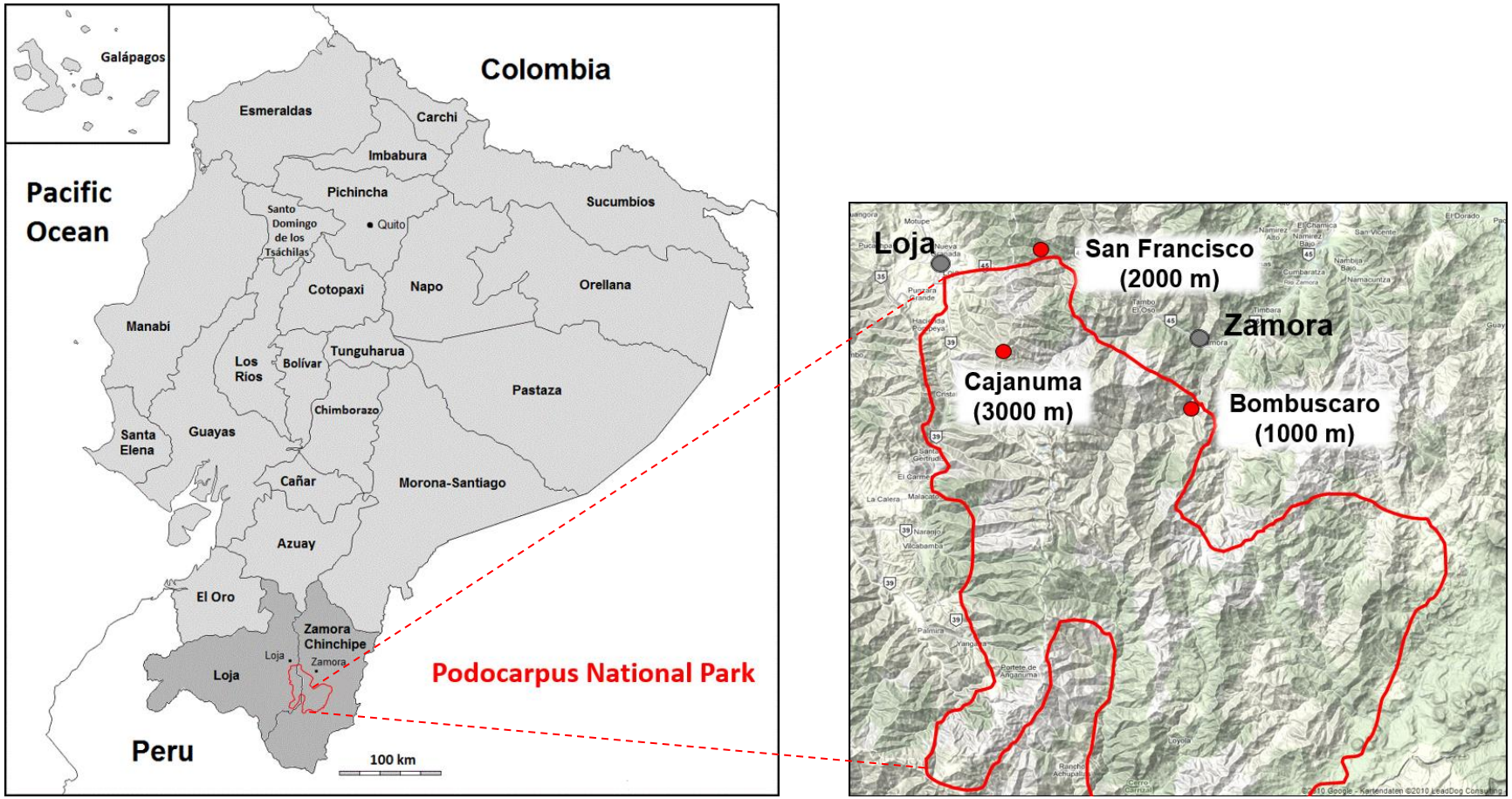


Figure 1.4 Map of Ecuador with the Podocarpus National Park situated in the South (left) and a map with the locations of the three study sites in and adjacent to the Podocarpus National Park (right). Maps adapted from: http://d-maps.com (left) and http://maps.google.de (right).

For our study, a nutrient manipulation experiment (NUMEX) was established as a complete block design, with four replicate blocks at each of the three study sites (elevations). Each block contained four treatment plots: N addition, P addition, N+P addition and untreated control (Figure 1.5). Nutrient application started in 2008 and amounts were split into two equal applications per year at moderate rates of 50 kg N ha^{-1} yr^{-1} (as urea) and 10 kg P ha^{-1} yr^{-1} (as sodium hydrogen phosphate). More detailed information about the study sites and experimental setup is given in the materials and methods sections of Chapters 2 to 4.

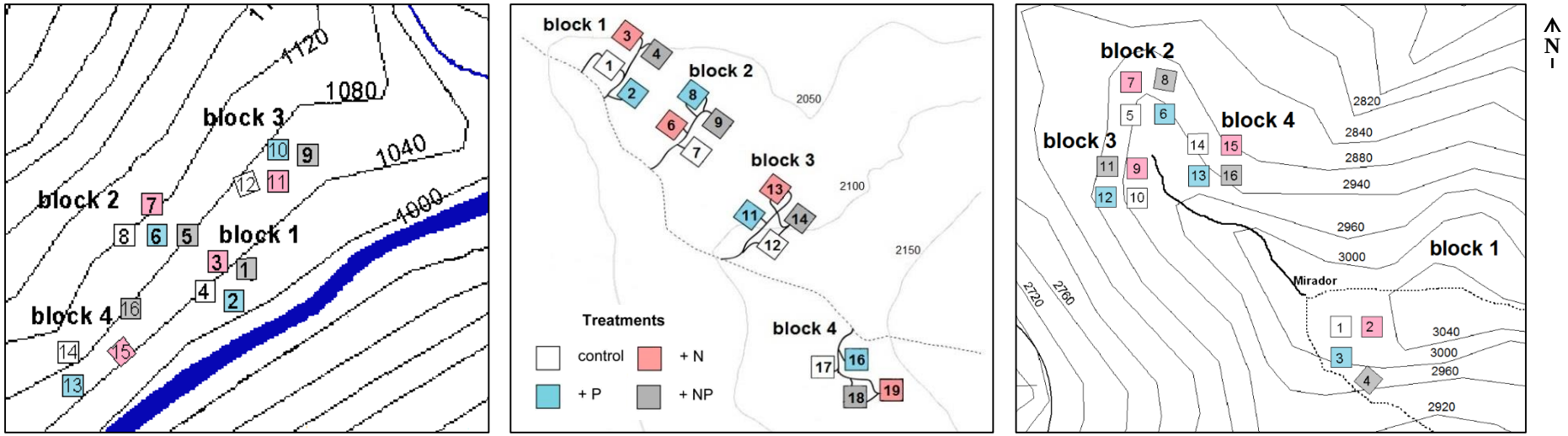


Figure 1.5 Topographic maps showing the plot layout of the nutrient manipulation experiment (NUMEX) along the elevation gradient at 1000 m, 2000 m and 3000 m (left to right) in tropical montane forests of southern Ecuador. Diagrams adapted from J. Homeier.

1.5.2 Methodological overview

We measured soil CO_2, N_2O and CH_4 fluxes once a month from November 2010 to August 2012 (years three to five of nutrient addition) using static vented chambers. G.O. Martinson (Martinson 2011) provided data of soil CO_2 and CH_4 fluxes measured from January 2008 to September 2009, using the same methodological approach. Gas samples were analyzed using gas chromatographs equipped with an electron capture detector and flame ionization detector and gas fluxes were calculated from the linear increase of gas concentrations in the chamber headspace over time. Parallel to gas sampling, soil temperature, gravimetric soil moisture and extractable mineral ammonium (NH_4^+) and nitrate (NO_3^-) of the top 5 cm of soil were determined.

During 2011 and 2012, several additional measurements were performed to distinguish between different sources of soil CO_2 fluxes: a small-scale litter removal and trenching experiment was established within NUMEX and monthly gas flux measurements were carried out for 1.5 years. Net N cycling rates were also measured in-situ on three occasions, using the 'buried bag method'. Finally, the relative contribution of NH_4^+ and NO_3^- to soil N_2O fluxes were quantified in control and N-amended plots on two occasions, using short-term ^{15}N tracing to $^{15}N_2O$. An overview on processes and stocks measured within this study is shown in Figure 1.1 and more detailed methodological descriptions are given in the materials and methods sections of Chapters 2 to 4.

1.6 References

Batjes NH, Bridges EM (1992) *A review of soil factors and processes that control fluxes of heat, moisture and greenhouse gases*. Technical Paper 23, International Soil Reference and Information Centre, Wageningen.

Beck E, Makeschin F, Haubrich F, Richter M, Bendix J, Valarezo C (2008) The ecosystem (Reserva Biológica San Francisco). In: Beck E, Bendix J, Kottke I, Makeschin F, Mosandl R (eds) *Gradients in a tropical mountain ecosystems of Ecuador.* Ecological Studies, Vol. 198, Springer-Verlag, Berling Heidelberg, pp. 1-14.

Beck E, Richter M (2008) Ecological aspects of a biodiversity hotspot in the Andes of southern Ecuador. In: Gradstein SR, Homeier J, Gansert D (eds) *The Tropical Mountain Forest – Patterns and Processes in a Biodiversity Hotspot*. Göttinger Centre for Biodiversity and Ecology, Biodiversity and Ecology Series 2: 195-217.

Bodelier PLE, Laanbroek HJ (2004) Nitrogen as a regulatory factor of methane oxidation in soils and sediments. *FEMS Microbiology Ecology 47*: 265–277.

Boehmer HJ (2011) Vulnerability of tropical montane rain forest ecosystems due to climate change. In: Brauch HG, Spring ÚO, Mesjasz C, et al. (eds) *Coping with global environmental change, disasters and security: threats, challenges, vulnerabilities and risks.* Hexagon Series on Human and Environmental Security and Peace, Vol. 5, Springer-Verlag Berlin, Heidelberg, New York. pp. 789-802.

Bowden RD, Newkirk KM, Rullo GM (1998) Carbon dioxide and methane fluxes by a forest soil under laboratory-controlled moisture and temperature conditions. *Soil Biology and Biochemistry 30*: 1591–1597.

Brehm G, Pitkin LM, Hilt N, Fiedler K (2005) Montane Andean rain forests are a global diversity hotspot of geometrid moths. *Journal of Biogeography 32*: 1621-1627.

Bruijnzeel LA (2005) Tropical montane cloud forest: a unique hydrological case. In: Bonell M, Bruijnzeel LA (eds) *Forests, Water and People in the Humid Tropics: Past, Present and Future Hydrological Research for Integrated Land and water Management.* Cambridge University press, Cambridge, UK, pp 462-483 (international hydrology series).

Bruijnzeel LA, Hamilton LS (2000) *Decision time for cloud forests. Water-related issues and problems of the humid tropics and other warm humid regions.* IHP Humid tropics program series No. 13. IHP-UNESCO, Paris.

Brummitt N, Lughadha EN (2003) Biodiversity: where's hot and where's not. *Conservation Biology 17*(5): 1442-1448.

Bubb P, May I, Miles L, Sayer J (2004) *Cloud forest agenda*. UNEP-WCMC, Cambridge, UK, 33 pages; Online: http://www.unep-wcmc.org/resources/publications/UNEP_WCMC_bio_series/20.htm.

Chapuis-Lardy L, Wrage N, Metay A, Chotte J-L, Bernoux M (2007) Soils, a sink for N_2O? A review. *Global Change Biology 13*: 1-17.

Carillo JH, Galanter Hastings M, Sigman DM, Huebert BJ (2002) Atmospheric deposition of inorganic and organic nitrogen and base cations in Hawaii. *Global Biogeochemical Cycles 16*: 1076.

Cleveland CC, Townsend AR (2006) Nutrient additions to a tropical rain forest drives substantial soil carbon dioxide losses to the atmosphere. *PNAS 203*: 10316-10321.

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.

Dalal RC, Allen DE (2008) Turner Review No. 18 Greenhouse gas fluxes from natural ecosystems. *Australian Journal of Botany 56*: 369-407.

Denman KL, Brasseur G, Chidthaisong A, et al. (2007) Couplings between changes in the climate system and biogeochemistry. In: Solomon S, Qin D, Manning M, Marquis M, Averyt K, Tignor MMB, Miller Jr HL, Chen Z (eds) *Climate change 2007: The physical science basis, contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change*. Cambridge University Press, Cambridge, UK and New York, USA. pp 499-587.

Dentener F, Derwent, R, Dlugokencky E, et al. (2001) Atmospheric chemistry and greenhouse gases. In: Houghton JT, Ding Y, Griggs DJ, et al. (eds) *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York. pp 241-287

Dörr H, Katruff L, Levin I (1993) Soil texture parameterization of the methane uptake in aerated soils. *Chemosphere 26*: 697–713.

Dutaur L, Verchot LV (2007) A global inventory of the soil CH_4 sink. *Global Biogeochemical Cycles 21*: GB4013.

Etheridge D, Steele L, Francey R, Langenfelds R (1998) Atmospheric methane between 1000 A.D. and present: Evidence of anthropogenic emissions and climatic variability. *Journal of Geophysical Research 103*: 15979–15993.

FAO (1993) *Forest resources assessment 1990 - Tropical countries*. FAO Forestry Paper 112, Food and Agriculture Organization of the United Nations, Rome, Italy. http://www.fao.org/docrep/007/t0830e/t0830e00.htm

Firestone MK, Davidson EA (1989) Microbiological basis of NO and N_2O production and consumption in soil. In: Andreae MO, Schimel DS (eds) *Exchange of trace gases between terrestrial ecosystems and the atmosphere*. John Wiley and Sons, New York, pp. 7-21.

Fisher JB, Malhi Y, Torres IC, et al. (2012) Nutrient limitation in rainforests and cloud forests along a 3,000-m elevation gradient in the Peruvian Andes. *Oecologia 172*: 889-902.

Flessa H, Pfau W, Dörsch P, Beese F (1996) The influence of nitrate and ammonium fertilization on N2O release and CH4 uptake of a well-drained topsoil demonstrated by a soil microcosm experiment. *Journal of Plant Nutrition and Soil Science 159*: 499-503.

Galloway JN, Townsend AR, Erisman JW, et al. (2008) Transformation of the nitrogen cycle: Recent trends questions, and potential solutions. *Science 320*: 889-892.

Galloway JN, Dentner 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.

Hamilton LS (1995) Mountain cloud forest conservation and research: a synopsis. *Mountain Research and Development 15*: 259-266.

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.

Hobbie SE, Vitousek PM (2000) Nutrient limitation of decomposition in Hawaiian forests. *Ecology 81*: 1867-1877.

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. *PLoS ONE 7*: e-47128.

Homeier J, Leuschner C, Bräuning A, et a.l (2013) Effects of nutrient addition on the productivity of montane forests and implications for the carbon cycle. 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, Springer, Heidelberg, pp. 315-329.

Houghton RA (2007) Balancing the global carbon budget. *Annual Review of Earth and Planetary Sciences 35*: 313-347.

IPCC (2013) *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Stocker TF, Qin D, Plattner G-K, et al. (eds). Cambridge University Press, Cambridge, United Kindom and New York.

Jørgensen PM, Ulloa Ulloa C, León B, et al. (2011) Regional patterns of vascular plant diversity and endemism. In: Herzog SK, Martínze R, Jørgensen PM, Tiessen H (eds) *Climate change and biodiversity in the Tropical Andes*. São José dos Campos: Inter-American Institute for Global Change Research, pp. 192-203.

Kaspari M, Garcia MN, Harms KE, Santana M, Wright SJ, Yavitt JB (2008): Multiple nutrient limit litterfall and decomposition in a tropical forest. *Ecology Letters 11*: 35-43.

Keith H, Jacobsen KL, Raison RJ (1997) Effects of soil phosphorus availability, temperature and moisture on soil respiration in *Eucalyptus pauciflora* forest. *Plant and Soil 190*: 127-141.

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

Koehler N, Corre MC, Veldkamp E, Sueta JP (2009b) Chronic nitrogen addition causes a reduction in soil carbon dioxide efflux during the high stem-growth period in a tropical montane forest but no response from a tropical lowland forest on a decadal time scale. *Biogeosciences 6*: 2973-1983.

Kuzyakov Y (2006) Sources of CO_2 efflux from soil and review of partitioning methods. *Soil Biology and Biochemistry 38*: 425-448.

Le Mer J, Roger P (2011) Production, oxidation, emission and consumption of methane by soils: A review. *European Journal of Soil Biology 37*: 25-50.

Luo Y, Zhou X (2006) *Soil respiration and the environment*. Academic press, San Diego.

Luyssaert S, Inglima I, Jung M, et al. (2007) CO_2 balance of boreal, temperate, and tropical forests derived from a global database. *Global Change Biology 13*: 2509-2537.

Mahowald NM, Artaxo P, Baker AP, 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.

Malhi Y, Baldocchi DD, Jarvis PG (1999) The carbon balance of tropical, temperate and boreal forests. *Plant, Cell and Environment 22*: 715-740.

Martinson GO, Werner FA, Scherber C, et al. (2010) Methane emissions from tank bromeliads in neotropical forests. *Nature Geoscience 3*: 766-769.

Martinson GO (2011) *Trace gas fluxes from tropical montane forests of Southern Ecuador*. PhD thesis, Georg-August-Universität Göttingen, Göttingen.

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.

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

Myers N, Mittermeier RA, Mittermeiet CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature 403*: 853- 858.

Naughton-Treves L, Alvarez-Berríos N, Brandon K, et al. (2006) Expanding protected areas and incorporating human resource use: a study of 15 forest parks in Ecuador and Peru. *Sustainability: Science Practice and Policy 2*: 32-44.

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

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.

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. *Global Biogeochemical Cycles 20*: GB3010.

Raich JW (1998) Aboveground productivity and soil respiration three Hawaiian rain forests. *Forest Ecology and Management 107*: 309-318.

Richter M (2008) Tropical mountain forests – distribution and general features. In: Gradstein SR, Homeier J, Gansert D (eds) *The Tropical Mountain Forest – Patterns and*

Processes in a Biodiversity Hotspot. Göttinger Centre for Biodiversity and Ecology, Biodiversity and Ecology Series 2: 7-24.

Rollenbeck R, Fabian P, Bendix J (2008) Temporal heterogeneities – matter deposition from remote areas. In: Beck E, Bendix J, Kottke I, Makeschin F, Mosandl R (eds) *Gradients in a tropical mountain ecosystems of Ecuador*. Ecological Studies, Vol. 198, Springer-Verlag, Berlin Heidelberg, pp. 303-309.

Saari A, Martikainen PJ, Ferm A, Ruuskanen J, de Boer W, Troelstra SR, Laanbroek HJ (1997) Methane oxidation in soil profiles of Dutch and Finnish coniferous forests with different soil texture and atmospheric nitrogen deposition. *Soil Biology and Biochemistry 29*: 1625-1632.

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.

Schlesinger WH, Andrews JA (2000) Soil respiration and the global carbon cycle. *Biogeochemistry 48*: 7-20.

Smith VH, Tilman GD, Nekola JC (1999) Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution 100*: 179-196.

Schwendenmann L, Veldkamp E, Brenes T, O'Brien JJ, Mackensen J (2003) Spatial and temporal variation in soil CO_2 efflux in an old-growth neotropical rain forests, La Selva, Costa Rica. *Biogeochemistry 64*: 111-128.

Spracklen DV, Righelato R (2014) Tropical montane forests are a larger than expected global carbon store. *Biogeosciences 11:* 2741-2754.

Stadtmüller T (1987) *Cloud forests in the humid tropics: A bibliographic review*. United Nations University Press, Tokyo, Japan and Centro Agronómico Tropical de Investigación y Enseñanza, Turrialba.

Steffen W, Sanderson A, Tyson PD, et al. (2004) *Global change and the earth system – A planet under pressure*. Global Change IGBP - Executive Summary, IGBP Secretariat, Stockholm.

Tanner EVJ, Vitousek PM, Cuevas E (1998) Experimental investigation of nutrient limitation of forest growth on wet tropical mountains. *Ecology 79*: 10-22.

Teklay T, Nordgren A, Malmer A (2006) Soil respiration characteristics of tropical soils from agricultural and forestry land-uses at Wondo Genet (Ethiopia) in response to C, N and P amendments. *Soil Biology and Biochemistry 38*: 125-133.

Townsend AR, Asner GP, Cleveland CC (2008) The biogeochemical heterogeneity of tropical forests. *Trends in Ecology and Evolution 23*: 424-431.

Wei Z, Jiangming M, Yunting F, Xiankai L, Hui W (2008) Effects of nitrogen deposition on the greenhouse gas fluxes from forest soils. *Acta Ecologica Sinica 28*: 2309-2319.

Weier KL, Doran JW, Power JF, Walters DT (1993) Denitrification and the dinitrogen/nitrous oxide ratio as affected by soil water, available carbon, and nitrate. *Soil Science Society of America Journal 57*: 66-72.

Weslien P, Klemedtsson ÅK, Börjesson G, Klemedtsson L (2009) Strong pH influence on N_2O and CH_4 fluxes from forested organic soils. *European Journal of Soil Science 60*: 311-320.

Wilcke W, Yasin S, Abramowski U, Valarezo C, Zech W (2002) Nutrient storage and turnover in organic layers under tropical montane rain forest in Ecuador. *European Journal of Soil Science 53*: 15-27.

Wolf K, Flessa H, Veldkamp E (2012) Atmospheric methane uptake by tropical montane forest soils and the contribution of organic layers. *Biogeochemistry 111*: 469-483.

Wright SJ, Yavitt JB, Wurzburger N, et al. (2011) Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest. *Ecology 92*: 1616-1625.

Zhang T, Zhu W, Mo J, Liu L, Dong S (2011) Responses of CH_4 uptake to the experimental N and P additions in an old-growth tropical forest, southern China. *Biogeosciences 8*: 2805-2813.

CHAPTER 2

Differential responses of soil CO_2 fluxes to nutrient inputs along an elevation gradient of Andean tropical montane forests

2.1 Abstract

Tropical forests play an important role in the global carbon cycle and are increasingly affected by elevated atmospheric nutrient inputs. We assessed the five-year impact of moderate nitrogen (N) and phosphorus (P) additions on total soil carbon dioxide (CO_2) fluxes and its components across an elevation gradient (1000, 2000 and 3000 m) of tropical montane forests in south Ecuador. In a nutrient manipulation experiment with control, N (50 kg N ha^{-1} $year^{-1}$), P (10 kg P ha^{-1} $year^{-1}$) and N+P additions, soil CO_2 fluxes were measured using static chambers. Soil CO_2 fluxes from controls decreased from 8.8 ± 0.2, 5.4 ± 0.4 to 2.4 ± 0.7 Mg C ha^{-1} $year^{-1}$ from 1000 m to 3000 m. Nutrient additions showed differential effects on soil CO_2 fluxes with elevation and duration of addition. At 1000 m, N addition did not affect soil CO_2 fluxes, whereas P and N+P additions decreased fluxes in the first and fourth-fifth years. At 2000 m, N and N+P additions increased CO_2 fluxes in the first year; thereafter, N addition decreased fluxes whereas N+P addition no longer showed any effect; P addition showed no effect. At 3000 m, N addition increased CO_2 fluxes consistently; P and N+P additions increased fluxes only in the first year showing no effect thereafter. Differential nutrient effects were related to initial soil N and P status and varied responses of soil respiration components. Our results illustrate that elevated N and P depositions can strongly affect the belowground C cycle in these Andean tropical montane forests.

2.2 Introduction

Tropical forests play an important role in the global carbon (C) cycle, storing over a fifth of global terrestrial C stocks [*Jobbagy and Jackson*, 2000; *Prentice et al.*, 2001] and cycling about 12% $year^{-1}$ of the atmospheric carbon dioxide (CO_2) through photosynthesis as well as plant/microbial respiration [*Malhi*, 2005]. In the global C cycle, CO_2 emissions from soils are the second-largest flux with an estimated 68-77 Pg C $year^{-1}$ [*Schlesinger and*

Andrews, 2000]. Despite their importance, soil CO_2 fluxes are one of the least understood fluxes in the C cycle [*Malhi et al.,* 1999; *Houghton*, 2007]. Soil CO_2 fluxes measured at the soil surface are mainly produced by autotrophic or root respiration (from roots, rhizosphere and associated mycorrhiza) and heterotrophic or microbial respiration, which can be subdivided into fresh litter respiration and soil organic matter (SOM) respiration [*Raich and Schlesinger*, 1992; *Hanson et al.*, 2000].

Although tropical montane forests (TMFs) cover more than 11% of the world's tropical forest area [*FAO,* 1993, 2001] and contain larger amounts of soil C than lowland tropical forests [*Dieleman et al.*, 2013], soil CO_2 fluxes are poorly studied in these diverse ecosystems. TMFs occur over large altitudinal gradients and experience a variety of environmental conditions, which at high elevations can impede decomposition processes, leading to thick organic layers [*Grubb*, 1977; *Stadtmüller*, 1987]. Soil CO_2 fluxes are controlled by many different factors. Among the more direct or proximal factors are soil temperature and moisture, whereas among the more indirect or distal factors are: soil type, vegetation, landscape position and nutrient availability. In TMFs, the combination of these controlling factors causes a reduction in soil CO_2 fluxes with increasing elevation (e.g. in Indonesia [*Purbopuspito et al.*, 2006] and Ecuador [*Wolf,* 2011]). Since soil CO_2 emissions are produced by different sources, which react differently to controlling factors [*van Straaten et al.*, 2011; *Tan et al.*, 2013], the contributions of these different sources to the total soil CO_2 flux may also vary greatly across an elevation gradient [*Zimmermann et al.*, 2010], with changing soil types [*Purbopuspito et al.*, 2006], climatic variables [*Fisher et al.*, 2013], nutrient availability [*Drake et al.*, 2012] and forest types [*Wang and Yang*, 2007].

Human activities like cultivation of nitrogen (N)-fixing plants, biomass burning, industrialization, fossil fuel use and fertilizer use, have more than doubled the amount of reactive N cycling globally [*Galloway et al.*, 2008]. As a result, N deposition in the tropics is

presently increasing, with further increases predicted in the next decade [*Galloway et al.*, 2004; *Phoenix et al.*, 2006; *Hietz et al.*, 2011]. Deposition of phosphorus (P) is also predicted to increase in tropical South America through increased input from biomass burning, anthropogenic mineral aerosols and biogenic particles from the neighboring Amazon Basin [*Mahowald et al.*, 2005].

How soil CO_2 flux will react to elevated nutrient deposition depends on whether the additional nutrients are limiting processes involved in CO_2 production. Traditionally, net primary production (NPP) of TMFs, which typically occur on soils that are not strongly weathered, was assumed to be N-limited whereas NPP of tropical lowland forests, which occur on heavily weathered soils, was assumed to be P-limited [*Vitousek and Farrington*, 1997]. However, in recent years, several studies have been published that do not support this generalization, developed originally in mono-species stands in Hawaii. It appears that in diverse tropical forests, multiple nutrient limitations are the rule rather than the exception [*Kaspari et al.*, 2008; *Wright et al.*, 2011; *Homeier et al.*, 2012], and there is increasing evidence that in many TMFs co-limitation of N and P occurs [*Tanner et al.*, 1998; *Homeier et al.*, 2012]. Furthermore, NPP may be limited by one specific nutrient, while other ecosystem processes may be limited by other nutrients [*Hobbie and Vitousek*, 2000; *Corre et al.*, 2010; *Homeier et al.*, 2012]. Several responses of the different components of soil CO_2 fluxes have been proposed when N and P are added to tropical forests. (1) For fresh litter respiration, the direct alleviation of nutrient limitations on microbial activity and community composition can accelerate decomposition of light soil C fractions, leading to an increase (short-term) in heterotrophic respiration [*Cleveland et al.*, 2002; *Cleveland and Townsend*, 2006; *Cusack et al.*, 2010; *Liu et al.*, 2013]. Since nutrient addition will often increase plant productivity, this will increase substrate quantity and quality, which may in turn increase (long-term) fresh litter respiration [*Sayer et al.*, 2007, 2011]. (2) N addition can suppress decomposition of more

decayed soil C fractions, present in large quantity in thick organic layers due to reduced activity of lignin-degrading enzymes [*Berg and Matzner*, 1997; *Janssens et al.*, 2010], reducing soil organic matter respiration [*Giardina et al.*, 2004]. (3) N addition can increase root maintenance respiration [*Ryan et al.*, 1996; *Jia et al.*, 2013] and root dynamics, especially in severely N-limited soils [*Nadelhoffer*, 2000; *Cleveland and Townsend*, 2006; *Yuan and Chen*, 2012). However, decreases in fine-root biomass and soil CO_2 fluxes with N addition to TMFs have frequently been reported as well [*Gower and Vitousek*, 1989; *Koehler et al.*, 2009; *Cusack et al.*, 2011], which are explained by a shift in C allocation in trees from below- to aboveground with increasing nutrient availability, and thus N addition may potentially lower root respiration [*Ågren and Franklin*, 2003; *Treseder*, 2004]. Moreover, long-term effects of N addition include decreases in soil pH and base saturation, and increases in aluminum saturation [*Matson et al.*, 1999, 2002; *Koehler et al.*, 2009], which can reduce microbial biomass and root growth and consequently soil respiration. Although P availability is often considered to have a stronger effect on root dynamics than N availability [*Ostertag*, 2001; *Treseder*, 2004; *Fisher et al.*, 2013], this was not confirmed in a recent meta-analysis that included TMF soils [*Yuan and Chen*, 2012]. Finally, the combined addition of N and P causes strong positive synergistic responses of plant productivity that exceed stimulations by single nutrient element addition [*Elser et al.*, 2007; *Harpole et al.*, 2011]; thus, N and P addition may have the potential both to amplify fresh litter respiration and to decrease root and soil organic carbon respiration.

Here, we report the effects of moderate N and P additions on soil CO_2 fluxes and its components across an elevation gradient of TMFs in southern Ecuador in the first five years of nutrient manipulation. We tested the following hypothesis: (1) CO_2 fluxes will decrease with increasing elevation, (2) the response of soil CO_2 fluxes to nutrient addition will change over time since different components of CO_2 fluxes will react with different magnitudes and

directions, and (3) the combined addition of N and P will lead to stronger effects than the addition of single nutrients. Our study is the only one so far, that investigated long-term (five years) changes in soil respiration with rates of N and P inputs that are realistic for tropical regions and moderate in comparison to other nutrient manipulation studies conducted in tropical forests [e.g. *Cleveland and Townsend*, 2006; *Fisher et al.*, 2013]. Thus, our findings provide critical information to predict and model future changes in C cycling of TMFs due to changes in nutrient deposition.

2.3 Material and Methods

2.3.1 Study area

We conducted this study on the eastern slope of the Cordillera del Consuelo in the provinces of Loja and Zamora Chinchipe, southern Ecuador. Three old-growth forest sites were selected along an elevation gradient of 1000 - 3000 m above sea level (asl) within the Podocarpus National Park and the adjacent private Biological Reserve San Francisco. A detailed description of these sites is given by *Martinson et al.* [2013] and summarized in Table S2.1.

The lowest site is located at 990-1100 m asl (referred to as 1000 m; 4.115° S, 78.968° W) and consists of a premontane tropical forest [*Homeier et al.*, 2008]. The soil (Cambisol) is developed on deeply weathered granitic rock [*Litherland et al.*, 1994] and has a sandy texture with only a thin layer of decomposing leaves. The mid-elevation site is located at 1950-2100 m asl (referred to as 2000 m; 3.982° S, 79.083° W) and consists of a lower montane rain forest [*Homeier et al.*, 2012]. The high elevation site is located at 2900-3050 m asl (referred to as 3000 m; 4.110° S, 79.178° W) and consists of an upper montane rain forest. The soils at 2000 m (Cambisol) and 3000 m (Histosol) have loamy texture, developed from metamorphic schists [*Litherland et al.*, 1994] and are covered by thick organic layers.

Mean annual temperature decreased with elevation from 19.4 at 1000 m to 15.7 at 2000 m and 9.4° C at 3000 m whereas mean annual precipitation was lowest at 2000 m with 1950 mm, followed by 1000 m with 2230 mm and highest at 3000 m with 4500 mm [*Moser et al.*, 2007]. The climate in the study area shows only slight seasonal variability with the driest and warmest month in November and both high rainfall and cold temperatures around July [*Bendix et al.*, 2006; *Emck*, 2007].

Ambient annual nutrient bulk and dry deposition in the study region has been increasing between 1998-2010 and ranged from 14 to 45 kg N and 0.4 to 4.9 kg P ha^{-1} [*Fabian et al.*, 2005; *Boy et al.*, 2008; *Homeier et al.*, 2012].

2.3.2 Experimental design

A full factorial nutrient manipulation experiment (NUMEX) was established in 2008 with N, P and N+P additions and untreated control plots [*Homeier et al.*, 2012; *Martinson et al.*, 2013]. At each site, 16 plots (20 m x 20 m each; ≥ 10 m distance from each other) were allocated to four blocks in a complete block design. Blocks covered topographic gradients which can influence soil characteristics and result in differing gas fluxes [*Wolf*, 2011]. Treatments were assigned randomly within a block with the restriction that unfertilized control treatments were located upslope and the combined treatment of N+P addition were located downslope in each block. In this steep terrain, this was necessary to avoid nutrient leaching from fertilized to control plots and from N+P plots to a plot fertilized with only one of these elements.

Fertilizers were applied manually in solid form at moderate rates of 50 kg N ha^{-1} $year^{-1}$ as urea ($CO(NH_2)_2$) and 10 kg P ha^{-1} $year^{-1}$ as sodium hydrogen phosphate ($NaH_2PO_4 \cdot H_2O$ and $NaH_2PO_4 \cdot 2H_2O$, in analytic grade quality). Fertilizer was split into two equal applications per year (February/March and August/September) starting in 2008. The second fertilization in

2010 was delayed four months due to logistical problems of shipping high-grade P fertilizer from Germany to Ecuador.

2.3.3 Litter removal and trenching experiment

To obtain additional information on possible mechanisms involved in differential responses of soil CO_2 fluxes observed within the first years of nutrient addition, we conducted a small-scale fresh litter removal and trenching experiment in the last 1.5 years of nutrient manipulation. We measured fresh litter respiration and root-related respiration, which encompass not only autotrophic respiration but also possible additional heterotrophic respiration from decomposition of severed roots, as discussed and defined by *Hanson et al.* [2000]. We selected an undisturbed and homogenous area (~ 2 m x 2 m; > 2 m from plot edges) in each plot of NUMEX, in which three chamber bases for soil CO_2 flux measurements (see description below) were installed close (< 1 m) to each other in November-December 2010 and were assigned randomly as undisturbed reference (R) chamber, litter removal (-L) chamber and trenched (T) chamber. Within –L chamber bases (0.04 m^2) all freshly fallen litter was removed once a month by hand, starting from February 2011 until August 2012.

Trenched areas (~0.15 m^2 area) were prepared in May 2011 by cutting a circular trench about 0.1 m from the previously installed T chamber base, using a hand saw. We did not trench larger areas around the chamber bases because that would have required severing many large roots. Trenching included at least 0.1 m of mineral soil and the minimum depths from the surface were 0.3 m at 1000 m, 0.4 m at 2000 m and 0.6 m at 3000 m. In these sites, more than 75% of the total fine and coarse roots within a depth of 0.8 m is located in the top 0.3 m [*Leuschner et al.*, 2007]; thus, our trenching depths guaranteed that most roots were cut [*Leuschner and Moser*, 2008]. No rooting barriers were inserted into the soil in order to avoid large disturbance and reduce possible changes in soil moisture [*Hanson et al.*, 2000].

Trenches were carefully re-cut bimonthly (directly after gas measurements) to prevent lateral root in-growth; seedlings within the isolated block of soil were also removed at least once a month.

2.3.4 Soil CO_2 flux, temperature and moisture measurements

Soil CO_2 fluxes were measured once a month from January 2008 to September 2009 and from November 2010 to August 2012, using static vented chambers. Chambers bases were circular in shape and made of polyvinyl chloride (Figure S2.1: 0.04 m² area, 0.15 m height, ~ 0.03 m insertion depth). In 2007, four chamber bases per treatment plot were permanently installed in three replicate blocks. In each plot, the chamber bases were located along two perpendicular random transects (≥ 2 m from plot edges). On each sampling day, these bases were covered with polyethylene hoods equipped with a Luer-lock sampling port and a vent for pressure equilibrium (totaling 12 L chamber volume). Four gas samples were taken at 2, 14, 26 and 38 minutes after chamber closure.

For the litter removal experiment, CO_2 fluxes were first measured in January 2011, a month prior to the start of fresh litter removal, for a background check of fluxes between –L and R chambers, and then once a month during the litter removal phase (February 2011 to August 2012). For the trenching experiment, background CO_2 fluxes were also measured prior to the start of trenching in May 2011, and then once a month following trenching (June 2011 to August 2012). On each sampling day, four gas samples were taken at 3, 13, 23 and 33 minutes after chamber closure.

Until April 2011, gas samples stored in pre-evacuated 60-ml glass containers equipped with stopcocks were analyzed in Ecuador. Thereafter, gas samples were stored (as overpressured samples) in 12 ml Labco Exetainers® (Labco Limited, Lampeter, UK) with rubber septa and shipped to Germany for analysis. Gas samples were analyzed using gas

chromatographs equipped with an electron capture detector (a Shimadzu GC-14B, Duisburg, Germany with an autosampler [*Loftfield et al.*, 1997], and a GC 6000 Vega Series 2, Carlo Erba Instruments, Milan, Italy with an ASPEC autosampler (Gilson SAS, Villiers, Le Bel, France)). CO_2 concentrations were determined from the comparison of integrated peak areas of samples to three or four standard gases (with concentrations from 350 to 5000 ppm; Deuste Steininger GmbH, Mühlhausen, Germany). Analysis was done in Ecuador not later than one day after sampling and in Germany up to several months after sampling. Exetainers® were tested for their good quality during extended sample storage and aircraft transport [*Glatzel and Well*, 2008] and their performance was controlled by crosschecking pressure and concentration of calibration gases stored and transported in the same way as the samples.

Gas fluxes were calculated from the linear increase of CO_2 concentrations in the chamber headspace over time; the headspace air volume was estimated based on measurements of the chamber height at three places around the chamber. We corrected the fluxes with air pressure and temperature measured during the time of sampling. The linear fit of data was checked using the coefficient of determination.

For the litter removal experiment, fresh litter respiration was calculated as the difference in CO_2 fluxes between R and -L chambers in the same plot. For the trenching experiment, root-related respiration was calculated as the difference in CO_2 fluxes between R and T chambers in the same plot. We excluded any measurement of total soil CO_2 flux within three weeks after fertilization since we were not interested in the short-term effect of nutrient addition [e.g. *Koehler et al.*, 2009]. Annual soil CO_2 fluxes were approximated using the trapezoidal rule on time intervals between measured flux rates, assuming constant daily flux rates as shown by a lack of systematic diurnal courses within the study area [*Iost et al.*, 2008].

Soil temperature was measured parallel to gas sampling at a depth of 0.05 m in each plot and from 2010 onward it was monitored more intensively (close to each chamber base

where gas samples were taken) using a GTH 175/Pt-E digital precision thermometer (Greisinger electronics GmbH, Regenstauf, Germany). Also, soil moisture was determined parallel to soil CO_2 flux measurements from samples taken at the top 0.05 m within 1 m of the chamber; the soil sample for each plot was either pooled from four sub-samples (taken near the four regular chambers) or one single sample taken for the litter removal and trenching experiments. Soil moisture was determined gravimetrically by oven-drying at 105° C for at least 24 h and expressed as percentage of water-filled pore space (WFPS), assuming a particle density of 2.65 g cm^{-3} for mineral soil [*Linn and Doran*, 1984] and 1.4 g cm^{-3} for the organic layer [*Breuer et al.*, 2002].

2.3.5 Statistical analysis

Data were checked for normality and homoscedasticity and either a square root or logarithmic transformation (adding a constant value if the dataset included negative values) was applied when required. First, we assessed if there were pre-existing differences in CO_2 fluxes among plots at each elevation prior to nutrient manipulation; we used the measurements conducted one month prior to the start of fertilization and conducted one-way analysis of variance with block effect. Similarly, we assessed if there were pre-existing differences in CO_2 fluxes between R, -L and T chambers at each elevation and treatment prior to litter removal and trenching experiments using Paired *t* tests.

Second, we assessed the influence of soil moisture and temperature on soil CO_2 flux from the control forests using Pearson's correlation test and multiple regression analyses. These analyses were conducted on the means of the three replicate plots on each sampling day, considering the measurements conducted in the last three years of the study (2010 to 2012). The maximal regression models (including linear or quadratic model with interaction terms) were reduced to the minimal adequate model through a series of single-term deletions

based on *F* tests [*Crawley*, 2007]. Multicollinearity was corrected by mean-centering explanatory variables so that the variance inflation factors were < 3 in all models. This was done for WFPS at 1000 m and soil temperature at 2000 m, for which regression functions are given for mean-centered data. Model significance was assessed by regression analysis of variance.

Third, we assessed the nutrient-addition effects on total soil CO_2 fluxes over a certain period of time, using the linear mixed effects (LME) models for these time series data [*Piepho et al.*, 2004; *Crawley*, 2007]. These analyses were conducted using the means of four to five chambers per plot on each sampling day (chambers are not replicates but plots and hence n = 3) and considering all the sampling days within each year separately as well as for the cumulative years of the experiment. Nutrient-addition effects on fresh litter respiration and root-related respiration as well as differences in soil temperature and moisture among treatments were also analyzed using LMEs for the time series data across the entire experimental period. Analyses of nutrient-addition effects were conducted separately for each elevation. Nutrient-addition treatments were considered fixed effects whereas sampling days and plots were included as random effects. The following structures were included in the LME model if these improved the relative goodness of the model-fit based on the Akaike information criterion: (1) a first-order temporal autoregressive process to account for decreasing correlation of measurements with increasing time difference [*Zuur et al.*, 2009], and (2) a variance function to account for heteroscedasticity of residual variances [*Crawley*, 2007]. The significance of the fixed effects was then determined by analysis of variance at $P \leq 0.05$. Mean values in the text are given with ± standard error (SE). Statistical analyses were conducted using R 2.14.0 [*R Development Core Team*, 2012].

2.4 Results

2.4.1 Soil temperature and water-filled pore space and effects of nutrient additions

Soil temperature and WFPS in the top 0.05 m varied with elevation during the entire study period of 2008-2012 ($P < 0.001$, Table 2.1). Mean soil temperature decreased with increasing elevation. WFPS was lowest at 1000 m, where soils had no organic layers, followed by 3000 m and then 2000 m with the highest WFPS. Neither soil temperature nor WFPS displayed a clear seasonal pattern at any site.

We found no effect of nutrient addition on soil temperature ($P = 0.184$ to 0.808) but soil WFPS in P and N+P plots were different from control plots at 1000 m and 3000 m (Table 2.1). At 1000 m, soil moisture was higher in P and N+P plots ($P < 0.001$) while at 3000 m soil moisture was lower in P and N+P plots ($P < 0.001$) compared to control plots. Compared to P plots, N+P plots were more strongly affected by nutrient manipulation with higher WFPS at 1000 m ($P = 0.009$) and lower WFPS at 3000 m ($P = 0.024$).

Table 2.1 Mean[a] (±SE, n = 3) soil temperature and water-filled pore space (WFPS) in the top 0.05 m of soil in montane forests across a 1000- to 3000-m elevation gradient during the first five years (encompassing 37 monthly measurements from January 2008 to August 2012) of nutrient manipulation

Elevation (m)	Treatment	Soil temperature (°C)	WFPS (%)
1000	Control	17.7 ± 0.1[a]	48.6 ± 4.7[c]
	Nitrogen (N)	17.8 ± 0.1[a]	53.8 ± 4.8[c]
	Phosphorus (P)	18.0 ± 0.1[a]	58.3 ± 10.2[b]
	N + P	18.0 ± 0.0[a]	65.3 ± 7.7[a]
2000	Control	13.8 ± 0.0[a]	80.4 ± 4.1[a]
	N	14.1 ± 0.1[a]	80.2 ± 1.2[a]
	P	13.9 ± 0.2[a]	80.3 ± 2.1[a]
	N + P	14.0 ± 0.2[a]	78.9 ± 1.3[a]
3000	Control	7.2 ± 0.2[a]	59.6 ± 0.8[a]
	N	7.2 ± 0.3[a]	57.4 ± 2.3[a]
	P	7.4 ± 0.3[a]	52.2 ± 2.0[b]
	N + P	7.3 ± 0.1[a]	48.2 ± 6.6[c]

[a] For each elevation, means followed by different letters indicate significant differences among treatments (linear mixed effects model at $P \leq 0.05$)

2.4.2 Soil CO_2 fluxes from control forests and their controlling factors

Annual soil CO_2 fluxes decreased with increasing elevation from 1000 m to 3000 m by a factor of nearly four in the entire measurement period from 2008 to 2012 (Table 2.2). Daily soil CO_2 fluxes showed a weak seasonality at 1000 m with increasing fluxes around October/November, highest fluxes in February/March and decreasing fluxes thereafter (Figure 2.1a). The variation of daily soil CO_2 fluxes in a year was small at 2000 m (Figure 2.1b) and 3000 m (Figure 2.1c).

Table 2.2 Mean[a] (± SE, n = 3) annual soil CO_2 fluxes (Mg C ha^{-1} $year^{-1}$) from montane forests across a 1000- to 3000-m elevation gradient in the first five years (2008-2012) of nutrient manipulation

Elevation (m)	Treatment	2008[b]	2009	2010/2011[b]	2012	2008-2012
1000	Control	8.93 ± 0.51	8.73 ± 0.28	8.92 ± 0.18	11.58 ± 0.49	8.85 ± 0.24
	Nitrogen (N)	9.06 ± 0.31	8.64 ± 0.63	8.41 ± 0.48	11.14 ± 0.07	8.19 ± 0.36
	Phosphorus (P)	8.04 ± 0.58	9.21 ± 0.53	7.56 ± 0.77	8.74 ± 0.92	7.99 ± 0.14
	N + P	7.64 ± 0.75	8.47 ± 0.92	8.73 ± 0.60	10.61 ± 0.54	8.26 ± 0.61
2000	Control	6.13 ± 0.71	5.45 ± 0.36	4.86 ± 0.19	6.82 ± 0.76	5.43 ± 0.37
	N	6.71 ± 0.68	6.13 ± 0.30	4.32 ± 0.15	6.23 ± 0.12	5.52 ± 0.18
	P	6.15 ± 0.42	5.98 ± 0.09	4.94 ± 0.30	7.17 ± 0.46	5.72 ± 0.16
	N + P	7.19 ± 0.24	5.81 ± 0.34	4.86 ± 0.31	7.04 ± 0.76	5.72 ± 0.25
3000	Control	2.87 ± 0.48	2.23 ± 0.70	1.98 ± 0.36	3.04 ± 0.43	2.41 ± 0.66
	N	4.09 ± 0.81	3.56 ± 1.03	3.72 ± 0.80	5.84 ± 1.46	3.94 ± 1.00
	P	3.51 ± 0.68	2.99 ± 0.69	2.58 ± 0.89	3.87 ± 1.29	2.87 ± 0.85
	N + P	3.37 ± 0.57	2.80 ± 0.43	1.88 ± 0.43	2.79 ± 0.24	2.50 ± 0.27

[a] Annual soil CO_2 fluxes were approximated by applying the trapezoid rule on time intervals between measured flux rates, assuming constant flux rates per day

[b] In 2008, annual values include one pre-treatment measurement; in 2010/2011, annual values include only two monthly measurements from 2010

Daily soil CO_2 fluxes, measured in 2010-2012, were correlated differently with soil temperature and soil moisture (Table 2.3): we found a positive correlation with WFPS at each elevation, and correlation coefficients as well as significant levels (P < 0.001 - 0.036) decreased with increasing elevation. At 1000 m, a minimal multiple regression model explaining the soil CO_2 flux contained only WFPS in a quadratic function (soil CO_2 flux = 120.40 + 1.94*WFPS – 0.12*$WFPS^2$, R^2 = 0.67, P < 0.001, n = 19). At 2000 m, we found no correlation of soil CO_2 flux with soil temperature but soil WFPS and temperature were negatively correlated (Table 2.3), and the minimum multiple regression model included this interaction (soil CO_2 flux = 19.07 + 5.45*T - 5.23*T^2 + 0.71*WFPS, R^2 = 0.66, P < 0.001, n = 19, where T is soil temperature). Only at 3000 m, were soil CO_2 fluxes positively correlated with soil temperature and WFPS (Table 2.3), and these were not correlated with each other; the minimal multiple regression model was a linear function (soil CO_2 flux = -35.37 + 3.54*T + 0.47*WFPS, R^2 = 0.36, P = 0.009, n = 20).

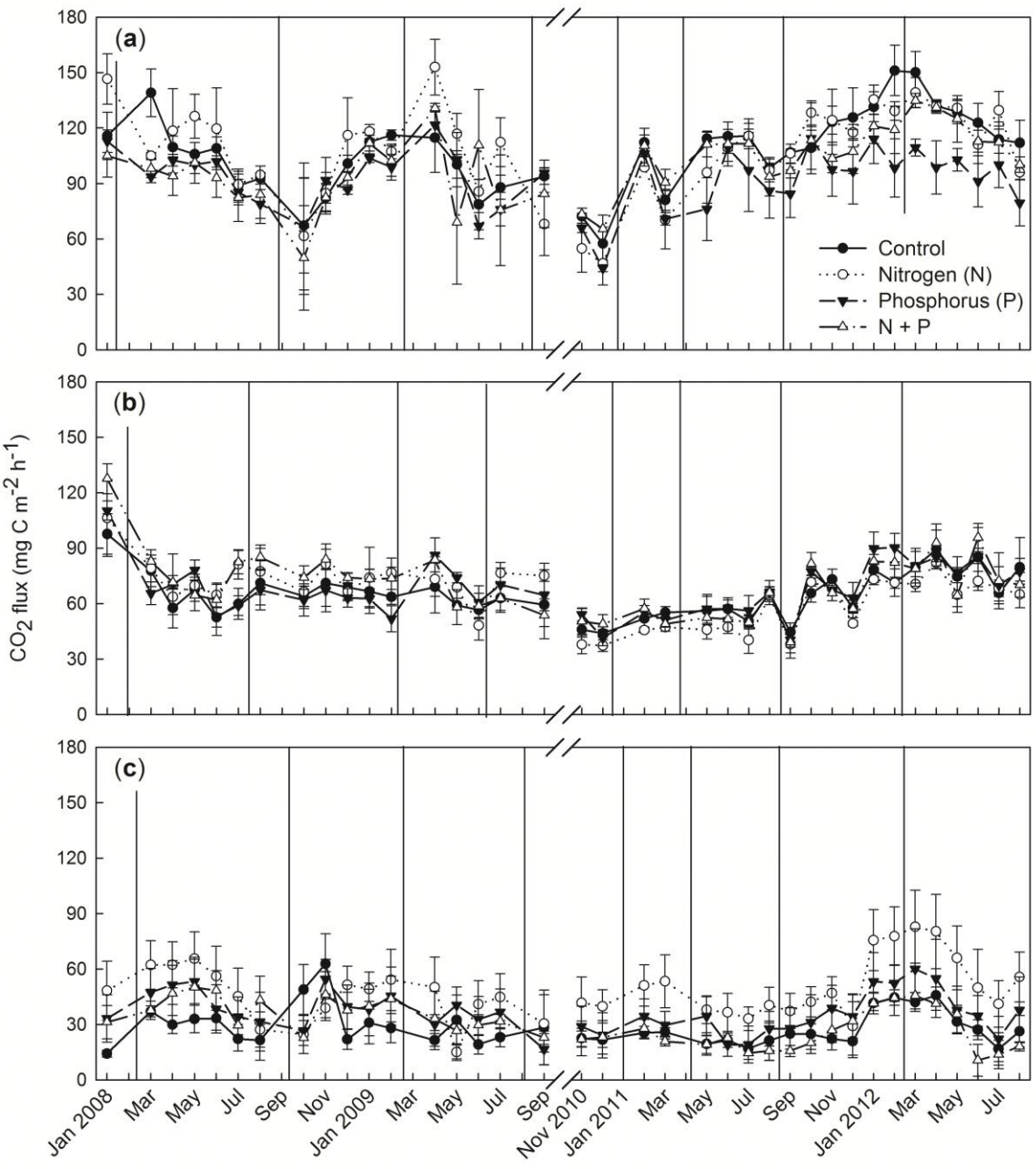


Figure 2.1 Mean (± SE, n = 3) soil CO_2 fluxes (mg C m^{-2} h^{-1}) from montane forests at (a) 1000 m, (b) 2000 m and (c) 3000 m elevations during five years of nutrient manipulation: control (*filled circle*), N addition (*open circle*), P addition (*filled triangle*) and N+P addition (*open triangle*). Vertical lines indicate fertilization events.

Table 2.3 Pearson correlation coefficients (n = 22) between soil CO_2 flux (mg C m^{-2} h^{-1}), soil temperature (°C, top 0.05-m depth) and water-filled pore space (WFPS, %, top 0.05-m depth) in montane forest control plots across a 1000- to 3000-m elevation gradient, measured from 2010 to 2012

Elevation (m)	Variable	Soil CO_2 flux	Soil temperature
1000	Soil temperature	-0.41	
	WFPS	0.78**	-0.28
2000	Soil temperature	-0.12	
	WFPS	0.67**	-0.68**
3000	Soil temperature	0.51*	
	WFPS	0.47*	0.14

*$P \leq 0.05$, **$P \leq 0.01$.

2.4.3 Effect of nutrient additions on soil CO_2 fluxes

One pre-treatment measurement conducted in January 2008 indicated that soil CO_2 fluxes did not differ between plots at any site (P = 0.121 to 0.999). Nutrient addition significantly affected soil CO_2 fluxes (Figure 2.1, Table 2.4). At 1000 m (Figure 2.1a), soil CO_2 fluxes from N plots did not differ from the control, regardless of whether they were analyzed for each year, for succeeding cumulative years or across five years of treatment (P = 0.223 to 0.980; Table 2.4). However, soil CO_2 fluxes were reduced both in N+P and especially in P plots in succeeding cumulative years and across five years of treatment (P < 0.001 to 0.038). In 2012, this resulted in a reduction of annual soil CO_2 fluxes by 8.4% (N+P addition) and 24.5% (P addition) compared to control plots (Table 2.2). In the first and second year, P addition did not affect soil CO_2 fluxes (P = 0.140 to 0.508) but reduced fluxes from the second year onward (P < 0.001 to 0.038) with the strongest effect in the fifth year (Table 2.4). Addition of N+P did reduce soil CO_2 fluxes in the first, last and across five years of treatment (P = 0.018 to 0.031), although there was no effect in the second and fourth treatment years (P = 0.425 to 0.730).

At 2000 m (Figure 2.1b), soil CO_2 fluxes were not affected by any of the nutrients added when analyzed for the entire five years of treatment (P = 0.061 to 0.836; Table 2.4). Looking at the treatment effects for each year, P addition had no effect on soil CO_2 fluxes at any year (P = 0.293 to 0.921) compared to the control whereas N and N+P additions showed high fluxes already in the first two years (P = 0.031 and P = 0.016, respectively). The N- and N+P-addition effects changed in the fourth and fifth year when soil CO_2 fluxes were the lowest in N plots (P < 0.001 and 0.084, respectively) whereas fluxes in N+P plots no longer differed from control plots (P = 0.712 to 0.939).

At 3000 m (Figure 2.1c), soil CO_2 fluxes across five years of nutrient addition were highest with N addition (P < 0.001), followed by P addition (P < 0.001) but were not affected by N+P addition (P = 0.368; Table 2.4). N addition showed higher soil CO_2 fluxes than the control in most years (P < 0.001 to 0.003) except in the second treatment year when the fluxes were only marginally higher than the control (P = 0.071). The annual fluxes from N plots exceeded those from control plots by 42.5 - 92.1% (average of 63.5%; Table 2.2). P plots emitted, on average, 22.3 - 34.1% (average of 19.1%) more CO_2 than control plots on an annual basis. Soil CO_2 fluxes from N+P plots were higher than control plots only when analyzed cumulatively for the first two years (P = 0.033) but in the succeeding years the fluxes were comparable with control plots (P = 0.108 to 0.828; Table 2.4).

Table 2.4 Mean[a] (± SE, n = 3) soil CO_2 fluxes (mg C m^{-2} h^{-1}) from montane forests across a 1000- to 3000-m elevation gradient in the first five years of nutrient manipulation

Elevation (m)	Treatment	Soil CO_2 flux (mg C m^{-2} h^{-1})			
1000		2008	2009	2010/11	2012
	Control	101.05 ± 5.19^{ab}	100.51 ± 2.64^{a}	102.58 ± 2.44^{a}	130.25 ± 5.95^{a}
	Nitrogen (N)	106.22 ± 5.59^{a}	108.71 ± 3.89^{a}	97.03 ± 4.86^{a}	125.34 ± 1.14^{ab}
	Phosphorus (P)	92.00 ± 5.75^{bc}	95.07 ± 4.33^{a}	87.37 ± 9.42^{b}	99.22 ± 10.91^{c}
	N + P	88.21 ± 8.38^{c}	97.68 ± 9.90^{a}	98.95 ± 6.78^{a}	119.42 ± 6.23^{b}
			2008-2009	2008-2011	2008-2012
	Control		100.83 ± 2.80^{a}	101.55 ± 2.61^{a}	107.76 ± 3.10^{a}
	N		107.25 ± 3.30^{a}	103.02 ± 3.94^{a}	107.84 ± 2.90^{a}
	P		93.27 ± 2.38^{b}	90.83 ± 3.58^{b}	92.64 ± 5.08^{c}
	N + P		92.11 ± 8.84^{b}	94.94 ± 7.60^{b}	100.23 ± 7.10^{b}
2000		2008	2009	2010/11	2012
	Control	68.79 ± 7.70^{b}	62.44 ± 4.26^{a}	55.59 ± 2.24^{a}	77.98 ± 9.06^{ab}
	N	75.63 ± 6.80^{ab}	70.35 ± 4.73^{a}	49.40 ± 1.53^{b}	70.87 ± 1.75^{b}
	P	69.57 ± 4.78^{b}	67.21 ± 1.06^{a}	57.20 ± 3.43^{a}	81.96 ± 5.48^{a}
	N +P	80.76 ± 2.85^{a}	66.21 ± 3.37^{a}	55.67 ± 3.62^{a}	79.86 ± 8.34^{a}
			2008-2009	2008-2011	2008-2012
	Control		66.18 ± 6.23^{b}	61.79 ± 4.56^{a}	65.29 ± 5.26^{a}
	N		73.46 ± 4.34^{a}	63.50 ± 3.12^{a}	65.09 ± 2.81^{a}
	P		68.60 ± 2.48^{ab}	63.88 ± 0.74^{a}	67.79 ± 1.27^{a}
	N + P		74.77 ± 1.74^{a}	66.86 ± 2.47^{a}	69.67 ± 3.74^{a}
3000		2008	2009	2010/11	2012
	Control	32.42 ± 5.30^{b}	26.15 ± 8.57^{a}	22.26 ± 4.06^{bc}	34.42 ± 5.20^{bc}
	N	48.12 ± 9.75^{a}	40.62 ± 10.30^{a}	41.08 ± 8.82^{a}	66.11 ± 16.38^{a}
	P	40.94 ± 7.68^{a}	34.28 ± 8.36^{a}	29.09 ± 10.08^{b}	44.08 ± 14.91^{b}
	N + P	39.24 ± 6.91^{ab}	32.39 ± 4.87^{a}	21.60 ± 5.01^{c}	31.24 ± 2.49^{c}
			2008-2009	2008-2011	2008-2012
	Control		29.84 ± 6.58^{b}	26.70 ± 5.40^{c}	28.37 ± 5.30^{c}
	N		45.04 ± 9.97^{a}	43.43 ± 9.20^{a}	48.38 ± 10.70^{a}
	P		38.20 ± 7.94^{a}	34.43 ± 8.83^{b}	36.52 ± 10.08^{b}
	N + P		36.42 ± 5.06^{a}	30.29 ± 4.93^{bc}	30.49 ± 4.15^{c}

[a] For each elevation, means followed by different superscript letters indicate significant differences among treatments within each year or time period (linear mixed effects model at $P \leq 0.05$).

2.4.4 Effect of nutrient additions on fresh litter and root-related respiration

One pre-manipulation measurement of soil CO_2 fluxes prior to fresh litter removal in January 2011 and trenching in May 2011, indicated, no differences between undisturbed reference and manipulation chambers in almost all treatments (P = 0.136 to 0.942). Only in N+P plots at 1000 m were soil CO_2 fluxes lower in pre-trenched chambers compared to the corresponding undisturbed reference chambers (P = 0.002).

Fresh litter respiration was significantly above zero in N plots at all elevations (P = 0.034 to 0.046) and in P plots at 1000 m (P = 0.015), with litter respiration in N and P plots at 1000 m elevation being significantly higher compared to controls (P = 0.026 and 0.011; Figure 2.2). Root-related respiration was only significantly higher than zero in N addition plots at 1000 and 3000 m (P = 0.013 and 0.026), with fluxes from 1000 m elevation being significantly different from controls (P = 0.004; Table 2.5). In all other cases, including all control plots, fresh-litter and root-related respiration were not different from zero due to the large variation among plots at each elevation (e.g. SE bars overlapped 0; Figure 2.2, Table 2.5).

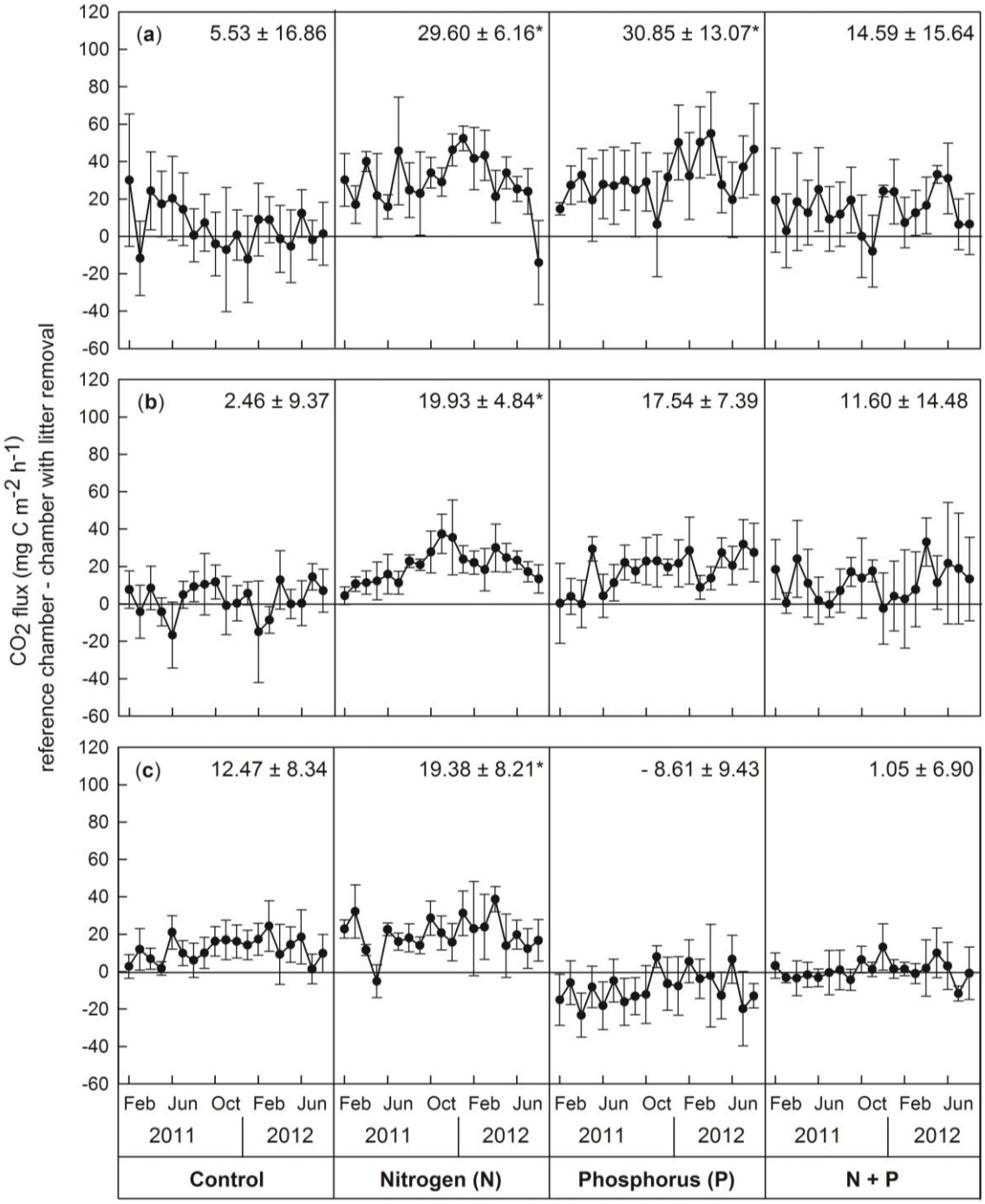


Figure 2.2 Difference in soil CO_2 fluxes (mean ± SE, n = 4) between reference chambers (undisturbed) and chambers with fresh litter removal in montane forests at (a) 1000 m, (b) 2000 m and (c) 3000 m during four to five years (February 2011 – August 2012) of nutrient manipulation. Values on the top of each panel are the means across the given measurement period, expressed in mg C m^{-2} h^{-1} (reference – fresh litter removal). Asterisks (*) indicate significant differences from zero (linear mixed effects model at $P \leq 0.05$).

Table 2.5 Mean (± SE, n = 4) soil CO_2 fluxes (mg C m^{-2} h^{-1}) from paired reference (undisturbed) and trenched chambers in each treatment plot, measured monthly in the fourth and fifth year (June 2011 – August 2012) of nutrient manipulation in montane forests across a 1000- to 3000-m elevation gradient

Elevation (m)	Treatment	Reference chamber	Trenched chamber	Root-related CO_2 flux[a] (Reference – Trenched)
1000	Control	105.28 ± 13.31	114.02 ± 8.70	-8.43 ± 5.20
	Nitrogen (N)	130.81 ± 11.74	94.29 ± 15.92	36.06 ± 21.31*
	Phosphorus (P)	120.24 ± 18.75	113.14 ± 21.30	7.08 ± 5.39
	N + P [b]	110.24 ± 12.33	99.50 ± 7.69	11.07 ± 10.93
2000	Control	53.86 ± 9.41	46.10 ± 7.34	7.76 ± 12.07
	N	57.11 ± 6.74	52.45 ± 6.44	4.80 ± 12.43
	P	58.60 ± 5.95	49.74 ± 1.14	8.99 ± 6.79
	N + P	64.06 ± 8.97	59.39 ± 2.03	4.67 ± 8.60
3000	Control	32.60 ± 6.59	29.44 ± 1.48	3.94 ± 7.38
	N	61.82 ± 5.85	36.78 ± 3.77	25.67 ± 8.31*
	P	40.59 ± 10.88	33.70 ± 12.60	6.86 ± 11.41
	N + P	35.13 ± 15.49	23.34 ± 11.04	11.38 ± 4.87

[a] Root-related CO_2 flux (e.g. from decomposition of cut roots and root respiration) for the given sampling day was calculated as: reference – trenched chambers. * - indicates significant difference from zero (linear mixed effects model at $P \leq 0.05$).

[b] One sampling day prior to trenching revealed lower fluxes in pre-trenched chambers compared to the corresponding reference chambers.

2.5 Discussion

2.5.1 Soil CO_2 fluxes from control forests and the controlling factors across the elevation gradient

The annual soil CO_2 fluxes across the elevation gradient (Table 2.2) were within the range of those reported from other studies that conducted in situ year-round measurements in TMFs at comparable elevations, e.g. Hawaii [*Raich*, 1998], Venezuelan Guyana [*Priess and Fölster*, 2001], Indonesia [*Purbopuspito et al.*, 2006; *van Straaten et al.*, 2011], Panama [*Koehler et al.*, 2009], Peru [*Zimmermann et al.*, 2010], Ecuador [*Wolf et al.*, 2012] and China [*Zhou et al.*, 2013].

Decreasing soil CO_2 fluxes with increasing elevation support our first hypothesis and are consistent with measurements from other elevation gradients of TMFs [*Purbopuspito et al.*, 2006; *Zimmermann et al.*, 2010; *Wolf et al.*, 2012]. Regression functions of soil CO_2 fluxes with WFPS and temperature show that the relationship between these important controlling variables changes slightly with elevation. However, we interpret the positive correlation of soil CO_2 fluxes with soil moisture across the elevation gradient as an indication that dry conditions generally limit microbial activity and thus microbial respiration, which was a major CO_2 source considering the low contributions of root-related respiration across all sites (Table 2.5). Temperature control, on the other hand, changed with elevation: soil temperature was not a controlling factor at 1000 m; although it was part of the regression function at 2000 m, it was not correlated with soil CO_2 fluxes on its own; only at 3000 m was it an explanatory variable in the regression equation, and it accounted for only 36% of the variation together with WFPS. We interpret this trend as increasing limitation of decreasing soil temperatures on microbial activity across the elevation gradient (Table 2.1). The explanatory power of soil moisture and temperature on soil CO_2 flux, however, became weaker with increasing elevation, implying the importance of other factors in controlling soil CO_2 fluxes. Such factors could include decreasing nutrient availability and substrate supply [*Grubb*, 1977; *Martinson et al.*, 2013] as shown in our study by a strong response of soil CO_2 efflux to N addition at 3000 m.

2.5.2 N-addition effects on soil CO_2 fluxes

At 1000 m, N addition had no effect on total soil CO_2 fluxes compared to the control, although it increased fresh litter respiration (Figure 2.2a) and root-related respiration (Table 2.5), which implies that a decrease in SOM respiration with N addition compensated for increases in fresh litter and root-related respirations. Similar findings

were reported for forests in Puerto Rico (on Acrisol soils at 260 m and 640 m elevations), where 5 years of N addition (with a rate of 50 kg N ha^{-1} $year^{-1}$ in the form of NH_4NO_3) caused differential effects on soil C pools, increasing decomposition of active soil C pools while slowing turnover of the slowly cycling C pools due to suppressed heterotrophic respiration [*Cusack et al.*, 2010]. Reduced heterotrophic respiration has also been claimed to be responsible for reduced soil CO_2 fluxes as a result of N addition, where the effect depended on the initial N status, duration of N addition and the quality of decomposing material [*Zhang et al.*, 2008; *Janssens et al.*, 2010]. In our study area, N availability decreased with increasing elevation, as reflected by decreasing aboveground plant productivity, litter quality (e.g. increasing C:N ratios), soil-N cycling rates, ^{15}N natural abundance signatures in the soil and increasing thickness of organic layers with increasing C:N ratios [*Wolf et al.*, 2011; *Homeier et al.*, 2013; *Martinson et al.*, 2013]. Chronic N addition to our site at 1000 m, which had larger rates of N cycling in the soil than the sites at 2000 m and 3000 m [*Martinson et al.*, 2013; *Baldos et al.*, in press], caused decreases in microbial biomass C and extractable organic C in the soil [*Baldos et al.*, in press], indicating a possible decrease in the amount of labile C. After 5 years of N addition to Puerto Rican forests, bacterial decomposers and hydrolytic enzyme activities increased and the amount of labile C compound decreased in the forest at 260 m elevation whereas fungal abundance and oxidative enzyme activities increased and the amount of poor-quality C compound decreased in the forest at 640 m elevation [*Cusack et al.*, 2011]. In our site, N addition may have resulted in decreased SOM respiration, possibly due to a decrease in the amount of labile C, which was compensated by the increases in fresh litter and root-related respirations, consequently resulting in unchanged soil CO_2 fluxes overall.

The effect of N addition at 2000 m changed with years of treatment. We attribute the elevated soil CO_2 fluxes in the first two years of N addition to a possible increase in fresh

litter and SOM respiration, considering that fine-root biomass and necromass did not change in the first year of N addition at this site [*Homeier et al.*, 2012], and thus possibly caused no change in root respiration. At this site, where the initial N availability is lower than at 1000 m [*Martinson et al.*, 2013; *Baldos et al.*, in press], fine litter quality increased in the first year of N addition [*Homeier et al.*, 2012]. Such positive feedback via plant input might have resulted in higher CO_2 fluxes in the short term [*Janssens et al.*, 2010; *Ramirez et al.*, 2012]. The decreased soil CO_2 fluxes in the fourth and fifth year of N addition could be due to a decrease in SOM respiration, since microbial biomass C and extractable organic C decreased in the fourth year of N addition, similar to the site at 1000 m [*Baldos et al.*, in press]. Also, root-related respiration was not affected by N addition (Table 2.5) and although proportions of fresh litter respiration from N plots were, with 33%, contributing significantly to total soil CO_2 fluxes (Figure 2.2b) they were not larger compared to control plots and therefore might not have compensated such a decrease in SOM respiration. The trend we observed at this site was similar to that at 1000 m, but the response was more delayed (i.e. SOM respiration decreased after 4-5 years of N addition at 2000 m as opposed to already occurring in the earlier years of N addition at 1000 m). Similar dynamic changes in soil CO_2 fluxes with chronic N addition (at rates between 37 and 150 kg N ha^{-1} $year^{-1}$ in the form of NH_4NO_3) were observed in temperate forest soils, which ultimately resulted in decreases in total soil respiration and SOM respiration [*Bowden et al.*, 2004].

At 3000 m, we attributed the immediate and continuous increases in soil CO_2 fluxes following N addition to significant fresh litter (Figure 2.2c) and root-related respiration (Table 2.5). Soil N availability at this site was initially the lowest among the three control forests [*Martinson et al.*, 2013; *Baldos et al.*, in press] and chronic N addition had stimulated fresh litter and root-related respiration in combined contribution to total soil

CO_2 fluxes of 85%. Since microbial biomass C and extractable organic C in the soil had decreased after four years of N addition compared to the control [*Baldos et al.*, in press] we can assume that such a decrease in the amount of labile C caused a decrease in SOM respiration. However, this might have been overly compensated by the increases in fresh litter and root-related respirations in the same period. Furthermore, fresh litter respiration increased with N addition by an average of 7 mg CO_2-C m^{-2} h^{-1} (Figure 2.2c) and root-related respiration by 22 mg CO_2-C m^{-2} h^{-1} (Table 2.5), which more than accounted for the overall increase in total soil respiration of 20 mg CO_2-C m^{-2} h^{-1} (Table 2.4) on top of the soil CO_2 fluxes from the control plots.

In summary, the initial N status of our forest sites might explain the different responses found with chronic N addition at different elevations of our study area. While there were indications that SOM respiration decreased differently with years of N addition (e.g. this probably occurred early on at 1000 m and more delayed at the higher elevations), the ultimate effects on the total soil respiration varied depending on whether or not the increase in litter and root-related respiration had compensated for the decrease in SOM respiration. These results support our second hypothesis. Such diverging responses of the components of soil respiration to N addition were similar to the findings in Puerto Rico, where 5 years of N addition with a rate similar to our application rate show differential effects on various fractions of soil organic C [*Cusack et al.*, 2011]. Similarly, the varying effects of N addition on soil respiration due to differing initial N status were similar to the findings in Panama, where >10 years of N addition at a rate double that of our application rate did not affect total soil CO_2 respiration in a lowland forest with high N availability but decreased total soil CO_2 respiration after 2-3 years of N addition in a montane forest with low N availability [*Koehler et al.*, 2009]. Our study further shows the importance of investigating the various components of soil respiration, despite limitations of the methods

used for quantifying these components in situ, in order to untangle the non-uniform effects of increased N availability on these components.

2.5.3 P-addition effects on soil CO_2 fluxes

The positive response of fresh litter respiration to P addition at 1000 m suggests P limitation on microbial decomposition of fresh sources of C. Similar findings were reported for old-growth lowland forests of Costa Rica, where 2-3 years of P addition (with a rate of 150 kg P ha^{-1} $year^{-1}$ in the form of KH_2PO_4) increased microbial respiration of easily available C [*Cleveland and Townsend*, 2006]. On the other hand, micronutrients can also limit decomposition [*Kaspari et al.*, 2008] and sodium (Na), which was contained in the sodium phosphate we added as P source (with a rate of 7.4 kg Na ha^{-1} $year^{-1}$), has been shown to increase decomposition in a tropical forest in Peru [*Kaspari et al.*, 2009]. Thus, the effect of P addition on litter respiration may have also been augmented by the effect of Na. Since fresh litter respiration was increased (Figure 2.2a) and root-related respiration was low and unaffected (Table 2.5), reduced total soil CO_2 fluxes with P addition at 1000 m are probably related to reduced SOM respiration. Although decreases in microbial respiration of SOM with P addition were reported for Chinese forest soils, *Ouyang et al.* [2008] did not have an explanation for their observation. We speculate that in our soils, such a decrease in SOM respiration might be caused by a decrease in easily available C related to a shift in C allocation from below- to above-ground plant parts with increasing P availability. Such a shift in C allocation by plants was indicated by a trend in decreasing fine-root biomass and increasing basal area increment after the first year of P addition at this site [*Homeier et al.*, 2013].

Although P addition did not change total soil CO_2 fluxes or any of its components at 2000 m, fine-root biomass decreased and fine-root necromass increased significantly in the

first year of P addition at this site [*Homeier et al.*, 2012]. Such changes in fine roots may indicate changes in root-related respiration, but we did not detect this in the fourth and fifth year of P addition. This was either because of the small contribution of root-related respiration (Table 2.5) as well as fresh litter respiration (Figure 2.2b) to total soil respiration in these plots (Table 2.4) or because of methodological limitations of the trenching technique.

Similarly, at 3000 m, we were unable to detect any effect of P addition on litter and root-related respirations because these components were quite small (Figure 2.2c, Table 2.5) compared to the total soil respiration in these P plots (Table 2.4). It was however clear that total soil CO_2 fluxes increased with P addition, which is probably related to increased SOM respiration. The initial P levels of the top 0.05 m of control forest soil at this elevation were the lowest (3 g P m^{-2} in organic layer) compared to the other two forest soils at the lower elevations (29 g P m^{-2} in mineral soil and 6 g P m^{-2} in organic layer at 1000 m and 2000 m, respectively; Table S2.1). Similar decreasing soil P concentrations along a 1000- to 3000-m elevation gradient in our study area were reported by *Wolf et al.* [2011]. With these extremely low soil P levels at 3000 m, P addition (and with it also Na) might have stimulated SOM respiration, which is in keeping with findings from tropical forests in China (with a rate of 150 kg P ha^{-1} $year^{-1}$ in the form of NaH_2PO_4) [*Liu et al.*, 2012]. A similar finding was reported for a submontane forest in Venezuelan Guyana, where total soil CO_2 fluxes increased with the addition of high doses of P (350 and 175 kg P ha^{-1}) in the form of $CaHPO_4$ [*Priess and Fölster*, 2001]. Finally, root-associated mycorrhizal fungi may have contributed to the higher CO_2 fluxes, since they can be directly limited by P availability and have been shown to proliferate following P addition in severely P-limited soils [*Treseder and Allen*, 2002; *Liu et al.*, 2013].

Taken together, we show that the responses of total soil CO_2 fluxes to P addition were related to the initial P status of the forest soils with increases at the highest elevation that had the lowest soil P levels and decreases or no effect at the lower elevations that had relatively high soil P levels. P addition at 1000 m increased litter respiration and this did not compensate for decreases in SOM respiration and potential decreases in autotrophic respiration, which were probably associated with a shift in C allocation.

2.5.4 Combined N+P-addition effects on soil CO_2 fluxes

The combined addition of N and P showed either a middling effect on soil CO_2 fluxes at 1000 m (i.e. 2008-2012, Table 2.4) in between the N- and P-addition effects or a comparable effect with N or P addition at 2000 m (i.e. 2008-2012, Table 2.4), and did not result in any stronger effect than the addition of a single nutrient. These trends were also mirrored by the pattern of responses of the litter and root-related respirations. Indeed, at 3000 m, the comparable effect of N+P addition with either N or P addition only occurred in the first two years while there were no more significant N+P effects relative to the control in the fourth to fifth year. It may be that N and P co-limitation on tree growth, as indicated by a trend toward increasing basal area at 3000 m with N+P addition compared to the control plots [*Homeier et al.*, 2013], had resulted in higher plant N or P uptake, lowering the observed effect of single elements on heterotrophic respiration over time at this site. These findings were in contrast with our third hypothesis, which is based on synergistic effects of nutrient co-limitation on plant growth [*Elser et al.*, 2007; *Harpole et al.*, 2011]. Nutrient co-limitations are often complex due to several limitations at the biochemical level as well as changes in nutrient limitations over time [*Davidson and Howarth*, 2007; *Harpole et al.*, 2011]. Furthermore, the amount of applied nutrient and duration of nutrient manipulation may influence results.

In conclusion, our results provide the much-needed data on responses of in situ soil CO_2 fluxes to moderate levels of nutrient inputs (at rates expected to occur in our study area) [*Homeier et al.,* 2012] on a multi-year temporal scale. We showed that addition of nutrients affects root and heterotrophic respiration, causing changes in total soil CO_2 fluxes that depended on the initial N and P status of the forest soils along our elevation gradient and on the duration of nutrient addition. This illustrates the need for long-term nutrient manipulation experiments and a mechanistic understanding of the variable responses of soil CO_2 flux components to nutrient addition in order to predict and model the effect of elevated nutrient deposition on soil respiration in these ecosystems. Our results suggest profound effects of elevated N and P input on the belowground C cycle, which illustrates the sensitivity of TMFs to increases in atmospheric N and P deposition.

2.6 References

Ågren, G. I., and O. Franklin (2003), Root:shoot ratios, optimization and nitrogen productivity, *Ann. Bot., 92*, 795-800, doi:10.1093/aob/mcg203.

Baldos A.P., Corre M.D., and E. Veldkamp (in press), Responses of N cycling to nutrient inputs in forest soils across 1000-3000-m elevation gradient in the Ecuadorian Andes, *Ecology*.

Bendix, J., J. Homeier, E. Cuva Ortiz, P. Emck, S.-W. Breckle, M. Richter, and E. Beck (2006), Seasonality of weather and tree phenology in a tropical evergreen mountain rain forest, *Int. J. Biometeorol.*, *50*(6), 370-384, doi: 10.1007/s00484-006-0029-8.

Berg, B., and E. Matzner (1997), Effect of N deposition on decomposition of plant litter and soil organic matter in forest systems, *Environ. Rev., 5*, 1-25.

Bowden, R. D., E. Davidson, K. Savage, C. Arabia, and P. Steudler (2004), Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the Harvard Forest, *For. Ecol. Manage., 196*, 43-56, doi:10.1016/j.foreco.2004.03.011.

Boy, J., R. Rollenbeck, C. Valarezo, and W. Wilcke (2008), Amazonian biomass burning-derived acid and nutrient deposition in the north Andean montane forest of Ecuador, *Global Biogeochem. Cycles, 22*, GB4011, doi:10.1029/2007GB003158.

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

Cleveland, C. C., and A. R. Townsend (2006), Nutrient additions to a tropical rain forest drive substantial soil carbon dioxide losses to the atmosphere, *Proc. Natl. Acad. Sci. U. S. A.*, *103*(27), 10316-10321, doi:10.1073/pnas.0600989103.

Cleveland, C. C., A. R. Townsend, and S. K. Schmidt (2002), Phosphorus limitation of microbial processes in moist tropical forests: evidence from short-term laboratory incubations and field studies, *Ecosystems*, *5*, 680-691.

Corre, M. D., E. Veldkamp, J. Arnold, and S. J. Wright (2010), Impact of elevated N input on soil N cycling and losses in old-growth lowland and montane forests in Panama, *Ecology*, *91*(6), 1715–1729, doi:10.1890/09-0274.1.

Crawley, M. J. (2007), The R book, John Wiley and Sons Ltd., West Sussex. England.

Cusack, D. F., M. S. Torn, W. H. McDowell, and W. L. Silver (2010), The response of heterotrophic activity and carbon cycling to nitrogen additions and warming in two tropical soils, *Global Change Biol.*, *16*, 2555-2572, doi: 10.1111/j.1365-2486.2009.02131.x.

Cusack, D. F., W. L. Silver, M. S. Torn, and W. H. McDowell (2011), Effects of nitrogen additions on above- and belowground carbon dynamics in two tropical forests, *Biogeochemistry*, *104*(1-3), 203-225, doi: 10.1007/s10533-010-9496-4.

Davidson, E. A., and R. W. Howarth (2007), Environmental science: nutrients in synergy, *Nature*, *449*, 1000-1001, doi: 10.1038/4491000a.

Dieleman, W. I. J., M. Venter, A. Ramachandra, A. K. Krockenberger, and M. I. Bird (2013), Soil carbon stocks vary predictably with altitude in tropical forests:

implications for soil carbon storage, *Geoderma, 204-205*, 59-67, doi:10.1016/j.geoderma.2013.04.005.

Drake, J. E., A. C. Oishi, M. A. Giasson, R. Oren, K. H. Johnsen, and A. C. Finzi (2012), Trenching reduces soil heterotrophic activity in a loblolly pine (*Pinus taeda*) forest exposed to elevated atmospheric [CO_2] and N fertilization, *Agric. For. Meteorol.*, *165*, 43-52, doi: 10.1016/j.agrformet.2012.05.017.

Elser, J. J., et al. (2007), Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems, *Ecol. Lett.*, *10*(12), 1135-1142, doi: 10.1111/j.1461-0248.2007.01113.x.

Emck, P. (2007), A climatology of south Ecuador with special focus on the major Andean ridge as Atlantic-Pacific climate divide, Ph.D. thesis, 275 pp., Friedrich-Alexander-Univ. Erlangen-Nürnberg, Erlangen-Nürnberg, Germany.

Fabian, P., M. Kohlpaintner, and R. Rollenbeck (2005), Biomass burning in the Amazon - fertilizer for the mountaineous rain forest in Ecuador, *Environ. Sci. Pollut. Res.*, *12*(5), 290-296, doi: 10.1065/espr2005.07.272.

FAO (1993), Forest resources assessment 1990 - Tropical countries, FAO Forestry Paper 112, Food and Agriculture Organization of the United Nations, Rome, Italy. http://www.fao.org/docrep/007/t0830e/t0830e00.htm

FAO (2001) FRA 2000 - Global ecological zoning for the global forest resources assessment 2000 - Final report, Forest Resources Assessment Programme, Working Paper 56, Food and Agriculture Organization of the United Nations, Rome, Italy.

Fisher, J. B., et al. (2013) Nutrient limitations in rainforests and cloud forests along a 3,000-m elevation gradient in the Peruvian Andes, *Oecologia*, *172*, 889-902, doi: 10.1007/s00442-012-2522-6.

Galloway, J. N., et al. (2004), Nitrogen cycles: past, present, and future, *Biogeochemistry*, *70*(2), 153-226, doi: 10.1007/s10533-004-0370-0.

Galloway, J. N., et al. (2008), Transformation of the nitrogen cycle: recent trends, questions, and potential solutions, *Science*, *320*, 889-892, doi:10.1126/science.1136674.

Giardina, C. P., D. Binkley, M. G. Ryan, J. H. Fownes, and R. S. Senock (2004), Belowground carbon cycling in a humid tropical forest decreases with fertilization, *Oecologia*, *139*, 545-550, doi: 10.1007/s00442-004-1552-0.

Glatzel, S., and R. Well (2008), Evaluation of septum-capped vials for storage of gas samples during air transport, *Environ. Monit. Assess.*, *136*, 307-311, doi: 10.1007/s10661-007-9686-2.

Gower, S. T., and P. M. Vitousek (1989), Effects of nutrient amendments on fine root biomass in a primary successional forest in Hawai'I, *Oecologia*, *81*, 566-568.

Grubb, P. J. (1977), Control of forest growth and distribution on wet tropical mountains: with special reference to mineral nutrition, *Annu. Rev. Ecol. Syst.*, *8*, 83-107.

Hanson, P. J., N. T. Edwards, C. T. Garten, and J. A. Andrews (2000), Separating root and soil microbial contributions to soil respiration: a review of methods and observations, *Biogeochemistry*, *48*(1), 115-146.

Harpole, W. S., et al. (2011), Nutrient co-limitation of primary producer communities, *Ecol. Lett.*, *14*, 852-862, doi: 10.1111/j.1461-0248.2011.01651.x.

Hietz, P., B. L. Turner, W. Wanek, A. Richter, C. A. Nock, and S. J. Wright (2011), Long-term change in the nitrogen cycle of tropical forests, *Science*, *334*, 664-666, doi: 10.1126/science.1211979.

Hobbie, S. E., and P.M. Vitousek (2000), Nutrient limitation of decomposition in Hawaiian forests, *Ecology*, *81*(7), 1867-1877.

Homeier, J., F. A. Werner, S. R. Gradstein, S. W. Breckle, and M. Richter (2008), Potential vegetation and floristic composition of Andean forests in south Ecuador, with a focus on the RBSF, in *Gradients in a Tropical Mountain Ecosystem of Ecuador*, Ecol. Stud., Vol. 198, edited by E. Beck, J. Bendix, I. Kottke, F. Makeschin and R. Mosandl, pp. 87-100, Springer, Berlin Heidelberg, Germany, doi: 10.1007/978-3-540-73526-7_10.

Homeier, J., 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, *PLoS One*, *7*(10), e-47128, doi:10.1371/journal.pone.0047128.

Homeier, J., et al. (2013), Effects of nutrient addition on the productivity of montane forests and implications for the carbon cycle, in *Ecosystem Services, Biodiversity and Environmental Change in a Tropical Mountain Ecosystem of South Ecuador*, Ecol. Stud. 221, edited by J. Bendix, E. Beck, A. Bräuning, F. Makeschin, R. Mosandl, S. Scheu and W. Wilcke, pp. 315-329, Springer, Berlin Heidelberg, Germany, doi:10.1007/978-3-642-38137-9_23.

Houghton, R. A. (2007), Balancing the global carbon budget, *Annu. Rev. Earth Planet Sci.*, *35*, 313-347, doi:10.1146/annurev.earth.35.031306.140057.

Iost, S., F. Makeschin, M. Abiy, and F. Haubrich (2008), Biotic soil activities, in *Gradients in a tropical mountain ecosystem of Ecuador*, Ecol. Stud., vol. 198, edited by E. Beck, J. Bendix, I. Kottke, F. Makeschin and R. Mosandl, pp. 217-227, Springer, Berlin Heidelberg, Germany, doi:10.1007/978-3-540-73526-7_21.

Janssens, I. A., et al. (2010), Reduction of forest soil respiration in response to nitrogen deposition, *Nat. Geosci.*, *3*, 315-322, doi: 10.1038/ngeo844.

Jia, S., N. B. McLaughlin, J. Gu, X. Li, and Z. Wang (2013), Relationships between root respiration rate and root morphology, chemistry and anatomy in *Larix gmelinii* and *Fraxinus mandshurica*, *Tree Physiol.*, *33*(6), 579-589, doi: 10.1093/treephys/tpt040.

Jobbagy, E. G., and R. B. Jackson (2000), The vertical distribution of soil organic carbon and its relation to climate and vegetation, *Ecol. Appl.*, *10*(2), 423-436.

Kaspari, M., M. N. Garcia, K. E. Harms, M. Santana, S. J. Wright, and J. B. Yavitt (2008), Multiple nutrients limit litterfall and decomposition in a tropical forest, *Ecol. Lett*, *11*, 35-43, doi:10.1111/j.1461-0248.2007.01124.x.

Kaspari, M., S. Yanovlak, R. Dudley, M. Yuan, and N. Clay (2009), Sodium shortage as a constraint on the carbon cycle in an inland tropical rainforest, *Proc. Natl. Acad. Sci. U. S. A.*, *106*(46), 19405-19409, doi:10.1073_pnas.0906448106.

Koehler, B., M. D. Corre, E. Veldkamp, and J. P. Sueta (2009), Chronic nitrogen addition causes a reduction in soil carbon dioxide efflux during the high stem-growth period in a tropical montane forest but no response from a tropical lowland forest on a decadal time scale, *Biogeosciences*, *6*, 2973-2983, doi:10.5194/bg-6-2973-2009.

Leuschner, C., G. Moser, C. Bertsch, M. Röderstein, and D. Hertel (2007), Large altitudinal increase in tree root/shoot ratio in tropical mountain forests of Ecuador, *Basic Appl. Ecol.*, *8*, 219-230, doi: 10.1016/j.baae.2006.02.004.

Leuschner, C., and G. Moser (2008), Carbon allocation and productivity in tropical mountain forests, in *The Tropical Mountain Forest. - Patterns and Processes in a Biodiversity Hotspot*, Biodiversity and Ecology Series, vol. 2, edited by S. R. Gradstein, J. Homeier and D. Gansert, pp. 109-128, Universitätsverlag Göttingen, Göttingen, Germany.

Linn D. M., and J. W. Doran (1984), Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils, *Soil Sci. Soc. Am. J.*, *48*, 1267-1272.

Litherland, M., J. A. Aspden, and R. A. Jemielita (1994), The metamorphic belts of Ecuador, Overseas Memoir 11, British Geological Survey, Nottingham, UK.

Liu, L., P. Gundersen, T. Zhang, and J. Mo (2012), Effects of phosphorus addition on soil microbial biomass and community composition in three forest types in tropical China, *Soil Biol. Biochem.*, *44*, 31-38, doi:10.1016/j.soilbio.2011.08.017.

Liu, L., T. Zhang, F. S. Gilliam, P. Gundersen, W. Zhang, H. Chen. Amd J. Mo (2013), Interactive effects of nitrogen and phosphorus on soil microbial communities in a tropical forest, *PLoS One*, *8*(4), e61188, doi: 10.1371/journal.pone.0061188.

Loftfield, N., H. Flessa, J. Augustin, and F. Beese (1997), Automated gas chromatographic system for rapid analysis of the atmospheric trace gases methane, carbon dioxide, and nitrous oxide, *J. Environ. Qual.*, *26*(2), 560-564.

Mahowald, N. M., P. Artaxo, A. R. Baker, T. D. Jickells, G. S. Okin, J. T. Randerson, and A. R. Townsend (2005), Impacts of biomass burning emissions and land use change on Amazonian atmospheric phosphorus cycling and deposition, *Global Biogeochem. Cycles*, *19*, GB4030, doi:10.1029/2005GB002541.

Malhi, Y. (2005), The carbon balance of the tropical forest biome, in *The Carbon Balance of Forest Biomes*, edited by H. Griffiths and P. G. Jarvis, pp. 232-251, Taylor and Francis Group, Abingdon, New York.

Malhi, Y., D. D. Baldocchi, and P.G. Jarvis (1999), The carbon balance of tropical, temperate and boreal forests, *Plant, Cell Environ.*, *22*, 715-740, doi: 10.1046/j.1365-3040.1999.00453.x.

Martinson, G. O., M. D. Corre, and E. Veldkamp (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*(1-3), 625-636, doi: 10.1007/s10533-012-9753-9.

Matson, P. A., W. H. McDowell, A. R. Townsend, and P. M. Vitousek (1999), The globalization of N deposition: ecosystem consequences in tropical environments, *Biogeochemistry*, *46*(1-3), 67-83.

Matson, P., K. A. Lohse, and S. J. Hall (2002), The globalization of nitrogen deposition: consequences for terrestrial ecosystems, *Ambio*, *31*(2), 113-119, doi:10.1579/0044-7447-31.2.113.

Moser, G., D. Hertel, and C. Leuschner (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, doi: 10.1007/s10021-007-9063-6.

Nadelhoffer, K. J. (2000), The potential effects of nitrogen deposition on fine-root production in forest ecosystems, *New Phytol.*, *147*, 131-139.

Ostertag, R. (2001), Effects of nitrogen and phosphorus availability on fine-root dynamics in Hawaiian montane forests, *Ecology*, *82*(2), 485-499.

Ouyang, X., G. Zhou, Z. Huang, C. Zhou, J. Li, J. Shi, and D. Zhang (2008), Effect of N and P addition on soil organic C potential mineralization in forest soils in South China, *J. Environ. Sci. (Beijing, China)*, *20*, 1082-1089.

Phoenix, G. K., et al. (2006), Atmospheric nitrogen deposition in world biodiversity hotspots: the need for a greater global perspective in assessing N deposition impacts, *Global Change Biol.*, *12*, 470-476, doi:10.1111/j.1365-2486.2006.01104.x.

Piepho, H. P., A. Büchse, and C. Richter (2004), A mixed modelling approach for randomized experiments with repeated measures, *J. Agron. Crop. Sci.*, *190*, 230-247, doi: 10.1111/j.1439-037X.2004.00097.x.

Prentice, I. C., et al. (2001), The carbon cycle and atmospheric carbon dioxide, in *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*, edited by J. T. Houghton, Y. Ding, D. J. Griggs *et al.*, pp. 183-237, Cambridge University Press, Cambridge.

Priess, J. A., and H. Fölster (2001), Microbial properties and soil respiration in submontane forests of Venezuelian Guyana: characteristics and response to fertilizer treatments, *Soil Biol. Biochem.*, *33*, 503-509.

Purbopuspito, J., E. Veldkamp, R. Brumme, and D. Murdiyarso (2006), Trace gas fluxes and nitrogen cycling along an elevation sequence of tropical montane forests in Central Sulawesi, Indonesia, *Global Biogeochem. Cycles*, *20*, GB3010, doi:10.1029/2005GB002516.

Raich, J. W. (1998), Aboveground productivity and soil respiration in three Hawaiian rain forests, *For. Ecol. Manage.*, *107*, 309-318.

Raich, J. W., and W. H. Schlesinger (1992), The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate, *Tellus*, *44B*, 81-99.

Ramirez, K. S., J. M. Craine, and N. Fierer (2012), Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes, *Global Change Biol.*, *18*(6), 1918-1927, doi:10.1111/j.1365-2486.2012.02639.x.

R Development Core Team (2012), R: a language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. http://www.R-project.org/

Ryan, M. G., R. M. Hubbard, S. Pongracic, R. J. Raison, and R. E. McMurtrie (1996), Foliage, fine-root, woody-tissue and stand respiration in *Pinus radiata* in relation to nitrogen status, *Tree Physiol.*, *16*, 333-343.

Sayer, E. J., J. S. Powers, and E. V. J. Tanner (2007), Increased litterfall in tropical forests boosts the transfer of soil CO_2 to the atmosphere, *PLoS One*, *2*(12), e1299, doi:10.1371/journal.pone.0001299.

Sayer, E. J., M. S. Heard, H. K. Grant, T. R. Marthews, and E. V. J. Tanner (2011), Soil carbon release enhanced by increased tropical forest litterfall, *Nat. Clim. Change*, *1*, 304–307, doi: 10.1038/nclimate1190.

Schlesinger, W. H., and J. A. Andrews (2000), Soil respiration and the global carbon cycle, *Biogeochemistry*, *48*(1), 7-20.

Stadtmüller, T. (1987), Cloud forests in the humid tropics - a bibliographic review, United Nations University, Tokyo, Japan.

Tan, Z. H., et al. (2013), Soil respiration in an old-growth subtropical forest: patterns, components, and controls, *J. Geophys. Res. Atmospheres*, *118*, 2981-2990, doi: 10.1002/jgrd.50300.

Tanner, E. V. J., P. M. Vitousek, and E. Cuevas (1998), Experimental investigation of nutrient limitation of forest growth on wet tropical mountains, *Ecology*, *79*(1), 10-22.

Treseder, K. K. (2004), A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies, *New Phytol.*, *164*(2), 347-355, doi: 10.1111/j.1469-8137.2004.01159.x.

Treseder, K. K., and M. F. Allen (2002), Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: a model and field test, *New Phytol.*, *155*(3), 507-515, doi: 10.1046/j.1469-8137.2002.00470.x.

van Straaten, O., E. Veldkamp, and M. D. Corre (2011), Simulated drought reduces soil CO_2 efflux and production in a tropical forest in Sulawesi, Indonesia, *Ecosphere*, *2*(10), art119, doi: 10.1890/ES11-00079.1.

Vitousek, P. M., and H. Farrington (1997), Nutrient limitation and soil development: experimental test of a biogeochemical theory, *Biogeochemistry*, *37*(1), 63-75.

Wang, C., and J. Yang (2007), Rhizospheric and heterotrophic components of soil respiration in six Chinese temperate forests, *Global Change Biol.*, *13*, 123-131, doi: 10.1111/j.1365-2486.2006.01291.x.

Wolf, K. (2011), Trace gas fluxes and belowground carbon allocation in tropical montane forest soils of Southern Ecuador, Ph.D. thesis, 122 pp., Georg-August University of Göttingen, Göttingen, Gemarny.

Wolf, K., E. Veldkamp, J. Homeier, and Martinson G. O. (2011), Nitrogen availability links forest productivity, soil nitrous oxide and nitric oxide fluxes of a tropical montane forest in southern Ecuador, *Global Biogeochem. Cycles*, *25*, GB4009, doi:10.1029/2010GB003876.

Wolf, K., H. Flessa, and E. Veldkamp (2012), Atmospheric methane uptake by tropical montane forest soils and the contribution of organic layers, *Biogeochemistry*, *111*(1-3), 469–483, doi: 10.1007/s10533-011-9681-0.

Wright, S. J., et al. (2011), Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest, *Ecology*, *92*(8), 1616-1625, doi: 10.1890/10-1558.1.

Yuan, Z. Y., and H. Y. H. Chen (2012), A global analysis of fine root production as affected by soil nitrogen and phosphorus, *Proc. R. Soc. B*, *279*(1743), 3796-3802, doi: 10.1098/rspb.2012.0955.

Zhang, D., D. Hui, Y. Luo, and G. Zhou (2008), Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors, *J. Plant Ecol.*, *1*(2), 85-93, doi: 10.1093/jpe/rtn002.

Zhou, Z., L. Jiang, E. Du, H. Hu, Y. Li, D. Chen, and J. Fang (2013), Temperature and substrate availability regulate soil respiration in the tropical mountain rainforests, Hainan Island, China, *J. Plant Ecol.*, *6*(5), 325-334, doi:10.1093/jpe/rtt034.

Zimmermann, M., P. Meir, M. I. Bird, Y. Malhi, and A. J. Q. Ccahuana (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, doi:10.1029/2010GB003787.

Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith (2009), Mixed effects models and extensions in ecology with R, Springer, New York.

Table S2.1 Site, forest and soil characteristics across the elevation gradient.

Characteristics	Elevation (m) - site		
	1000 Bombuscaro	2000 San Francisco	3000 Cajanuma
Site			
Latitude (°S)	4.115	3.982	4.110
Longitude (°W)	78.968	79.083	79.178
Range of elevation (m asl)	990 - 1,100	1,950 - 2,100	2,900 - 3,050
Annual mean air temperature (°C) [a]	19.4	15.7	9.4
Precipitation (mm $year^{-1}$) [a]	2,230	1,950	4,500
Bulk N deposition (kg N ha^{-1} $year^{-1}$) [b]		14 - 45	
Bulk P deposition (kg P ha^{-1} $year^{-1}$) [b]		0.4 - 4.9	
Forest [c]			
Rain forest type	Premontane	Lower montane	Upper montane
Most abundant tree families	*Moraceae, Sapotaceae*	*Melastomaceae, Euphorbiaceae*	*Cunoniaceae, Clusiaceae*
Stand height (m)	20 - 25	10 - 14	6 - 8
Mean basal areas (m^2 ha^{-1})	33.4	22.8	25.5
Tree density (trees ha^{-1})	748	1143	1305
Soil			
Soil profiles[d]			
Soil type (FAO)	Dystric Cambisol	Stagnic Cambisol	Stagnic Histosol
Soil texture	Sandy loam	Medium loam	Medium loam
Organic layer thickness (cm)	0	10 - 30	10 - 40
Bulk density (g cm^{-3}), top 5 cm	0.84 ± 0.10	0.18 ± 0.05	0.11 ± 0.01
Soil characteristics, top 5 cm[e]			
Soil material	Mineral soil	Organic layer	Organic layer
pH-H_2O	4.3 ± 0.2	4.0 ± 0.1	3.7 ± 0.2
Total C (kg C m^{-2})	2.5 ± 0.7	4.4 ± 0.1	2.6 ± 0.1
Total N (g N m^{-2})	168 ± 35	167 ± 12	75 ± 4
C/N ratio	14 ± 1	26 ± 2	35 ± 1
Total P (g P m^{-2})	29 ± 7	6 ± 1	3 ± 1

[a] *Moser et al.* [2007], measured at 1.5 m height within the stands in the study area

[b] *Homeier et al.* [2012]

[c] *Homeier et al.* [2013] and *J. Homeier* unpublished data; excluding trees ≤ 0.1 m in diameter at breast height

[d] *Martinson et al.* [2013]; soil characteristics were determined from soil profiles (mean ± SE, n = 3) in November 2007 before the first nutrient application.

[e] *Baldos et al.*, unpublished data; soil characteristics (mean ± SE, n = 4) were determined in control plots in 2012 except for total P contents at 1000 m elevation, which were determined by *Wolf et al.* [2011] in a nearby and comparable forest. Sample preparation and analytical methods applied are described in detail by *Corre et al.* [2010].

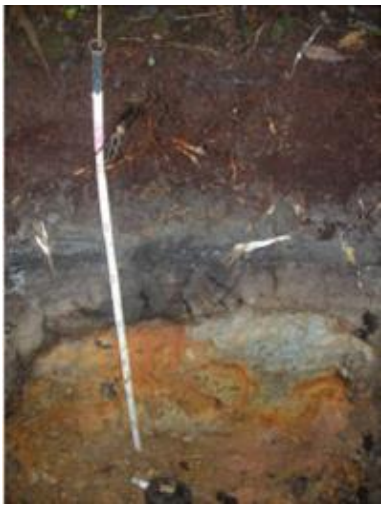
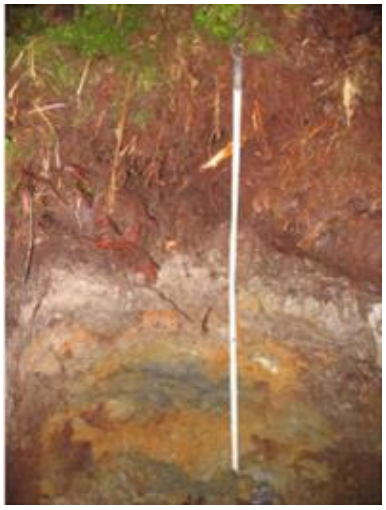
(**a**) 1000 m (Bombuscaro)	(**b**) 2000 m (San Francisco)	(**c**) 3000 m (Cajanuma)
		
		
		

Figure S2.1 Tropical montane forests, permanently-installed chamber bases for soil CO_2 flux measurement and soil profiles at (a) 1000 m, (b) 2000 m and (c) 3000 m in the Podocarpus National Park and San Francisco Biological Reserve, southern Ecuador. Photo credit: G.O. Martinson, A. K. Müller.

CHAPTER 3

Soil N_2O fluxes along a 1000- to 3000-m elevation gradient of Ecuadorian montane forests with five years of nitrogen and phosphorus input

3.1 Abstract

Although deposition of nutrients to tropical forests is increasing, few studies investigate the effects of multiple-year nutrient manipulations on fluxes of nitrous oxide (N_2O), a potent greenhouse gas. Our objectives were (1) to determine the effects of three to five years of moderate nitrogen (N) and phophorus (P) additions on soil N_2O fluxes and net soil-N cycling rates, and (2) to quantify the relative contributions of nitrification and denitrification to N_2O fluxes. In 2008, a nutrient manipulation experiment was established along a 1000- to 3000-m elevation gradient of montane forests in southern Ecuador. Each elevation had four replicate blocks, with subplots (20 m x 20 m each) of control, N (50 kg $N \cdot ha^{-1} \cdot yr^{-1}$), P (10 kg $P \cdot ha^{-1} \cdot yr^{-1}$) and N+P additions. We report measurements that we conducted from November 2010 to August 2012. Annual N_2O fluxes from the control plots decreased along the elevation gradient (from 0.57±0.26 to 0.17±0.06 to 0.05±0.04 kg N_2O-N ha^{-1} yr^{-1} at 1000 m, 2000 m and 3000 m, respectively). Measurements were low for a tropical montane forest, which we attributed to our sites' conservative soil N cycling. Denitrification was the main N_2O source at 1000 m and the only N_2O source in organic layers at 2000 m and 3000 m. In contrast to the first two years of this experiment, N addition did not affect N_2O fluxes during our 2010-2012 measurements; we attribute the lack of response to the relatively low soil moisture contents during this period. Across the elevation gradient, P addition decreased N_2O fluxes and mineral N concentrations, presumably because it alleviated P limitations to net primary production, increasing plant N uptake. N+P addition showed similar trends to N addition, but less pronounced because of the counteracting effects of P addition. Results from the whole experiment (2008-2012) showed that effects of N and P addition on soil N_2O fluxes were not linear with time of exposure, highlighting the importance of long-term studies.

3.2 Introduction

Globally, soils are the biggest natural source of nitrous oxide (N_2O), a potent greenhouse gas and a dominant ozone-depleting substance (Denman et al. 2007). Tropical forest soils alone contribute most to this source, accounting for 30% of the global natural emissions from terrestrial soils (Ehhalt et al. 2001). In soils of temperate forest ecosystems, it has been demonstrated that elevated N deposition has the potential to accelerate soil N cycling and increase soil N availability causing substantial N losses in the form of N_2O emissions (e.g. Butterbach-Bahl et al. 1998; Gundersen et al. 1998). Since nitrogen (N) deposition in tropical regions is rapidly increasing due to biomass burning, fertilizer use and industrialization (Hietz et al. 2011), it is expected that N_2O emissions from tropical forest soils will also increase (Koehler et al. 2009; Martinson et al. 2013). Additionally, it has been suggested that the N_2O response of tropical forests to elevated N input might be stronger than in other ecosystems (Liu and Graever 2009). In soils, N_2O is mainly produced and consumed together with other N-oxide gases by the microbial processes of nitrification and denitrification (Chapius-Lardy et al. 2007). While nitrification is an obligate aerobic process which depends on ammonium (NH_4^+) and organic N as substrates, denitrification is an anoxic process which is controlled mainly by the soil aeration/oxygen status or conversely soil water content, nitrate (NO_3^-) availability, microbially-available organic carbon and soil pH (Firestone and Davidson 1989). Thus, soil N availability and water content play a crucial role in controlling the amounts and relative ratios of N-oxide fluxes from soils. These relations have been described in the conceptual 'hole-in-the-pipe' (HIP) model (Firestone and Davidson 1989), which has been shown to be applicable across a wide range of ecosystems and climates, including tropical forests (Davidson et al. 2000).

Tropical montane forests (TMFs) represent over 11% of the world's tropical forests (Bubb et al. 2004; FAO 1993) and occur across large elevation gradients with a variety of

environmental conditions. TMFs tend to have a so-called "conservative" soil N cycle, with low N losses relative to tropical lowland forest soils which tend to have a more "leaky" soil N cycle, as indicated by their higher soil-N cycling rates (Corre et al. 2010; Vitousek and Matson 1988) and consequently larger N-oxide (NO, N_2O) fluxes (e.g. Keller et al. 2005; Koehler et al. 2009; Matson and Vitousek 1987; Purbopuspito et al. 2006) and NO_3^- leaching (e.g. Dechert et al. 2005; Hedin et al. 2003; Schwendenmann and Veldkamp 2005). N addition experiments in TMFs of Hawaii (Hall and Matson 2003), Panama (Corre et al. 2014; Koehler et al. 2009) and Ecuador (Martinson et al. 2013) have shown increases in soil mineral N production, especially nitrification rates, and in N-oxide fluxes as early as 1-2 years after the onset of N addition. In the Panamanian TMF, soil N_2O emissions during the third and fourth year of N addition were even as high as the emissions from the lowland forest, which already had 11-12 years of N addition (Corre et al. 2014); this shows the potential of TMF soil to be a significant N_2O source when subjected to chronic N input.

How soil N cycling in TMFs reacts to elevated phosphorus (P) input, another nutrient that often limits plant growth in TMFs (Homeier et al. 2012; Tanner et al. 1998), has been little studied, even though atmospheric P deposition is also increasing in tropical South America mainly due to biomass burning (Mahowald et al. 2005). Results from two years of moderate P addition to TMF soils along an elevation gradient in southern Ecuador showed no effect on net rates of soil N cycling and N_2O emissions (Martinson et al. 2013). In a plantation of N-fixing trees in Indonesia, however, a one-time application of 100 kg P ha^{-1} followed by two years of measurements, decreased soil total N contents and N_2O emissions but increased plant N uptake (Mori et al. 2013).

Since responses of ecosystem processes to chronic nutrient addition are nonlinear with time (e.g. Aber et al. 1998), quantifying changes in N-oxide fluxes and their controlling factors need to be conducted in long-term and large-scale nutrient manipulation experiments.

Here, we report the changes in soil N_2O fluxes, contributions of nitrification and denitrification to N_2O fluxes, and net rates of soil N cycling (an index of plant-available N) in the third to fifth year of nutrient addition with moderate N (50 kg N ha^{-1} yr^{-1}), P (10 kg P ha^{-1} yr^{-1}) and combined N+P additions along an elevation gradient of TMFs in southern Ecuador. These measurements are a continuation from the first two years of nutrient manipulation reported by Martinson et al. (2013). We thereby provide information on mid-term, moderate-level nutrient manipulation in TMFs, which may serve as a basis to model and predict future effects of nutrient depositions on soil N_2O fluxes from these ecosystems. Martinson et al. (2013) showed that after two years of moderate N and N+P additions, soil N_2O fluxes increased compared to the control plots along the 1000- to 3000-m elevation gradient. N and N+P additions at 1000 m did not significantly change the relatively high net nitrification rate already present at that elevation, whereas the same treatments at 2000 m and 3000 m resulted in small increases in the previously-undetectable net nitrification activity, but only in the second year of treatment. Addition of P affected neither soil N_2O fluxes nor net soil-N cycling rates at any elevation.

The objectives of the present study were to (1) determine the effect of three to five years of moderate nutrient additions on soil N_2O fluxes and net soil-N cycling rates, and (2) assess the contributions of nitrification and denitrification to soil N_2O fluxes. We hypothesized that (1) soil N_2O fluxes together with net soil-N cycling rates will further increase with years of continued N and N+P additions whereas the moderate P addition will have a minimal effect or will even decrease soil N_2O emissions and (2) denitrification will be the major source of N_2O fluxes in these moist TMF soils.

3.3 Material and Methods

3.3.1 Study area

The study area was located in the eastern part of the Andes of southern Ecuador in the provinces of Loja and Zamora Chinchipe. The experiment was conducted in three old-growth forest sites, spanning across an elevation gradient from 1000 to 3000 m above sea level, and located within and close to the Podocarpus National Park (Table S2.1; Homeier et al. 2012; Martinson et al. 2013).

Forest types across the elevation gradient ranged from premontane tropical forest at '1000 m' (990-1100 m; 4.115° S, 78.968° W), to lower montane rain forest at '2000 m' (1950-2100 m; 3.982° S, 79.083° W) to upper montane rain forest at '3000 m' (2900-3050 m; 4.110° S, 79.178° W) (Homeier et al. 2012). At 1000 m, Cambisol soil with sandy texture (covered only by a thin layer of decomposing leaves) developed from deeply weathered granitic rock (Litherland et al. 1994). At 2000 m and 3000 m, loamy textured Cambisol soil and Histosol soil, respectively, developed from metamorphic schists (Litherland et al. 1994); these soils were covered by 10-40 cm of thick organic layers (Table S2.1).

Climatic parameters in the study area displayed only slight seasonal variability (Emck 2007). Mean annual air temperature decreased with elevation from 19.4 °C at 1000 m and 15.4 °C at 2000 m to 9.4 °C at 3000 m. Mean annual precipitation ranged from 1950 to 4500 mm yr^{-1} with intermediate rainfall of 2230 mm yr^{-1} at 1000 m, lowest amount at 2000 m and highest rainfall at 3000 m (Moser et al. 2007).

Ambient bulk and dry deposition of N and P in the study region ranged between 14 and 45 kg N ha^{-1} yr^{-1} and 0.4 and 4.9 kg P ha^{-1} yr^{-1}, with an increasing tendency for deposition over the years of measurement from 1998 to 2010 (Boy et al. 2008; Homeier et al. 2012) and thereafter (personal communication, W. Wilcke).

3.3.2 Experimental design

At each elevation, we established nutrient manipulation experiments (NUMEX) with 16 plots (20 m x 20 m each) equally distributed to four blocks. The four blocks served as replicates and included little topographic difference (50-100 m) within each elevation. Each block consisted of four plots: N, P and N+P additions, and untreated controls; these plots were separated by at least 10 m (Homeier et al. 2013; Martinson et al. 2013). Fertilization started in 2008 with two equal applications per year (February/March and August/September), with the exception of 2010 when there was a four month delay of the second fertilization due to logistical problems in shipping the high-grade P fertilizer from Germany to Ecuador. Fertilizers were applied manually in solid form at moderate rates of 50 kg N ha^{-1} yr^{-1} in the form of urea (CH_4N_2O) and 10 kg P ha^{-1} yr^{-1} in form of sodium hydrogen phosphate ($NaH_2PO_4 \cdot H_2O$ and $NaH_2PO_4 \cdot 2H_2O$, with analytic grade quality).

3.3.3 Measurements

Soil N_2O flux, temperature, moisture and mineral N concentrations

Measurements of soil N_2O flux, temperature, moisture and mineral N concentrations followed the same procedure described in detail by Martinson et al. (2013). Measurements were performed monthly from November 2010 to August 2012 in three out of the four blocks, with a minimum distance of 2 m to plot borders for the nutrient-addition plots. In each plot, measurements were conducted at four locations that were laid out in a stratified random pattern (Martinson et al. 2013); after January 2011, we added one additional location per plot as part of a small-scale manipulation study, to measure soil CO_2 fluxes (Chapter 2). Since we were interested in long-term effects of nutrient deposition rather than the transitory peaks of N_2O that occur directly after N addition (Koehler et al. 2009), we only included measurements that were taken at least three weeks after fertilization. This timespan was

chosen based on a study from our working group in Panama, where mineral N concentrations and N_2O emissions peaked within 3 weeks following N application in a TMF (Koehler et al. 2009).

Soil N_2O fluxes were measured using static vented chambers with permanently installed round polyvinyl chloride chambers bases (area 0.04 m², height 0.15 m, ~ 0.03 m inserted into the soil) and polyethylene chamber hoods with a Luer lock sampling port and a vent for pressure equilibrium (0.16 m height of chamber cover, 0.03 m overlapping width with chamber base for tight cover, and 12 L total volume). Gas samples were taken at 2, 14, 26 and 38 minutes or at 3, 13, 23 and 33 minutes after chamber closure and stored in pre-evacuated glass containers (60 ml vials until April 2011, and 12 ml Exetainers® afterwards). Gas samples were either analyzed in Ecuador or in Germany, after shipping as over-pressured samples in Labco Exetainers® (Labco Limited, UK). We have tested these Exetainers® for their quality during extended sample storage and aircraft transport (see also Glatzel and Well 2008). Gas samples were analyzed using gas chromatographs (Shimadzu GC-14B, Duisburg, Germany for samples analyzed in Ecuador and GC 6000 Vega Series 2, Carlo Erba Instruments, Milan, Italy for samples analyzed in Germany; both of these and the standard gases are owned by our group and were calibrated regularly) equipped with an electron capture detector and autosamplers. N_2O concentrations were determined from the comparison of integrated peak areas from samples to three or four standard gases (ranging from 350 ppb to 3,000 ppb; Deuste Steininger GmbH, Mühlhausen, Germany). Fluxes of N_2O, expressed as N_2O-N flux per area ($\mu g\ N\ m^{-2}\ h^{-1}$) were calculated from the linear increase of N_2O concentration in the chamber headspace over time, corrected for the air pressure and temperature measured at the time of field sampling.

Soil temperature was measured parallel to gas sampling at a 0.05-m depth close to each of the four chamber bases per plot using a GTH 175/Pt-E digital precision thermometer

(Greisinger electronics GmbH, Regenstauf, Germany). Soil moisture and mineral N concentrations were determined parallel to gas sampling for each plot from pooled soil samples of the top 0.05 m of soil, consisting of four samples taken within 1 m of each chamber. Soil moisture was determined by oven-drying subsamples at 105° C for at least 24 h and was expressed as percentage of water-filled pore space (WFPS) using the measured soil densities in the top 0.05 m of soil (reported by Martinson et al. 2013) and particle densities of 2.65 g cm^{-3} for mineral soil at 1000 m and 1.4 g cm^{-3} for organic layers at 2000 m and 3000 m (Breuer et al. 2002; Linn and Doran, 1984). Soil extraction for mineral N concentration determination was carried out in-situ in order to avoid alterations of actual N concentrations during transport (Arnold et al. 2008). A subsample of soil was added into a prepared extraction bottle with 150 ml of 0.5 mol L^{-1} K_2SO_4-solution. After returning to the laboratory on the same day, samples were shaken (1 h), filtered and kept frozen until arrival in Germany. Soil extraction of mineral NH_4^+ and NO_3^- concentration was done at the University of Goettingen, using continuous flow injection colorimetry (Cenco/Skalar Instruments, Breda, Netherlands); NH_4^+ was analyzed by the Berthelot reaction method (Skalar Method 155-000) and NO_3^- by the copper-cadmium reduction method with NH_4Cl buffer but without ethylenediamine tetraacetic acid (Skalar Method 461-000).

Net soil-N cycling rates: ammonification and nitrification

Net rates of soil N cycling were determined twice in 2011 (February and December; just over 1 month and about 4 months following fertilization) and once in 2012 (April; about 2 months following fertilization), using the in-situ buried bag method (Hart et al. 1994). Two pairs of intact soil cores were taken from the top 0.05 m of soil/organic matter in each plot of all four blocks. One soil core of each pair was extracted immediately in the field with 0.5 mol L^{-1} K_2SO_4 (as described above); the other soil core was put in a plastic bag, inserted back into

the soil to incubate for 10 days and afterwards extracted. Net soil-N cycling rates of each sampling pair were calculated by subtracting the initial soil mineral N concentrations from mineral N concentrations of incubated soils. Net ammonification is the difference in NH_4^+ concentrations and net nitrification is the difference in the NO_3^- concentrations.

^{15}N tracing to $^{15}N_2O$: contribution of nitrification and denitrification to soil N_2O flux

Short-term tracing from $^{15}NH_4^+$ or $^{15}NO3^-$ to $^{15}N_2O$ was used to determine the contributions of nitrification and denitrification to soil N_2O fluxes; we used the same method in a montane forest in Panama (it is described in detail in Corre et al. 2014). Tracing was conducted in all four replicate plots of the control and N-addition treatments at all three elevations and was carried out in July 2011 and January 2012, four and five months after the last fertilization. In each of the four control or N-addition plots, two additional chamber bases (same dimensions and material as described above) were installed > 10 m apart, at least 3 weeks prior to sampling. In N-addition plots, the bases were > 2 m from plot edges. For the second sampling, the chamber bases were located close to the previous chambers of the same ^{15}N source but always upslope to prevent influences from previously applied ^{15}N. Each of the two chambers in a plot was labelled separately with either $^{15}NO_3^-$ or $^{15}NH_4^+$.

The amounts of added ^{15}N (either NH_4^+ or NO_3^-) were calculated based on the extant soil mineral N levels in the control and N-addition plots such that the added ^{15}N would be at most 50% of the native levels and the volume of solution would not increase the soil moisture contents. In each plot, 0.52 mg ^{15}N-KNO_3 in 50 ml distilled water was applied to the soil surface within one chamber (0.04 m^2) and 13.29 mg of ^{15}N-$(NH_4)_2SO_4$ in 50 ml distilled water was applied to the soil surface within the other chamber. Half of the amount of the ^{15}N solution was injected about 0.025 m deep with a side-port spinal needle at several points inside the chamber. The other half was sprayed with a hand sprayer onto the surface of the

soil after removal of the leaf litter layer (which was returned afterwards). Transparent plastic covers (0.85 x 0.6 m) were put 0.5 m above the chamber bases one to two days before labeling to prevent immediate leaching losses of the applied ^{15}N isotope tracers in case of rainfall. These applied amounts of $^{15}NO_3^-$ and $^{15}NH_4^+$ were the same for all plots and represented 3 – 30 % and 7 – 46 % of the native NO_3^- and NH_4^+ levels, respectively in the top 0.05 m of soil across all control and N-addition plots

Thirty minutes after ^{15}N application, gas samples were taken with a syringe at 2, 17 and 32 minutes following chamber closure and stored as overpressured samples in 100 ml pre-evacuated glass vials with butyl rubber septa. These glass vials were tested as leak proof in an earlier study (Corre et al. 2014). Gas samples were brought to Germany where N_2O concentrations were analyzed using the same gas chromatograph described above and $^{15}N_2O$ was determined using isotope ratio mass spectrometry (IRMS; Finnigan Delta^{Plus} XP, Thermo Electron Corporation, Bremen, Germany). Following the final gas sampling, we took a soil sample of the top 0.05 m in the center of each chamber base to determine soil moisture and mineral N concentrations following the procedures described above. Additionally, ^{15}N from NH_4^+ and NO_3^- was determined by the ^{15}N diffusion procedures described in detail by Corre and Lamersdorf (2004) and analyzed using IRMS (Delta C, Finnigan MAT, Bremen, Germany). Contributions of nitrification and denitrification to soil N_2O fluxes were calculated following the same calculations given by Corre et al. (2014).

3.3.4 Statistical analysis

Data were checked for normality and homoscedasticity, and we used either square root or logarithmic transformation (adding a constant value if the dataset included negative values) for data with non-normal distribution and unequal variance. If after transformation the data were still non-normally distributed we used non-parametric statistical tests.

The influence of soil factors (moisture, temperature, mineral N concentrations) on soil N_2O fluxes was assessed using Pearson's correlation tests: first, across the elevation gradient considering the control plots only to assess which of these soil factors control N_2O fluxes under ambient nutrient conditions, and second for each elevation considering all treatment plots to determine if changes in these soil factors due to nutrient amendment influence changes in N_2O fluxes. These analyses were conducted for the entire 2010-2012 measurements on the treatment means (average of three replicate plots) on each sampling day.

Effects of elevation and nutrient addition on time series data (soil N_2O flux, temperature, WFPS, mineral N concentration and net N-cycling rates) were assessed using linear mixed effects (LME) models (Crawley 2012; Piepho et al. 2004). Analyses were based on plot means (the average of four or five chamber measurements for N_2O and two measurements for net soil N cycling) with three replicate plots (for all parameters) or four replicate plots (for soil N-cycling rates). Elevation effects were assessed for control plots only and nutrient-addition effects were assessed separately for each of the three elevations. Elevation or treatments were considered fixed effects whereas sampling month and plot (as spatial replication) were included as random effects. The following structures were included in the LME model if this improved the relative goodness of the model fit based on the Akaike information criterion: (1) a first-order temporal autoregressive process accounting for decreasing correlation of measurements with increasing time difference (Zuur et al. 2009) and (2) a variance function varIdent to model heteroscedasticity of residual variances (Crawley 2012). The significance of the fixed effects was then determined by analysis of variance and stepwise model simplification.

For the short-term ^{15}N tracing method of N_2O sources, we first assessed the effects of added ^{15}N solution on soil parameters (mineral N concentrations, WFPS, NO_3^-/NH_4^+ ratio) and soil N_2O fluxes for each measurement campaign, elevation and treatment. We compared

$^{15}NH_4^+$- with $^{15}NO_3^-$-labeled chambers and both with reference (without ^{15}N) chambers that were measured in the nearest sampling months, using Paired T tests. Second, we tested the differences in relative contributions of nitrification and denitrification to N_2O fluxes between years for each elevation and treatment, between elevations for the control plots only and between treatments for each elevation, using either T tests (paired and unpaired) or a Mann Whitney U test.

The significance level was defined at $P \leq 0.050$ and mean values in the text are given with ± standard error (SE). Statistical analyses were conducted using R 2.14.0 (R Development Core Team 2012).

3.4 Results

3.4.1 Control plots along the elevation gradient: soil N_2O fluxes and controlling factors

Soil N_2O fluxes from control plots were influenced by elevation ($P < 0.001$; Table 3.1) with annual fluxes at 1000 m more than three times larger than at 2000 m and more than eleven times larger than at 3000 m over the entire measurement period (Table 3.2). However, in 2012 the difference between soil N_2O fluxes at 1000 m and 2000 m was not significant ($P = 0.103$; Table 3.1). Temporal variability of N_2O fluxes from the control plots was largest at 1000 m although without a clear seasonal trend (Figure 3.1).

Table 3.1 Mean (±SE, n = 3) soil temperature, water-filled pore space (WFPS) and N_2O fluxes in montane forests along a 1000- to 3000-m elevation gradient, measured monthly between November 2010 and August 2012

Elevation (m)	Treatment	Soil temperature (°C)	WFPS (%)	Soil N_2O fluxes (μg N m^{-2} h^{-1})		
		2010-2012	2010-2012	2010/2011	2012	2010-2012
1000	Control	18.43 ± 0.10^{A}	43.35 ± 3.87^{C}	$5.75 \pm 1.97^{A,a}$	7.37 ± 4.99^{A}	$6.40 \pm 3.17^{A,ab}$
	Nitrogen (N)	18.50 ± 0.04	45.26 ± 6.91	6.73 ± 0.99^{a}	8.67 ± 1.54	7.50 ± 1.20^{a}
	Phosphorus (P)	18.60 ± 0.09	51.41 ± 9.69	2.25 ± 1.62^{b}	5.71 ± 1.18	3.63 ± 1.31^{b}
	N + P	18.66 ± 0.07	54.06 ± 6.68	6.14 ± 0.84^{a}	7.60 ± 2.13	6.73 ± 0.67^{a}
2000	Control	14.67 ± 0.28^{B}	71.12 ± 4.21^{A}	1.79 ± 0.89^{B}	$2.43 \pm 1.91^{AB,ab}$	$2.05 \pm 0.64^{B,ab}$
	N	14.77 ± 0.19	71.64 ± 2.00	2.20 ± 0.28	4.18 ± 0.61^{a}	2.99 ± 0.42^{a}
	P	14.81 ± 0.17	72.88 ± 2.41	0.83 ± 0.26	1.47 ± 0.71^{b}	1.09 ± 0.40^{b}
	N + P	14.64 ± 0.18	69.00 ± 1.76	2.17 ± 0.24	4.21 ± 0.97^{a}	2.98 ± 0.51^{a}
3000	Control	$9.80 \pm 0.26^{C,b}$	$58.58 \pm 0.82^{B,a}$	0.85 ± 0.47^{B}	-0.10 ± 0.92^{B}	0.47 ± 0.62^{B}
	N	9.61 ± 0.26^{b}	57.00 ± 2.25^{a}	1.31 ± 0.29	0.56 ± 0.58	1.00 ± 0.21
	P	10.03 ± 0.26^{a}	50.34 ± 2.13^{ab}	0.46 ± 0.91	1.27 ± 0.70	0.78 ± 0.75
	N + P	9.74 ± 0.10^{b}	44.61 ± 6.91^{b}	1.04 ± 0.49	1.95 ± 1.53	1.40 ± 0.33

Means followed by different capital letters indicate significant differences across the elevation gradient for the control plots, and means followed by small letters indicate significant differences among treatments within each elevation and year(s) (linear mixed effects model at $P \leq 0.05$)

Table 3.2 Mean (± SE, n = 3) annual N_2O fluxes (kg N ha^{-1} yr^{-1}) from montane forest soils along a 1000- to 3000-m elevation gradient, measured 12 and 8 times at monthly interval in 2010/2011 and 2012, respectively

Elevation (m)	Treatment	2010/2011	2012	2010-2012
1000	Control	0.51 ± 0.15	0.68 ± 0.48	0.57 ± 0.26
	Nitrogen (N)	0.56 ± 0.04	0.77 ± 0.13	0.64 ± 0.08
	Phosphorus (P)	0.22 ± 0.16	0.53 ± 0.11	0.33 ± 0.13
	N + P	0.54 ± 0.09	0.72 ± 0.23	0.59 ± 0.06
2000	Control	0.15 ± 0.05	0.20 ± 0.17	0.17 ± 0.06
	N	0.22 ± 0.03	0.37 ± 0.07	0.27 ± 0.04
	P	0.08 ± 0.03	0.11 ± 0.05	0.09 ± 0.03
	N + P	0.19 ± 0.02	0.36 ± 0.09	0.25 ± 0.05
3000	Control	0.07 ± 0.04	0.00 ± 0.08	0.05 ± 0.04
	N	0.12 ± 0.02	0.04 ± 0.04	0.10 ± 0.02
	P	0.05 ± 0.06	0.13 ± 0.04	0.07 ± 0.05
	N + P	0.08 ± 0.04	0.21 ± 0.14	0.12 ± 0.02

Annual soil N_2O fluxes were approximated by applying the trapezoid rule on time intervals between measured flux rates, assuming constant flux rates per day

In the control plots at each elevation, addition of ^{15}N-solutions for the short-term ^{15}N tracing of N_2O did not affect soil N_2O fluxes, WFPS or mineral N concentrations ($P \geq 0.060$) compared to the reference (without ^{15}N) chambers, except at 2000 m in 2011 where addition of $^{15}NH_4^+$ solution increased soil NH_4^+ concentrations ($P < 0.009$) relative to the reference chambers. The relative contributions of nitrification and denitrification to N_2O fluxes did not differ between the two measurement campaigns in July 2011 and January 2012 ($P \geq 0.500$), and hence we report the means (± SE, $n = 4$) of these two periods. Denitrification dominated N_2O fluxes in control plots along the elevation gradient with contributions of 67 ± 26% at 1000 m, 100 ± 0% at 2000 m and 98 ± 3% at 3000 m. There was a larger contribution of nitrification at 1000 m than at 2000 m ($P = 0.029$). The amounts of $^{15}N_2O$ emitted during 30 minutes of chamber closure were very small: maximally 0.003% of soil $^{15}NH_4$ and 0.755% of soil $^{15}NO_3$ in the top 0.05 m across the elevation gradient.

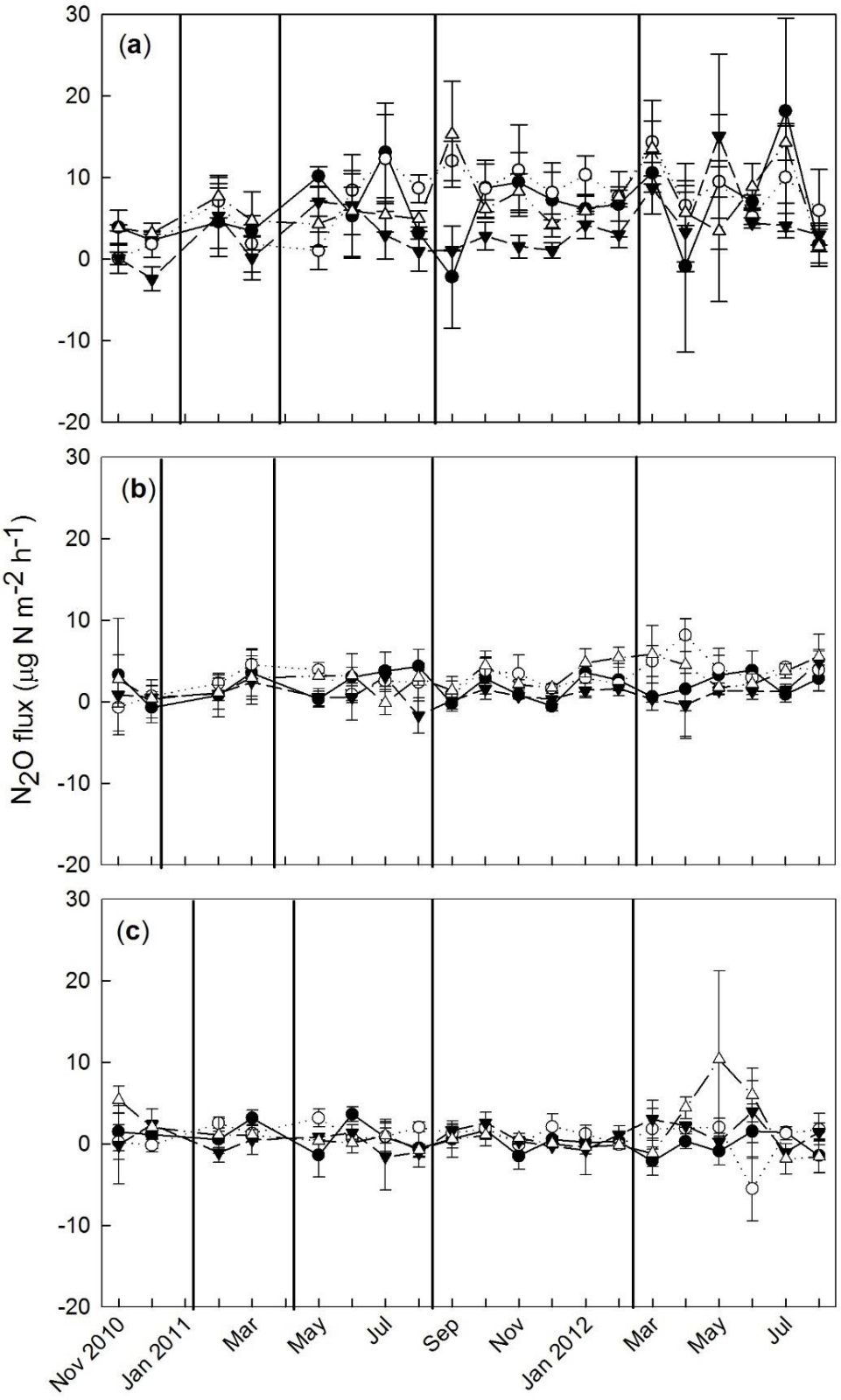


Figure 3.1 Mean (± SE, n = 3) soil N_2O fluxes (μg N m^{-2} h^{-1}) from montane forests at (a) 1000 m, (b) 2000 m and (c) 3000 m during 3-5 years of nutrient manipulation: control (*filled circle*), N addition (*open circle*), P addition (*filled triangle*) and N+P addition (*open triangle*). Vertical lines indicate fertilization events.

Across the 2010-2012 measurements, soil temperature and WFPS in the top 0.05 m of soil in control plots differed between elevations (Table 3.1) but showed no clear seasonal pattern at any elevation. Soil temperature decreased with increasing elevation ($P < 0.001$). WFPS was highest at 2000 m followed by 3000 m and 1000 m ($P < 0.001$).

In the control plots, net ammonification rates did not differ across the elevation gradient ($P = 0.126$; Table 3.3), which was caused by the large spatial variability (i.e. large SE), but net nitrification rates were larger at 1000 m than at 2000 m and 3000 m ($P < 0.001$; Table 3.3). At all elevations, the dominant form of mineral N in the top 0.05 m of soil was NH_4^+ (Table 3.3). NH_4^+ was higher at 1000 m and 2000 m compared to 3000 m ($P < 0.001$), and did not vary markedly within the measurement period. Similar to net nitrification, NO_3^- concentrations decreased with increasing elevation ($P < 0.001$; Table 3.3), and seasonally elevated soil NO_3^- concentrations at 1000 m and 2000 m coincided with months of large litterfall (Homeier et al. unpublished data on litterfall).

Across the elevation gradient, soil N_2O fluxes from control plots were positively correlated with soil temperature and NO_3^- (Table 3.4). Soil temperature was negatively correlated with WFPS and positively correlated with NH_4^+ and NO_3^- concentrations. WFPS was negatively correlated with NO_3^- concentration. NO_3^- and NH_4^+ concentrations were also positively correlated. Correlation tests performed for each elevation only revealed correlations at 1000 m; positive correlations between soil N_2O fluxes and WFPS ($R = 0.59$; $P = 0.004$; $n = 22$) and between soil temperature and NH_4^+ concentrations ($R = 0.52$; $P = 0.013$; $n = 22$). At 2000 m and 3000 m, we did not detect any significant correlations.

Table 3.3 Mean (± SE) net soil-N cycling rates (n = 4) and soil mineral N concentrations (n = 3) in the top 0.05 m of montane forest soils along a 1000- to 3000-m elevation gradient. Net N-cycling rates were measured three times between 2011 and 2012 (end of the 3rd, end of the 4th and middle of the 5th year of nutrient manipulation) and soil mineral N concentrations were measured monthly between November 2010 and August 2012

Elevation (m)	Treatment	Net soil-N cycling rates (mg N m^{-2} d^{-1})		Soil mineral N concentration (mg N m^{-2})	
		ammonification	nitrification	NH_4^+	NO_3^-
1000	Control	15.24 ± 5.59^{a}	35.48 ± 6.82^{A}	335.79 ± 31.38^{A}	43.41 ± 26.01^{A,c}
	Nitrogen (N)	3.02 ± 1.73^{ab}	42.93 ± 1.83	308.96 ± 25.59	76.07 ± 15.40^{a}
	Phosphorus (P)	19.71 ± 8.45^{a}	52.95 ± 7.96	317.02 ± 15.62	19.44 ± 8.33^{d}
	N + P	-3.76 ± 2.23^{b}	38.56 ± 3.28	328.13 ± 55.86	58.60 ± 7.88^{b}
2000	Control	1.25 ± 5.64^{b}	0.25 ± 0.29^{B,b}	359.96 ± 17.12^{A,c}	9.59 ± 2.33^{B,b}
	N	44.97 ± 19.37^{a}	4.39 ± 3.02^{a}	745.60 ± 49.82^{a}	44.08 ± 14.81^{a}
	P	1.58 ± 4.12^{b}	0.34 ± 0.30^{b}	324.51 ± 20.85^{c}	8.78 ± 2.33^{b}
	N + P	19.04 ± 6.97^{a}	8.39 ± 2.45^{a}	563.17 ± 57.76^{b}	60.49 ± 6.46^{a}
3000	Control	-0.04 ± 1.93^{c}	-0.03 ± 0.09^{B,b}	217.95 ± 10.71^{B,b}	3.77 ± 0.83^{C,c}
	N	21.31 ± 5.48^{a}	1.24 ± 0.71^{a}	504.82 ± 99.84^{a}	22.25 ± 1.11^{a}
	P	5.69 ± 3.79^{bc}	0.08 ± 0.11^{b}	159.51 ± 9.94^{c}	2.53 ± 0.77^{d}
	N + P	14.57 ± 5.83^{ab}	1.76 ± 1.36^{a}	248.07 ± 64.80^{b}	6.77 ± 1.36^{b}

Means followed by different capital letters indicate significant differences across the elevation gradient for the control plots and means with superscript small letters indicate significant differences among treatments for each elevation (linear mixed effects model at $P \leq 0.05$)

Table 3.4 Pearson correlation coefficients for monthly average (n = 66) soil N_2O flux (μg N m^{-2} h^{-1}), soil temperature (°C), water-filled pore space (WFPS; %) and mineral N concentrations (mg N m^{-2}) in control plots of montane forests across a 1000- to 3000-m elevation gradient

	Soil temperature	WFPS	NH_4^+	NO_3^-
Soil N_2O flux	0.51*	-0.13	0.21	0.36*
Soil temperature		-0.40*	0.50*	0.67*
WFPS			0.05	-0.41*
NH_4^+				0.34*

* $P < 0.01$

3.4.2 Effects of nutrient additions on soil N_2O fluxes and controlling factors at each elevation

At 1000 m, soil N_2O fluxes from nutrient-amended plots were not different than control plots over the entire measurement period (P = 0.059 – 0.146; Figure 3.1a; Table 3.1). P plots, however, had lower soil N_2O fluxes compared to N (P = 0.001) and N+P plots (P = 0.004) during the 2010-2012 measurement period. Analyzing measurement years separately, in 2010/2011 P plots had N_2O fluxes that were > 60% smaller compared to all other treatments (P = 0.001 – 0.034) while in 2012 there was no detectable differences (P = 0.334). At 2000 m, nutrient additions over the entire 2010-2012 measurement period had similar effects as at 1000 m: N_2O fluxes from nutrient-amended plots were not significantly different than control plots (P = 0.119 – 0.128), but P plots had lower soil N_2O fluxes compared to N and N+P plots (both P = 0.002; Figure 3.1b; Table 3.1). Although in 2010/2011 there was no detectable treatment difference (P = 0.213), in 2012 treatment effects followed the same pattern as that of the entire measurement period (P = 0.019). At 3000 m, there were no detectable treatment differences in soil N_2O fluxes (P = 0.391 to 0.651) over any time period from 2010 to 2012 (Figure 3.1c; Table 3.1).

For the short-term ^{15}N tracing method of N_2O sources, addition of ^{15}N solutions did not affect soil N_2O fluxes, WFPS or mineral N concentrations ($P \geq 0.062$) as compared to the reference chambers in each N plot at each elevation. Relative contribution of nitrification and denitrification to soil N_2O fluxes did not differ between the two measurement campaigns in 2011 and 2012 for each treatment ($P \geq 0.500$) and hence we reported the average values of these two measurements. We did not detect a significant difference in the sources of N_2O fluxes between control and N plots at any elevation ($P = 0.625$) and mean (±SE, $n = 4$) contributions of denitrification to N_2O fluxes in N plots were 96 ± 12% at 1000 m, 100 ± 1% at 2000 m and 99 ± 2% at 3000 m. The amounts of $^{15}N_2O$ emitted during 30 minutes of chamber closure were maximally 0.004% of soil $^{15}NH_4^+$ and 0.065% of soil $^{15}NO_3^-$ in the top 0.05 m across the elevation gradient.

Nutrient addition affected net soil-N cycling rates across all elevations (Table 3.3). At 1000 m, net ammonification rates decreased in N+P plots ($P = 0.017$) compared to control and P plots ($P = 0.004$) whereas the N plots showed intermediate rates (Table 3.3). There was no treatment difference detected for net nitrification rates ($P = 0.357$). At 2000 m, net ammonification and nitrification rates increased in N and N+P plots compared to control and P plots ($P = 0.001$ - 0.033; Table 3.3). At 3000 m, net ammonification rates increased in N ($P = 0.001$) and N+P plots ($P = 0.007$) compared to control plots, and P plots did not differ from the control ($P = 0.196$; Table 3.3). Furthermore, net ammonification rates in N plots were higher than in P plots ($P = 0.029$). Net nitrification rates increased in N ($P = 0.011$) and N+P plots ($P = 0.005$) whereas P plots were comparable with the control ($P = 0.536$).

Soil mineral N concentrations measured monthly between 2010 and 2012 were also influenced by nutrient addition (Table 3.3). At 1000 m, NH_4^+ concentrations did not differ between treatments ($P = 0.601$) whereas NO_3^- concentrations decreased in the order N, N+P, control and P plots ($P \leq 0.017$; Table 3.3). At 2000 m, NH_4^+ concentrations in N and N+P

plots were larger compared to control plots ($P < 0.005$) with concentrations in N plots being larger than in N+P plots ($P = 0.007$). NO_3^- concentrations displayed a similar pattern (Table 3.3). At 3000m, NH_4^+ concentrations were higher in N plots and lower in P plots compared to control and N+P plots ($P < 0.001$), while NO_3^- concentrations at 3000 m displayed the same pattern described for 1000 m with descending concentrations in the order of N, N+P, control and P plots ($P < 0.001$; Table 3.3). For soil temperature and WFPS measured between 2010 and 2012, only at 3000 m were there differences between treatments in soil temperature and moisture, measured between 2010 and 2012 ($P < 0.001$; Table 3.1); soil temperature was higher in P plots compared to all other treatments ($P = 0.006$ - 0.033) while WFPS was lower in N+P plots compared to control ($P = 0.013$) and N plots ($P = 0.026$).

Across all treatment plots, correlations between soil N_2O fluxes and soil temperature, WFPS and mineral N varied for each elevation (Table S3.1). At 1000 m N_2O fluxes were positively correlated with WFPS. At 2000 m, there was a positive correlation of N_2O fluxes with WFPS, NH_4^+ and NO_3^- concentrations. At 3000 m, we did not detect any significant correlation with N_2O fluxes.

3.5 Discussion

3.5.1 Control plots along the elevation gradient: soil N_2O fluxes and controlling factors

Annual soil N_2O fluxes from control plots measured for nearly two years across the elevation gradient (Table 3.2) were lower than those from other TMFs at comparable elevations that reported in-situ, year-round measurements, e.g. in Brazil (Sousa Neto et al. 2011), Panama (Koehler et al. 2009), Peru (Teh et al. 2014), and Indonesia (Purbopuspito et al. 2006). Our annual N_2O fluxes were, however, within the range of those reported for the same study area (Wolf et al. 2011) and for the same control forests measured earlier (in 2008 and 2009 by Martinson et al. 2013).

According to the HIP model, the amount of gaseous N losses from soils is primarily controlled by soil N availability, which is commonly measured using soil-N cycling rates (Davidson et al. 2000; Firestone and Davidson 1989). We compared the net soil-N cycling rates from our control plots (Table 3.3) with published data from other old-growth TMFs that used in-situ incubation of intact soil cores. Our net N cycling rates in the top 0.05 m of soil at 1000 m were lower than values reported for TMFs on Andosol soils located between 700 m and 1500 m in northwestern Ecuador and Costa Rica (Arnold 2008; Arnold et al. 2009). Similarly, net nitrification rates in the top 0.05 m of the organic layer in an Andosol soil at 1200 m in Panama (Koehler et al. 2009) were more than 10 times higher than values from the same depth of organic layers in our Cambisol soil at 2000 m and our Histosol soil at 3000 m. A separate study carried out at our study sites showed that gross rates of mineral N production (N mineralization and nitrification) in our control plots were low and closely coupled with microbial N immobilization (Baldos et al. in press), which is typical for conservative soil N cycling and supports our low net soil-N cycling rates. Therefore it is not surprising, that N_2O losses from our study sites were very low, with mean daily N_2O fluxes (Table 3.1) accounting for only 0.02% to 0.06% of gross N mineralization rates (used as an index of soil available N; ranging from 60 ± 10 mg N m^{-2} d^{-1} at 3000 m to 235 ± 30 mg N m^{-2} d^{-1} at 1000 m in the top 0.05 m of soil; Baldos et al. in press). This was comparable with the 0.06% N_2O loss in proportion to gross N mineralization rate in the top 0.05 m of soil reported for a TMF in Panama (Corre et al. 2014). The low levels of N availability (and corresponding low N_2O fluxes) in our soils were also partly controlled by temperature. Along our elevation gradient, the decreasing N_2O fluxes from the control plots were correlated with the decreasing soil temperatures (Table 4). Temperature also influenced the decreasing gross rates of soil mineral N production across our elevation gradient (Baldos et al. in press) as was shown by the positive correlations of soil temperature with NH_4^+ and NO_3^- (Table 3.4).

The second level of control on gaseous N losses from soils in the HIP model is the soil aeration status, usually represented by the soil WFPS, which influences the relative contributions of nitrification and denitrification to gaseous N losses. Denitrification is proposed to become the dominant source of N_2O fluxes above a threshold value of 60% WFPS (Davidson et al. 2000) and to become the only N_2O source at WFPS >70% (Bateman and Baggs 2005; Davidson 1991; Machefert and Dise 2004). Across our elevation gradient, WFPS in the top 0.05 m of soil only surpassed these threshold values at 2000 m and not at 1000 m or 3000 m (Table 3.1). However, the ^{15}N tracing method showed that denitrification was the dominant source of N_2O at 1000 m and the only N_2O source at 2000 m and 3000 m. In previous studies, it has been shown that WFPS threshold values can vary substantially depending on soil texture; for example, in acid brown earth (Cambisol) with 48% sand in Northern Ireland 60-80% of N_2O was derived from denitrification at 40% WFPS (Stevens et al. 1997). This is comparable to our results from the sandy loam mineral soil (Cambisol) at 1000 m. At 2000 m and 3000 m, the 59-71% WFPS in the top 0.05 m of the organic layer cannot explain the absence of nitrification-derived N_2O fluxes. However, these organic layers had very high gravimetric soil moisture contents (3-5 g g^{-1}) due to the high water holding capacity of the organic matter (Hudson 1994). To illustrate this: approximately 27-30 kg H_2O m^{-2} was stored in the top 0.05 m organic layer, which was much more than the approximately 15 kg H_2O m^{-2} stored in the top 0.05 m of mineral soil at 1000 m. Such high gravimetric water contents in organic layers can create plenty of anaerobic microsites in which denitrification can occur despite the relatively low WFPS. Indeed, the positive correlations between N_2O flux and NO_3^- (Table 3.4) also supported our results from the ^{15}N tracing method that denitrification was the dominant N_2O source. Thus, our findings illustrate that in contrast to mineral soils, different threshold values of WFPS should be used for organic layers in estimating limits of the relative importance of nitrification and denitrification as N_2O

sources. The low N_2O losses from our study sites, especially at the higher elevations with thick organic layers, may be further attributed to the combination of low NO_3^- concentrations (Table 3.3) and presumably high labile carbon in organic layers, which have been shown to result in high N_2/N_2O ratios, causing N losses via denitrification to be dominated by N_2 (Weier et al. 1993).

In summary, our study sites were characterized by low N_2O fluxes as reflected by their conservative soil N cycling (i.e. small net (Table 3.3) and gross (Baldos et al. in press) rates of mineral N production with closely coupled microbial N immobilization). This has also been observed in other TMFs in Puerto Rico, Hawaii and Panama (Corre et al. 2010, 2014; Hall and Matson 2003; Silver et al. 2001), and was in line with the previously demonstrated strong limitation of N_2O fluxes by N availability in our study area (Martinson et al. 2013; Wolf et al. 2011). The high gravimetric water content of organic layers strongly favored denitrification as the source of N_2O at 2000 m and 3000 m. Moreover, the low N_2O losses probably also resulted from the combination of low soil NO_3^- concentrations and high carbon in organic layers, which would have favored the already low gaseous N losses to be dominated by N_2 via denitrification.

3.5.2 N-addition effects on N_2O fluxes

Along our elevation gradient, N addition did not increase N_2O fluxes relative to the control (Table 3.1, Figure 3.1) although net ammonification and nitrification rates increased with N addition at 2000 m and 3000 m (Table 3.3). Along the same elevation gradient, N addition also led to increases in gross rates of N mineralization and nitrification and decreases in microbial immobilization of NH_4^+ and NO_3^- (measured in the third and fourth year of nutrient manipulation; Baldos et al. in press), which supported our observed increases in net ammonification, net nitrification, NH_4^+ (at 2000 m and 3000 m) and NO_3^-

concentrations (at all elevations) in N plots (Table 3.3). Such increases in net and gross rates of mineral N production and mineral N levels in the N plots, however, did not lead to increases in N_2O fluxes. This may be due to produced N_2O being further reduced to N_2. The idea that N_2 rather than N_2O was not the dominant gaseous N loss, was supported by the results of the ^{15}N tracing experiment; although denitrification was the main N_2O source, emitted $^{15}N_2O$ within 30 minutes of chamber closure accounted for maximally 0.065% of soil $^{15}NO_3^-$ (see *Results: effects of nutrient addition*), suggesting further reduction of N_2O to N_2 given favorable anaerobic microsites, high carbon and low NO_3^- levels (as we discussed above). Chronic N addition can cause increases in NO_3^- levels and decreases in soil pH, which then inhibit N_2O to N_2 reduction, as was observed in an Andosol soil from a montane forest on in Panama (Corre et al. 2014; Koehler et al. 2009, 2012). The increases in NO_3^- levels in our N plots (Table 3.3) were much lower than those observed from the Panamanian montane forest soil of course, since that soil received four years of 125 kg N ha^{-1} yr^{-1} (with NO_3^- levels as high as 50-60 mg N m^{-2} in the organic layer and 112-183 mg N m^{-2} in the mineral). Our moderate levels of nutrient addition are probably also the reason why soil pH in our N plots did not yet differ significantly from the control plots even after four years (Baldos et al. in press).

Whether an increase in soil N availability (e.g. mineral N concentrations, net/gross rates of mineral N production) results in an increase in N_2O fluxes also depends on inter-annual variations in climate. Corre et al. (2014) showed that the N_2O response to chronic N addition in tropical forest soils was strongly controlled by inter-annual variability of rainfall and thus soil moisture. In the first two years of N addition to our sites, Martinson et al. (2013) reported that net nitrification at all elevations increased slightly and that these increases were accompanied by small increases in N_2O fluxes in the second year of N addition. However, WFPS during our 2010-2012 measurement period (Table 3.1) was lower than that measured

during the first two years (2008-2009) of nutrient manipulation (63-88% WFPS; Martinson et al. 2013) when N addition led to increased N_2O fluxes. The positive correlation of N_2O fluxes with WFPS at 1000 m, both in control plots (see results) and across all treatments (Table S3.1), clearly indicates moisture limitations to N_2O fluxes. At 2000 m, a combination of changes in mineral N concentrations and soil moisture contents were controlling N_2O fluxes, as indicated by the significant positive correlation across all treatments (Table S3.1) but lack of significant effects of WFPS and mineral N concentrations in control plots. Thus, the relatively low soil moisture contents during our measurement period likely contributed to the generally low N_2O fluxes, and combined with its possible further reduction to N_2 in anaerobic microsites, resulted in insignificant effects of N addition on soil N_2O fluxes.

3.5.3 P-addition effects on N_2O fluxes

In contrast to the initial two years (2008-2009) of nutrient manipulation in our sites, when no effect of P addition on soil N_2O fluxes was detected (Martinson et al. 2013), we did detect several changes during our study period (2010-2012). As compared to control plots, P addition plots exhibited decreases in: soil N_2O fluxes at 1000 m (Table 3.1; Figure 3.1a), NH_4^+ at 3000 m and NO_3^- at 1000 m and 3000 m (Table 3.3) Across our elevation gradient, aboveground net primary production (ANPP) is limited by P and/or co-limited by N+P as indicated by the trend towards higher basal area increment already evident after one year of nutrient addition in P-addition plots (1000 and 2000 m) and in N+P-addition plots (all elevations) (Homeier et al. 2012, 2013). Increased ANPP with P addition could mean that there was an increase in plant uptake of soil nutrients, including soil mineral N. P addition did not change net (Table 3.3) or gross (Baldos et al. in press) rates of mineral N production and hence, increase in uptake of soil mineral N by plants without changes in rates of soil mineral N production, would lead to lower mineral N levels in P plots. Since NO_3^- was the main

substrate for N_2O production across our elevation gradient (see *Results on ^{15}N tracing method*, i.e. denitrification as the main N_2O source), decreased NO_3^- concentrations in P plots also led to reduced N_2O fluxes. A comparable mechanism was described for a 6 year old leguminous tree plantation in Indonesia, where P addition alleviated plant P limitation and increased root N uptake, resulting in decreased mineral N concentrations and N_2O fluxes (Mori et al. 2013). At 3000 m, we did not observe a significant reduction in N_2O fluxes in P addition plots, despite a reduction in mineral N, but at this site N_2O fluxes were already very low to start with (Figure 3.1c). Additionally, N_2O fluxes at 3000 m elevation were not correlated with any of the measured soil factors, neither for control plots nor across all treatments (Tables 3.4 and S3.1), which suggests that the N_2O fluxes were too low (mostly fluctuating around zero; Figure 3.1c) to generate any significant relationship with the soil factors known to control N_2O fluxes.

3.5.4 Combined N+P-addition effects on N_2O fluxes

As was the case for the N plots, N_2O fluxes from N+P plots did not differ from the control plots along the elevation gradient (Table 3.1; Figure 3.1). Again, this is in contrast to the second year (2009) of N+P addition to our sites, when Martinson et al. (2013) observed increases in N_2O fluxes from the N+P plots compared to the control plots. The net (Table 3.3) and gross (Baldos et al. in press) rates of mineral N production and the soil mineral N concentrations in the N+P plots during our 2010-2012 measurement period were comparable with the N plots, in that both were larger than the control plots. In P plots, however, these parameters either decreased, or did not change. Thus, changes in rates of mineral N production and mineral N concentrations in N+P plots were dominated by the added N. It can be noted that the effects of N+P addition on these parameters (Table 3.3: net ammonification and NO_3^- at 1000 m and 3000 m; NH_4^+ at 2000 m and 3000 m) were not as strong as the

effects of N addition alone, presumably because of the opposing effect of P addition on these parameters (as discussed above). Similar to the N plots, then soil N_2O fluxes from N+P plots did not differ from the control plots (despite an increase in soil N availability) due to the generally low N_2O fluxes and relatively low soil moisture contents during our study period compared to the 2008-2009 measurement period of Martinson et al. (2013).

In conclusion, we have shown that soil N_2O fluxes in our study sites were among the lowest measured in TMFs and that denitrification was the main (at 1000 m) or the only (at 2000 m and 3000 m) source of N_2O production, probably due to anaerobic microsites. These low levels of N_2O fluxes were the result of the conservative soil N cycling along our elevation gradient (Baldos et al. in press), and the combination of low NO_3^- concentrations and presumably high available C in the organic layers (at 2000 m and 3000 m) which probably favored the already low gaseous N losses to be dominated by N_2 via denitrification.

In contrast to the first two years of this study (Martinson et al. 2013), in the third to the fifth year we did not detect significant increases in N_2O fluxes despite increase in soil N availability. This can be attributed to the generally low N_2O fluxes during our 2010-2012 measurement period, which we in turn attribute to the relatively low rainfall and soil moisture contents during our study period However, we did detect a reduction in soil mineral N concentrations and N_2O fluxes with P addition in the third year, again in contrast to the first two years of P addition, when no effects on N_2O fluxes were observed (Martinson et al. 2013). The significant P effect was probably due to increased uptake of soil mineral N by vegetation after an extended period of P addition, since P is a limiting element for ANPP at our sites. N+P addition showed similar trends in net rates of mineral N production, mineral N concentrations and N_2O fluxes as those with N addition alone, although to a lesser degree because of the counteracting effects of P addition. Combined with the previous work of Martinson et al. (2013), our results show that effects of N and P addition on soil N_2O fluxes

were not linear with time of exposure to elevated nutrient inputs. We observed large inter-annual variation in N_2O responses, which we attributed to changes of soil moisture conditions. Without this multiple-year study we would not have been able to detect these annual variations of N_2O responses to N and P additions, highlighting the importance of long-term studies.

3.6 References

Aber J, McDowell W, Nadelhoffer K, et al. (1998) Nitrogen saturation in temperate forest ecosystems: hypotheses revisited. *BioScience 48:* 921-934.

Arnold J (2008) Internal nitrogen cycling in tropical forest soils. PhD thesis, Georg-August-Universität Göttingen, Göttingen.

Arnold J, Corre MD, Veldkamp E (2008) Cold storage and laboratory incubation of intact soil cores do not reflect in-situ nitrogen cycling rates of tropical forest soils. *Soil Biology and Biochemistry 40*: 2480-2483.

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

Baldos AP, Corre MD, Veldkamp E (in press) Responses of N cycling to nutrient inputs in forest soils across 1000-3000-m elevation gradient in the Ecuadorian Andes. *Ecology*.

Bateman EJ, Baggs EM (2005) Contributions of nitrification and denitrification to N_2O emissions from soils at different water-filled pore space. *Biology and Fertility of Soils 41*: 379-388.

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 Biogeochemical Cycles 22*: GB4011.

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.

Bubb P, May I, Miles L, Sayer J (2004) *Cloud forest agenda*. UNEP-WCMC, Cambridge.

Butterbach-Bahl K, Gasche R, Huber CH, Kreutzer K, Papen H (1998) Impact of N-input by wet deposition on N-trace gas fluxes and CH_4-oxidation in spruce forest ecosystems of the temperate zone in Europe. *Atmospheric Environment 32*: 559-564.

Chapuis-Lardy L, Wrage N, Metay A, Chotte J-L, Bernoux M (2007) Soils, a sink for N_2O? A review. *Global Change Biology 13*: 1-17.

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, Lamersdorf NP (2004) Reversal of nitrogen saturation after long-term deposition reduction: impact on soil nitrogen cycling. *Ecology 85*: 3090-3104.

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.

Crawley MJ (2012) *The R book*. Wiley, Chichester.

Davidson EA (1991) Fluxes of nitrous oxide and nitric oxide from terrestrial ecosystems. In: Rogers JE, Whitman WB (eds) *Microbial production and consumption of greenhouse gases: methane, nitrogen oxides and halomethanes*. American Society for Microbiology, Washington, pp 219-235.

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.

Dechert G, Veldkamp E, Brumme R (2005) Are partial nutrient balances suitable to evaluate nutrient sustainability of land use systems? Results from a case study in Central Sulawesi, Indonesia. *Nutrient Cycling in Agroecosystems 72*: 201-2012.

Denman KL, Brasseur G, Chidthaisong A, et al. (2007) Couplings between changes in the climate system and biogeochemistry. In: Solomon S, Qin D, Manning M, et al. (eds) *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, pp 499-588.

Ehhalt D, Prather M, Dentener F, et al. (2001) Atmospheric chemistry and greenhouse gases. In: Houghton JT, Ding Y, Griggs DJ, et al. (eds) *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge, pp 241-287.

Emck P (2007) *A climatology of south Ecuador with special focus on the major Andean ridge as Atlantic-Pacific climate divide*. PhD thesis, Friedrich-Alexander-Universität Erlangen-Nürnberg.

FAO (1993) *Forest resources assessment 1990 – Tropical countries. FAO Forestry Paper 112*, Food and Agricultural Organization of the United Nations, Rome.

Firestone MK, Davidson EA (1989) Microbiological basis of NO and N_2O production and consumption in soil. In: Andreae MO, Schimel DS (eds) *Exchange of trace gases between terrestrial ecosystems and the atmosphere*. Wiley, New York, pp 7-21.

Glatzel S, Well R (2008) Evaluation of septum-capped vials for storage of gas samples during air transport. *Environmental Monitoring and Assessment 136*: 307-311.

Gundersen P, Emmett BA, Kjønaas OJ, Koopmans CJ, Tietema A (1998) Impact of nitrogen deposition on nitrogen cycling in forests: as synthesis of NITREX data. *Forest Ecology and Management 101*: 37-55.

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 RW, Angle JS, Bottomley PJ, Bezdicek DF, Smith S, Tabatabai MA, Wollum AG. (eds) *Methods of soil analysis, Part 2: microbial and biochemical properties*. Soil Science Society of Amerika Book Series, Madison, Wisconson, pp 985-1018.

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

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

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. *PLoS ONE 7*: e47128. doi:10.1371/journal.pone.0047128.

Homeier J, Leuschner C, Bräuning A, et al. (2013) Effects of nutrient addition on the productivity of montane forests and implications for the carbon cycle. 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, Springer, Heidelberg, pp 315-329.

Hudson B (1994) Soil organic matter and available water capacity. *Journal of Soil and Water Conservation 49*: 189-194.

Keller M, Varner R, Dias JD, et al. (2005) Soil-atmosphere exchange of nitrous oxide, nitric oxide, methane, and carbon dioxide in logged and undisturbed forest in the Tapajos National Forest, Brazil. *Earth Interactions 9*: 1-28.

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. *Global Change Biology 15*: 2049-2066.

Litherland M, Aspden JA, Jemielita RA (1994) *The metamorphic belts of Ecuador*. Overseas Memoir 11. British Geological Survey, Nottingham.

Linn DM, Doran JW (1984) Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. *Soil Science Society of America Journal 48*: 1267-1272.

Liu L, Graever TL (2009) A review of nitrogen enrichment effects on three biogenic GHGs: the CO_2 sink may be largely offset by stimulated N_2O and CH_4 emission. *Ecology Letters 12*: 1103-1117.

Machefert SE, Dise NB (2004) Hydrological controls on denitrification in riparian ecosystems. *Hydrology and Earth System Sciences 8*: 686-694.

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.

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.

Matson PA, Vitousek PM (1987) Cross-system comparisons of soil nitrogen transformations and nitrous oxide flux in tropical forest ecosystems. *Global Biogeochemical Cycles 1*: 163-170.

Mori T, Ohta S, Ishizuka S, et al. (2013) Soil greenhouse gas fluxes and C stocks as affected by phosphorus addition in a newly established *Acacia mangium* plantation in Indonesia. *Forest Ecology and Management 310*: 643-651.

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.

Piepho HP, Büchse A, Richter C (2004) A mixed modelling approach for randomized experiments with repeated measures. *Journal of Agronomy and Crop Science 190*: 230-247.

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. *Global Biogeochemical Cycles 20*: GB3010. doi:10.1029/2005GB002516.

R Development Core Team (2012) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna. ISBN 3-900051-07-0. http://www.R-project.org/.

Schwendenmann L, Veldkamp E (2005) The role of dissolved organic carbon, dissolved organic nitrogen, and dissolved inorganic nitrogen in a tropical wet forest ecosystem. *Ecosystems 8*: 330-351.

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

Sousa Neto E, Carmo JB, Keller M, et al. (2011) Soil-atmosphere Exchange of nitrous oxide, methane and carbon dioxide in a gradient of elevation in the coastal Brazilian Atlantic forest. *Biogeosciences 8*: 733-742.

Stevens RJ, Laughlin RJ, Burns LC, Arah JRM, Hood RC (1997) Measuring the contributions of nitrification and denitrification to the flux of nitrous oxide from soil. *Soil Biology and Biochemistry 29*: 139-151.

Tanner EVJ, Vitousek PM, Cuevas E (1998) Experimental investigation of nutrient limitation of forest growth on wet tropical mountains. *Ecology 79*: 10-22.

Teh YA, Diem T, Jones S, et al. (2014) Methane and nitrous oxide fluxes across an elevation gradient in the tropical Peruvian Andes. *Biogeosciences 11*: 2325-2339.

Van Straaten O, Veldkamp E, Köhler M, Anas I (2010) Spatial and temporal effects of drought on soil CO_2 efflux in a cacao agroforestry system in Sulawesi, Indonesia. *Biogeosciences 7*: 1223–1235.

Vitousek PM, Matson PA (1988) Nitrogen transformations in a range of tropical forest soils. *Soil Biology and Biochemistry 20*: 361-367.

Weier KL, Doran JW, Power JF, Walters DT (1993) Denitrification and dinitrogen/nitrous oxide ratio as affected by soil water, available carbon, and nitrate. *Soil Science Society of America Journal 57*: 66-72.

Wolf K, Veldkamp E, Homeier J, Martinson GO (2011) Nitrogen availability links forest productivity, soil nitrous oxide and nitric oxide fluxes of a tropical montane forest in southern Ecuador. *Global Biogeochemical Cycles 25*: GB4009. doi:10.1029/2010GB003876.

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) *Mixed effects models and extensions in ecology with R*. Springer, New York.

Table S3.1 Pearson coefficients for monthly average (n = 80) soil N_2O flux (μg N m^{-2} h^{-1}), soil temperature (°C), water-filled pore space (WFPS; %) and mineral N concentrations (mg N m^{-2}) of all treatment plots of montane forests along a 1000- to 3000-m elevation gradient

Elevation (m)		Soil temperature	WFPS	NH_4^+	NO_3^-
1000	Soil N_2O flux	-0.21	0.25*	0.02	0.12
	Soil temperature		-0.29**	0.49**	0.15
	WFPS			-0.16	-0.24**
	NH_4^+				0.36**
2000	Soil N_2O flux	0.09	0.32**	0.21*	0.10**
	Soil temperature		-0.16	0.03	-0.04
	WFPS			0.01	-0.10
	NH_4^+				0.58**
3000	Soil N_2O flux	0.10	-0.04	0.09	0.03
	Soil temperature		-0.01	0.07	0.03
	WFPS			0.33**	0.08
	NH_4^+				0.56**

*$P < 0.05$, ** $P < 0.01$

CHAPTER 4

Nutrient addition increases soil CH_4 uptake across an elevation gradient in Andean tropical montane forests

4.1 Abstract

Tropical forest soils are important sinks for the greenhouse gas methane (CH_4). They are also increasingly affected by elevated nitrogen (N) and phosphorus (P) deposition. An increase in N and P availability has the potential to affect the CH_4 budget of these ecosystems. While inhibiting effects of nitrogen on CH_4 fluxes have been studied extensively, there is increasing evidence that nutrients can limit CH_4 uptake, especially in tropical montane forests. Here, we assess the impact of moderate N (50 kg N ha^{-1} yr^{-1}), P (10 kg P ha^{-1} yr^{-1}) and N+P additions on soil CH_4 fluxes across an elevation gradient of tropical montane forests in Ecuador. Using static vented chambers, we measured CH_4 fluxes in a nutrient manipulation experiment, at elevations of 1000 m, 2000 m and 3000 m over a period of five years. Control forest soils were net atmospheric CH_4 sinks with annual fluxes of -2.23 ± 0.52 kg C ha^{-1} at 1000m, -2.77 ± 0.15 kg C ha^{-1} at 2000m and -1.45 ± 0.63 kg C ha^{-1} at 3000 m elevation. During the first two years, nutrient addition did not affect soil CH_4 fluxes at any elevation, which we attributed to the combination of moderate amounts of added nutrients; the strong immobilization of added nutrients, and the separation of the highest CH_4 uptake capacity in the subsoil from the surface of the soil, where fertilizers were added. In years three to five, nutrient additions increased soil CH_4 uptake. However effects for N and P varied along the elevation gradient: at 1000 m, N and N+P addition increased annual CH_4 uptake by 20-60%; at 2000 m, P and N+P addition increase uptake by 21-50%; and at 3000 m, N addition increased CH_4 uptake by 34-40%. These differential effects of nutrient addition may be related to initial soil nutrient status and differential responses of ecosystem components to nutrient addition at each elevation. Our results are the first to show that CH_4 uptake across an elevation gradient of tropical montane forest was nutrient limited and could be stimulated by elevated N and P deposition. Whether increases in CH_4 uptake capacity will continue to be

elevated under chronic N deposition is uncertain since it is likely that after some time chronic N deposition will lead to inhibition of CH_4 uptake.

4.2 Introduction

Atmospheric methane (CH_4) concentrations have more than doubled since pre-industrial times due to human activities (Etheridge et al. 1998) and are steadily increasing despite a short period of stabilization between 1999 and 2006 (Kirschke et al. 2013). Nowadays, this makes CH_4, which is mainly produced by methanogenic archea, the second most important greenhouse gas causing global warming (Denman et al. 2007). Soils are both important natural biogenic sinks and sources of CH_4 (Le Mer and Roger 2001), in which CH_4 production in anaerobic zones and CH_4 consumption in aerobic zones can occur simultaneously. In aerated soils, CH_4 uptake dominated over CH_4 production, which makes these soils a net CH_4 sink. Globally, forest soils are strong net CH_4 sinks (Le Mer and Roger 2001), with tropical forest soils contributing about 28% (6.2 Tg CH_4 yr^{-1}) to the global annual CH_4 uptake by soils (Dutaur and Verchot 2007). Although tropical montane forest (TMF) soils cover more than 11% of the world's tropical forest area (Bubb et al. 2004; FAO 1993), they are presently neglected in global CH_4 budgets, despite large variance in CH_4 uptake across elevation gradients within this ecosystem (Purbopuspito et al. 2006; Teh et al. 2014).

Strength and direction of soil CH_4 fluxes in aerated soils are mainly controlled by soil moisture (Bowden et al. 1998), soil texture (Dörr et al. 1993) and presence of organic layers (Saari et al. 1998), which affect CH_4 production, consumption and transport via soil oxygen status and gas diffusion (Bradford et al. 2001). In addition, soil temperature (Bowden et al. 1998) and nitrogen (N) availability (Bodelier and Laanbroek 2004) have been shown to be important controlling factors, so that the interplay of different controlling factors finally determines CH_4 fluxes.

Human activities like biomass burning, fossil fuel consumption and fertilizer use have more than doubled the amount of reactive nitrogen (N) cycling globally (Galloway et al. 2008). The area affected by emitted reactive N is not only restricted to the region close to its origin, but also includes areas far from its source. Elevated N deposition has been observed in natural tropical forest regions, for example (Hietz et al. 2011), and further increases, which are expected in the next decade could exceed 25 kg N ha^{-1} yr^{-1} (Phoenix et al. 2006). Compared to lowland forests TMFs may be especially affected due to the importance of cloud water deposition (Carillo et al. 2002).

There are contradictory observations of the effect of N addition on soil CH_4 fluxes from forest soils, which has mainly been studied in temperate regions (Bodelier and Lannbroek 2004). For years, most studies discussed the inhibitory effect of N addition on soil CH_4 uptake. However, in the last decade an increasing amount of studies have shown potential N limitation of CH_4 uptake in soils (Bodelier and Laanbroek 2004) and recently, indications of N-limited CH_4 uptake came from TMF soils in Ecuador (Wolf et al. 2012) and Panama (Veldkamp et al. 2013). Several mechanisms may affect CH_4 uptake by soils following N addition: (1) competition of ammonium with CH_4 for reactive sites of the methane monooxygenase enzyme, which inhibit of methane oxidation (Bédard and Knowles 1989), (2) toxic effects of by-products of N transformation (e.g. NO_2^-, NO, N_2O) on methanogenic archea (Klüber and Conrad 1998) and methanotrophic bacteria (Schnell and King 1994), (3) inhibition of methanogenesis by nitrate since it is preferred as an electron-acceptor (Conrad 1989), (4) osmotic effects of high N doses (Schnell and King 1996), (5) microbial N limitation of methanotrophs (Bodelier and Laanbroek 2004) and (6) reduction of energy-intensive N assimilation by methanotrophic microorganisms that are also capable of N_2-fixation (Hanson and Hanson 1996). Only some of these processes have been demonstrated in the field.

In tropical South America, phosphorus (P) deposition is also predicted to increase through increased input from biomass burning, anthropogenic mineral aerosols and biogenic particles from the neighboring Amazon Basin (Mahowald et al. 2005), as well as from Saharan dust (Okin et al. 2004). How elevated P availability affects soil CH_4 fluxes from soils has not been studied extensively, however P addition in P-deficient soils may affect soil CH_4 fluxes (1) directly via increased microbial activity and growth if this includes methanogenic archaea and methanotrophic bacteria (Cleveland et al. 2002) or (2) indirectly via changes in soil oxygen and nutrient (i.e. N) status caused by changes in plant water and plant nutrient uptake (Zhang et al. 2011).

Recent studies indicate that multiple nutrient limitations are common in diverse tropical forests (Kaspari et al. 2008) and in diverse TMFs there is increasing evidence that co-limitation of N and P occurs (Homeier et al. 2012; Tanner et al. 1998). In (sub)tropical forests only two in-situ nutrient addition studies have been conducted in which year-round CH_4 fluxes were measured. These studies have shown contradictory effects of N addition on CH_4 fluxes. In Panama, addition of 125 kg urea-N ha^{-1} yr^{-1} did not significantly affect CH_4 uptake both on long-term (9-12 yrs) N amended lowland tropical Nitisols/Cambisols and mid-term (1-4 yrs) N amended tropical montane forest Andosols with a thick organic layer. However, there were indications that CH_4 uptake was N-limited (Veldkamp et al. 2013). In contrast, addition of 50 - 150 kg NH_4NO_3-N ha^{-1} yr^{-1} applied for up to 3 years in N-saturated subtropical forest Oxisols decreased CH_4 uptake in Southern China (Zhang et al. 2008, 2011). Addition of 150 kg NaH_2PO_4-P ha^{-1} yr^{-1} in the same forest increased CH_4 uptake, while addition of N+P with 150 kg ha^{-1} yr^{-1} each had no effect on CH_4 fluxes. The results from these experiments and those conducted in other climates suggest that N addition to soils with high soil N status and high amounts of added N tend to inhibit CH_4 uptake (Aronson and Helliker 2010) while N addition to soils with low N availability may stimulate CH_4 uptake.

Here we report the effects of 5 years of moderate nutrient addition on soil CH_4 fluxes across an elevation gradient in tropical montane forests of Southern Ecuador. We hypothesized that N, P and N+P addition would increase CH_4 uptake since these forests showed evidence of N and P co-limitation (Homeier et al. 2012, 2013) and an earlier study showed indications of N-limited CH_4 uptake in the same area (Wolf et al. 2012).

4.3 Material and Methods

4.3.1 Study area

The study area was located in a tropical mountain ecosystem in the provinces Loja and Zamora Chinchipe, on the eastern slope of the South Ecuadorian Andes. We selected three sites along an elevation gradient (1000-3000 m above sea level (asl)) of old-growth tropical montane rainforests within the Podocarpus National Park and the adjacent private Biological Reserve San Francisco.

The elevation gradient covers a premontane tropical forest (Homeier et al. 2008) on a Dystric Cambisol developed on deeply weathered granitic rock (Litherland et al. 1994; Martinson et al. 2013) at '1000 m' (990-1100 m asl; 4.115° S, 78.968° W), a lower montane rain forest on a Stagnic Cambisol at '2000 m' (1950-2100 m asl; 3.982° S, 79.083° W) and an upper montane rain forest on a Stagnic Histosol at '3000 m' (2900-3050 m asl; 4.110° S, 79.178° W). Soils at 2000 m and 3000 m were both formed from methamorphosed schists and covered by 0.1-0.4 m organic layers (Litherland et al. 1994). Detailed forest and soil characteristics are given by (Homeier et al. 2013; Martinson et al. 2013) and in Table S2.1.

Along the elevation gradient mean annual temperature decreased from 19.4 at 1000 m to 15.7 at 2000 m and 9.4° C at 3000 m, whereas mean annual precipitation was lowest at 2000 m with 1950 mm, followed by 1000 m elevation with 2230 mm and highest at 3000 m elevation with 4500 mm (Moser et al. 2007). Rainfall and temperature display only slight

seasonal variability in this region (Emck 2007). Ambient annual nutrient bulk and dry deposition in the study region increased between 1998 and 2010 ranging from 14 to 45 kg N and 0.4 to 4.9 kg P ha^{-1} (Boy et al. 2008; Homeier et al. 2012).

4.3.2 Experimental design

A full factorial nutrient manipulation experiment (NUMEX) with N-, P-, N+P-addition and untreated control treatments, was established along the elevation gradient in 2007 as described in previous studies (Homeier et al. 2013; Martinson et al. 2013). In short, we established at each site a stratified complete block design, comprising of four replicate blocks, each with four plots (20 x 20 m) with at least 10 m distance between plots. Blocks were established along topographic positions which could affect soil CH_4 fluxes (Wolf et al. 2012). Treatments were assigned randomly within a block with the restriction that unfertilized control treatments were located upslope and the combined treatment of N+P addition were located downslope in each block, to avoid unwanted fertilization effects due to nutrient leaching in this steep terrain.

Nutrients were applied manually at moderate rates of 50 kg N ha^{-1} yr^{-1} in the form of urea (CH_4N_2O) and 10 kg P ha^{-1} yr^{-1} in form of sodium hydrogen phosphate ($NaH_2PO_4 \cdot H_2O$ and $NaH_2PO_4 \cdot 2H_2O$, with analytic grade quality). Starting in early 2008, rates were split into two equal applications per year (February/March and August/September) with a four-month delay of the second fertilization in 2010 due to logistical problems related to the shipping of high-grade P fertilizer from Germany to Ecuador.

4.3.3 Soil CH_4 flux, temperature, moisture and mineral N measurements

Soil CH_4 fluxes were measured with static vented chambers consisting of permanently installed round polyvinyl chloride chamber bases (area 0.04 m², height 0.15 m, ~0.03 m inserted into the soil) and polyethylene chamber hoods (totaling the chamber volume to 12 L), equipped with a Luer-Lock sampling port and vent for pressure equilibrium. In three out of four blocks per elevation, four chamber bases per plot were installed in 2007 along two perpendicular random transects and one additional chamber per plot was installed before January 2010, ensuring a minimum distance of 2 m to the border for non-control plots. Four gas samples were drawn at 2, 14, 26 and 38 minutes after chamber closure in a monthly sampling frequency from January 2008 to September 2009 and from November 2010 to August 2012. Gas samples from the additional chamber were taken monthly from January 2010 to August 2012 at 3, 13, 23 and 33 minutes after chamber closure. Since we were not interested in short-term effects of nutrient manipulation, measurements within three weeks after fertilization were excluded. Equipment failure resulted in missing values in the time from June 2009 to July 2009 and May 2011 to July 2011.

For logistical reasons, we stored and transported gas samples in pre-evacuated 60 ml glass containers equipped with stop cocks until April 2011 and in 12 ml Labco Exetainer® (Labco Limited, Lampeter, UK) with pierceable rubber septa thereafter (as overpressured samples). Analysis of gas samples was done with gas chromatographs (Shimadzu GC-14B, Germany and Carlo Erba GC 6000 Vega Series 2; AllTech® packed GC column) equipped with a flame ionization detector (FID) and autosampler (Loftfield et al. 1997) and ASPEC auto-sampler, Gilson SAS; Villiers, Le Bel, France). Integrated peak areas of samples were compared to three to four standard gases (between 1000 and 20,000 ppb; Deuste Steininger GmbH, Mühlhausen, Germany). Analysis was performed either within one day in Ecuador or up to several months later in Germany. Extended storage and transport were only performed

for gas samples in Exetainers®, which are known for their good quality during extended sample storage and aircraft transport (Glatzel and Well 2008), and their performance was tested by crosschecking pressure and concentration of transported calibration gases. Gas flux per chamber was calculated from the linear increase of CH_4 concentration in the headspace over time, whereby the headspace air volume was estimated based on measurements of the chamber height at three places around the chamber base and linear fit of data was checked visually and via coefficient of determination. Gas fluxes were adjusted for air pressure and temperature measured during sampling and expressed as CH_4-C flux per area soil ($\mu g\ C\ m^{-2}\ h^{-1}$). Annual soil CH_4 fluxes were approximated using the trapezoid rule on time intervals between measured flux rates, assuming constant daily flux rates.

Parallel to gas sampling, we measured soil temperature, gravimetric soil moisture and extractable mineral N concentrations (in situ extraction with 0.5 M K_2SO_4 solution) of the top 0.05-m depth within each plot. Soil temperature was measured more intensively from 2010 onwards with measurements close to each chamber base, where gas measurements were taken. Soil moisture and mineral N concentrations were determined from pooled samples per plot and soil moisture was expressed as water-filled pore space (WFPS; for calculation see Chapter 2).

4.3.4 Statistical analysis

Statistical analyses of our data were conducted using R 2.14.0 (R Development Core Team, 2012). Data were checked for normality and homoscedasticity and either a square root or logarithmic transformation (adding a constant value if the dataset included negative values) was applied when required.

The relationship between monthly average soil CH_4 flux and soil parameters (WFPS, mineral NH_4^+ and NO_3^- concentrations, temperature) was tested for control plots with

Pearson's correlation. Analyses were conducted on the means of the three replicate plots on each sampling day, considering the measurements conducted in the last three years of the study (2010 to 2012).

Before assessing nutrient-addition effects, pre-existing differences in CH_4 fluxes among plots at each elevation were assessed for measurements conducted one month prior to the start of fertilization using a one-way analysis of variance with block effect. Nutrient-addition effects as well as elevation effects on time series and cumulative data of soil CH_4 fluxes were then assessed using linear mixed effects (LME) models (Crawley 2007, Piepho et al. 2004) on plot-mean CH_4 fluxes (four and five chambers) for each year separately as well as for different time period from the beginning of the experiment. Treatment effects on soil temperature and moisture of plot means were analyzed the same way without subdividing into shorter time periods. Analyses of treatment effects were conducted separately for each of the three elevations. Nutrient treatment was considered a fixed effect, whereas sampling day and spatial replicate were included as random effects. Elevation effects on time series data were tested only on control plots with elevation as fixed effect and sampling day as random effect. The following structures were included in the LME model if these improved the relative goodness of the model-fit based on the Akaike information criterion: (1) a first-order temporal autoregressive process to account for decreasing correlation of measurements with increasing time difference (Zuur et al. 2009), and (2) a variance function to account for heteroscedasticity of residual variances (Crawley 2007). The significance of the fixed effects was then determined by analysis of variance at $P \leq 0.05$. Mean values in the text are given with ± standard error (SE).

4.4 Results

4.4.1 Controlling factors and soil CH_4 flux of control forests along the elevation gradient

Mean soil temperature (±SE, n = 3) in control forests decreased with increasing elevation (1000 – 3000 m) from 17.7 ± 0.1 to 13.8 ± 0.0 and 7.2 ± 0.2°C during 2008 to 2012 ($P < 0.001$). Mean soil moisture (±SE, n = 3) was highest at 2000 m with 80.4 ± 4.1% WFPS, followed by 3000 m with 59.6 ± 0.8% WFPS and 1000 m with 48.6 ± 4.7% WFPS ($P < 0.001$). Neither soil temperature nor WFPS displayed a clear seasonal pattern at any site (data not shown).

Mineral N concentrations in the top 5 cm of control forest soils varied with elevation; NH_4^+ concentrations decreased from 422.85 ± 6.86 mg N m^{-2} at 2000 m to 333.76 ± 26.65 mg N m^{-2} at 1000 m elevation and 236.67 ± 12.13 mg N m^{-2} at 3000 m ($P \leq 0.027$); NO_3^- concentrations were highest at 1000 m ($P < 0.001$) with 38.35 ± 21.25 mg N m^{-2} and did not differ between 2000 m with 5.72 ± 0.96 mg N m^{-2} and 3000 m with 3.08 ± 0.29 mg N m^{-2} ($P = 0.405$).

Annual soil CH_4 uptake in control forests was 35 and 48 % higher at 1000 m and 2000 m compared to 3000 m over the entire measurement period from 2008 to 2012 ($P < 0.001$; Table 4.1), but there was considerable inter-annual variability; in 2008 CH_4 uptake did not differ between elevations ($P = 0.136$), while in 2012 CH_4 uptake at 2000 m was higher compared to the other two elevations that did not differ from each other (Table 4.2). There was no seasonal trend of CH_4 uptake at any of the sites, but temporal variability within and between years were largest at 1000 m (Figure 4.1).

Table 4.1 Mean (± SE, *n* = 3) annual soil CH_4 fluxes (kg C ha^{-1} yr^{-1}) from montane forests along an elevation gradient during five years (2008-2012) of nutrient manipulation. Annual soil CH_4 fluxes were approximated by applying the trapezoid rule on time intervals between measured flux rates, assuming constant flux rates per day.

Elevation (m)	Treatment	2008†	2009	2010/2011†	2012	2008-2012
1000	Control	-2.02 ± 0.41	-2.96 ± 0.38	-2.57 ± 0.71	-2.23 ± 0.75	-2.23 ± 0.52
	Nitrogen (N)	-2.12 ± 0.81	-2.38 ± 0.37	-4.11 ± 1.24	-3.55 ± 1.79	-2.94 ± 0.89
	Phosphorus (P)	-4.07 ± 1.73	-2.94 ± 0.48	-2.96 ± 1.30	-1.87 ± 1.11	-3.50 ± 0.96
	N + P	-3.29 ± 0.96	-2.43 ± 0.44	-3.77 ± 1.42	-2.68 ± 1.18	-3.27 ± 1.04
2000	Control	-2.42 ± 0.30	-2.59 ± 0.09	-2.49 ± 0.38	-2.99 ± 0.59	-2.77 ± 0.15
	N	-2.77 ± 0.73	-3.01 ± 0.44	-2.52 ± 0.19	-2.77 ± 0.15	-2.82 ± 0.39
	P	-2.23 ± 0.41	-2.67 ± 0.35	-3.39 ± 0.44	-3.61 ± 0.65	-3.16 ± 0.25
	N + P	-3.08 ± 0.15	-2.71 ± 0.12	-3.73 ± 0.24	-3.84 ± 0.50	-3.28 ± 0.20
3000	Control	-1.52 ± 0.50	-1.54 ± 0.75	-1.52 ± 0.52	-1.81 ± 0.54	-1.45 ± 0.63
	N	-1.81 ± 0.56	-1.31 ± 0.67	-2.03 ± 0.55	-2.54 ± 0.62	-1.79 ± 0.55
	P	-1.52 ± 0.29	-1.32 ± 0.33	-1.64 ± 0.49	-1.86 ± 0.50	-1.36 ± 0.39
	N + P	-1.29 ± 0.23	-1.29 ± 0.47	-1.28 ± 0.24	-1.33 ± 0.16	-1.25 ± 0.13

† In 2008, annual values include one pre-treatment measurement; in 2010/2011, annual values include only two monthly measurements from 2010.

Table 4.2 Mean (± SE, n = 3) soil CH_4 fluxes (μg C m^{-2} h^{-1}) in montane forests across an elevation gradient.

Elevation (m)	Treatment	Soil CH_4 flux (μg C m^{-2} h^{-1})			
1000		2008	2009	2010/11	2012
	Control	-22.17 ± 4.65	-32.85 ± 3.82	-28.13 ± 8.94[a]	-24.96 ± 8.93
	Nitrogen (N)	-44.00 ± 22.28	-32.56 ± 8.83	-44.99 ± 14.72[b]	-39.60 ± 20.51
	Phosphorus (P)	-26.85 ± 7.20	-31.41 ± 5.02	-33.31 ± 15.75[a]	-21.37 ± 12.90
	N + P	-39.53 ± 11.60	-31.75 ± 6.25	-40.54 ± 16.26[ab]	-29.31 ± 14.97
			2008-2009	2008-2011	2008-2012
	Control		-26.78 ± 2.4	-27.25 ± 4.61[a]	-26.67 ± 5.10[a]
	N		-39.26 ± 16.59	-41.21 ± 15.60[c]	-40.84 ± 16.63[b]
	P		-28.73 ± 6.23	-30.25 ± 9.63[ab]	-28.29 ± 10.40[a]
	N + P		-36.13 ± 8.80	-37.75 ± 10.90[bc]	-35.77 ± 11.88[b]
2000		2008	2009	2010/11	2012
	Control	-28.12 ± 4.17	-31.57 ± 1.51	-30.19 ± 4.68[a]	-34.45 ± 6.56[a]
	N	-32.10 ± 7.84	-34.42 ± 4.95	-29.27 ± 1.98[a]	-31.59 ± 1.56[a]
	P	-25.99 ± 4.89	-30.77 ± 3.31	-40.56 ± 5.73[b]	-41.53 ± 7.66[b]
	N +P	-35.67 ± 1.27	-30.62 ± 1.01	-43.96 ± 2.89[b]	-44.15 ± 5.18[b]
			2008-2009	2008-2011	2008-2012
	Control		-29.63 ± 2.79	-29.79 ± 1.33[a]	-30.91 ± 2.19[a]
	N		-33.12 ± 6.14	-31.64 ± 4.49[a]	-31.63 ± 3.76[ab]
	P		-28.08 ± 2.39	-32.88 ± 0.95[a]	-34.92 ± 2.48[b]
	N + P		-33.46 ± 1.10	-37.42 ± 1.39[b]	-39.00 ± 2.26[c]
3000		2008	2009	2010/11	2012
	Control	-16.88 ± 5.62	-16.97 ± 8.01	-16.98 ± 5.80[a]	-20.80 ± 6.49[a]
	N	-20.98 ± 6.72	-16.57 ± 7.03	-23.02 ± 6.02[b]	-29.60 ± 7.40[b]
	P	-17.60 ± 3.22	-15.73 ± 3.48	-17.99 ± 5.23[a]	-21.27 ± 5.78[a]
	N + P	-14.70 ± 2.57	-14.65 ± 4.43	-14.77 ± 3.20[a]	-15.59 ± 1.31[a]
			2008-2009	2008-2011	2008-2012
	Control		-16.91 ± 6.41	-16.94 ± 6.00[a]	-17.88 ± 6.12[a]
	N		-19.51 ± 6.80	-20.87 ± 6.49[b]	-23.01 ± 6.66[b]
	P		-16.97 ± 3.31	-17.38 ± 4.07[a]	-18.32 ± 4.48[a]
	N + P		-14.68 ± 2.13	-14.73 ± 2.51[a]	-14.92 ± 1.58[a]

Means within each year or time period followed by different superscript letters indicate significant differences among treatments within each elevation (linear mixed effects model at $P \leq 0.05$)

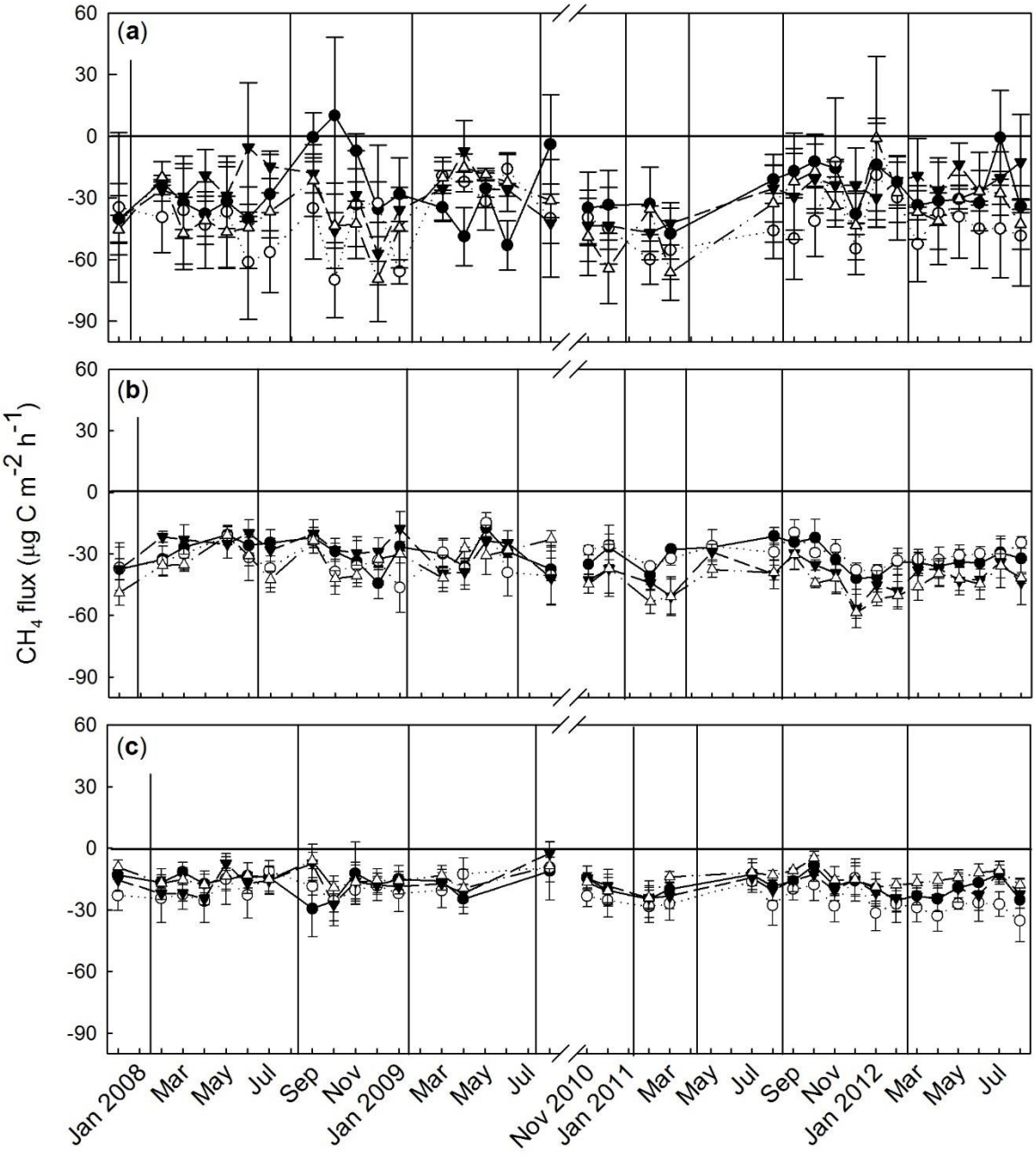


Figure 4.1 Mean (± SE, n = 3) soil CH_4 fluxes (μg C m^{-2} h^{-1}) from montane forests at (a) 1000 m, (b) 2000 m and (c) 3000 m during five years of nutrient manipulation: control (*filled circle*), N addition (*open circle*), P addition (*filled triangle*) and N+P addition (*open triangle*). Vertical lines indicate fertilization events.

Soil CH_4 fluxes from control forests between 2010 and 2012 that were found to be negatively correlated to soil parameters (Table 4.3), imply a positive correlation between net soil CH_4 uptake and soil parameters. Negative correlations were observed between CH_4 fluxes and soil temperature (P = 0.002) and soil NH_4^+ concentrations (P = 0.010) across the elevation gradient. While soil CH_4 flux at the two lower elevations were not significantly correlated with soil parameters (P = 0.068 - 0.951), CH_4 fluxes at 3000 m were significantly correlated with soil temperature (P = 0.033).

Table 4.3 Pearson correlation coefficients between soil CH_4 fluxes (μg C m^{-2} h^{-1}) and soil parameters of control plots in montane forests along an elevation gradient, measured in 2010-2012.

Elevation (m)	*n*	WFPS (%)	NH_4^+ (mg N m^{-2})	NO_3^- (mg N m^{-2})	Temperature (°C)
1000	19	0.43	-0.02	-0.05	-0.23
2000	20	0.10	-0.22	0.01	0.03
3000	20	-0.33	-0.15	-0.18	**-0.48***
1000-3000	59	-0.06	**-0.33****	-0.10	**-0.39****

*$P \leq 0.05$, ** $P \leq 0.01$

4.4.2 Effect of nutrient additions on soil CH_4 fluxes

During a pre-treatment measurement conducted in January 2008, soil CH_4 fluxes did not differ between plots of different prospective treatments at any site (P = 0.417 - 0.987). Furthermore, there was no effect of nutrient addition on soil CH_4 flux at any elevation within or during the first two years of nutrient addition (P = 0.064 - 0.997). However, after 2010, nutrient effects varied with added nutrient, elevation and time period (Figure 4.1; Table 4.2).

At 1000 m (Figure 4.1a), soil CH_4 uptake increased with N and N+P addition over the entire five years of nutrient addition, while addition of P alone did not affect soil CH_4 flux at any time (P = 0.489 - 0.903). Increased CH_4 uptake with N addition (P = 0.001) and a trend

towards increased uptake with N+P addition in 2010/11 resulted in increased CH_4 uptake with N and N+P addition within four years of nutrient addition ($P < 0.001$ and 0.015).

At 2000 m (Figure 4.1b), N addition did not affect soil CH_4 fluxes at any time over five years of nutrient addition (P = 0.170 - 0.678), whereas P addition and especially N+P addition increased CH_4 uptake over the five years of nutrient addition (P = 0.019 and < 0.001), being significant in 2010/11 ($P < 0.001$) and 2012 ($P = 0.014$ and 0.001). When considering four years of nutrient addition, however, the effect was only significant for N+P addition ($P < 0.001$).

At 3000 m (Figure 4.1c), N addition resulted in higher CH_4 uptake over five years of nutrient addition ($P < 0.001$), while addition of P and N+P did not affect soil CH_4 fluxes at any time (P = 0.360 - 0.977 and 0.150 - 0.863). Increased CH_4 uptake with N addition was significant in 2010/11 ($P = 0.007$) and 2012 ($P < 0.001$) as well as over the first four years of nutrient addition ($P = 0.013$).

4.5 Discussion

4.5.1 Soil CH_4 flux and controlling factors of control forests along the elevation gradient

Soils in our study area were net sinks for atmospheric CH_4. Annual soil CH_4 fluxes across the elevation gradient (Table 4.1) were within the range of studies reporting in-situ year-round measurements of tropical montane forests at comparable elevations in Brazil (Sousa Neto et al. 2011), Ecuador (Wolf et al. 2012), Indonesia (Purbopuspito 2006), Peru (Teh et al. 2014) and Panama (Veldkamp et al. 2013)

Our results support previous results from our study area (Wolf et al. 2012), since there was no evidence of net CH_4 production, and NH_4^+ concentrations and soil temperature, rather than soil moisture, correlated with CH_4 uptake across our elevation gradient. This indicates that despite the presence of thick organic layers, CH_4 uptake is not restricted by CH_4 diffusion

into the soil. Instead, the high porosity of organic layers with aerobic conditions favor net CH_4 uptake, which is controlled by mineral NH_4^+ availability and soil temperature, where soil temperature was especially important at 3000 m elevation ´(Table 4.3).

4.5.2 Nutrient-addition effects on soil CH_4 fluxes – unresponsive phase (year 1-2)

During the first tqo years of nutrient addition, the lack of nutrient effects on soil CH_4 fluxes across the elevation gradient (Table 4.2; Figure 4.1) was probably caused by the moderate amounts of nutrients added, strong immobilization of added nutrients and the location of the highest CH_4 uptake activity occuring in the subsoil (Wolf et al. 2012).

During the first two years of our experiment, the cumulative amounts of N and P applied in our experiment were 100 kg N ha^{-1} and 20 kg P ha^{-1}, which was considerably lower than the yearly doses applied in other nutrient manipulation studies performed in (sub)tropical forests (e.g. 150 kg N and 150 kg P ha^{-1} yr^{-1} in (Zhang et al. 2011); 125 kg N ha^{-1} yr^{-1} in (Veldkamp et al. 2013)). Even though laboratory incubations mostly result in immediate short-term responses of CH_4 fluxes to nutrient additions, nutrient additions to entire ecosystems have a different response time, since strong competition for added nutrients and other interactive processes (e.g. plant induced changes in soil moisture) can result in slowed responses of CH_4 fluxes to added nutrients (Bodelier and Laanbroek 2004). In the soil of our experiment the zone with the highest CH_4 oxidation activity was located just above the interface of the organic layers and the mineral soil (Wolf et al. 2012) and it is likely that most of the moderate amounts of nutrients applied to the surface did not reach this zone due to quick immobilization, as was demonstrated in a ^{15}N pulse-chase experiment at our sites (Baldos 2014).

4.5.3 N-addition effects on soil CH_4 fluxes – responsive phase (year 3-5)

Increased CH_4 uptake with N addition at 1000 m and 3000 m (Table 4.2; Figure 4.1a,c) confirms previous indications of N-limited CH_4 oxidation in our study area (Wolf et al. 2012) which were also found in tropical forest soils in Panama (Veldkamp et al. 2013). It is, however, in contrast to observations in an N-rich subtropical forest in China where N addition (150 kg N ha^{-1} yr^{-1} in form of NH_4NO_3) caused inhibition of CH_4 uptake (Zhang et al. 2011). The soils in our experiment were characterized by a conservative N cycle with low N cycling rates, large microbial N immobilization and N retention (Martinson et al. 2013). However, N addition decreased N retention, decoupled N cycling (Baldos 2014; Baldos et al. in press) and increased mineral N availability (Chapter 3), which probably led to alleviation of N limitation on methanotrophic activity and consequently increasing CH_4 uptake. Most methanotrophs can survive independent from mineral N sources due to their ability to fix N_2 (Auman et al. 2001) and recently a study from peatlands indicate that methanotrophy can be tightly linked with N_2 fixation (Larmola et al. 2014). However, increased availability of mineral N could allow them to switch from energy-demanding N_2 fixation to low energy N acquisition, which might increase their CH_4 oxidizing activity (Bodelier and Laanbroek 2004). The lack of an effect of N addition at 2000 m elevation (Table 4.2; Figure 4.1b) compared to the other elevations, despite similar trends in N cycling in the top 5 cm (Baldos et al. in press), might be related to strong P limitation of soil CH_4 oxidation at this elevation.

4.5.4 P-addition effects on soil CH_4 fluxes – responsive phase (year 3-5)

At 1000 m and 3000 m elevation, P addition had no effect on CH_4 fluxes (Table 4.2; Figure 4.1a,c). Since P addition probably increased N uptake by the vegetation (as illustrated by decreased extractable NO_3^- of the top 0.05 m and lower N_2O fluxes in year 3-4 of our experiment (Chapter 3)), this might have aggravated N limitation, potentially decreasing CH_4

uptake. However, the methanotrophic bacteria were either able to compete for available NH_4^+ or they compensated the lower N availability by increased N_2 fixation since the ability to fix N is widespread among methanotrophic bacteria (Auman et al. 2011).

Compared to the other elevations, P immobilization was probably highest in the subsoil (including the top 0.05 m of mineral soils) at 2000 m as indicated by C/P ratios (983 and 266 in the top 0.05 m of the organic and mineral soil; Martinson et al. 2013) and low rates of soil P-losses by leaching (Wullaert et al. 2010). Soil C/P ratios above 100 indicate strong microbial P immobilization (White 2006) and P-addition may have alleviated P limitation on methanotrophic activity, resulting in increased CH_4 uptake (Table 4.2; Figure 4.1b). Thus, the direction of the P effect was the same as hypothesized and reported in the nutrient addition experiment in China (Zhang et al. 2011), but the mechanisms responsible in our experiment were very different from their observations, where P addition increased plant water uptake, which in turn reduced soil moisture and increased CH_4 diffusivity of the soil.

4.5.5 Combined N+P-addition effects on soil CH_4 fluxes- responsive phase (year 3-5)

At 1000 m and 2000 m, the direction of changes in CH_4 uptake were similar for combined N+P additions and for N (at 1000m) or P (at 2000m) addition (Table 4.2; Figure 4.1a,b). Since at 2000 m, the effect of N+P addition was stronger than with P addition alone, and N addition had no effect, this may indicate a serial limitation of methanotrophs with synergistic responses, as has been described for nutrient co-limitation of primary producer communities (Harpole et al. 2011). With serial nutrient limitation, not only P (the primary limiting nutrient), but also N is limiting CH_4 uptake, whereby synergistic responses to N can only occur after P addition.

At 3000 m, combined N+P addition did not affect CH_4 uptake (Table 4.2; Figure 4.1c), although addition of N alone increased CH_4 uptake and addition of P had no effect. This

might be attributed to increased plant N uptake with N+P addition due to co-limitation of tree growth. Decreased soil moisture and a trend towards increasing basal area increment with N+P addition compared to the control plots at 3000 m elevation also suggested co-limitation of forest productivity (Homeier et al. 2013).

4.5.6 Implications for elevated nutrient deposition in TMFs

In summary, we showed the first field measurements of nutrient limited CH_4 uptake across an elevation gradient of TMFs. We detected differential nutrient limitation of CH_4 uptake across the elevation gradient with N limitation being dominant at 1000 m and 3000 m and P limitation being dominant at 2000 m. Increasing depositions of N and P thus has the potential to affect CH_4 fluxes in these ecosystems. However, the soils in these ecosystems, where elevated N and P deposition has only recently started increasing, have a considerable capacity to immobilize N and P which will delay such effects; this is especially the case in soils with thick organic layers where the highest capacity to oxidize CH_4 is located just above the interface of the mineral soil and organic layers. At present, N and P limitation is dominating effects of elevated nutrient inputs, but it is very likely that chronic N deposition will ultimately lead to inhibition of CH_4 uptake as has been shown in other laboratory and field experiments.

4.6 References

Aronson EL, Helliker BR (2010) Methane flux in non-wetland soils in response to nitrogen addition: a meta-analysis. *Ecology 91*: 3242–3251.

Auman AJ, Speake CC, Lidstrom ME (2001) *nifH* sequences and nitrogen fixation in Type I and Type II methanotrophs. *Applied and Environmental Microbiology 67*: 4009–4016.

Baldos AP (2014) *Soil nitrogen cycling and fate of nitrogen in montane forests along a 1000- to 3000-m elevation gradient in the Ecuadorian Andes.* PhD thesis, Georg August Universität, Göttingen.

Baldos AP, Corre MD, Veldkamp E (in press) Responses of N cycling to nutrient inputs in forest soils across 1000-3000-m elevation gradient in the Ecuadorian Andes. *Ecology*.

Bédard C, Knowles R (1989) Physiology, biochemistry, and specific inhibitors of CH_4, NH_4^+ and CO oxidation by methanotrophs and nitrifiers. *Microbiological Reviews 53*: 68–84.

Bodelier PLE, Laanbroek HJ (2004) Nitrogen as a regulatory factor of methane oxidation in soils and sediments. FEMS Microbiology *Ecology 47*: 265–277.

Bowden RD, Newkirk KM, Rullo GM (1998) Carbon dioxide and methane fluxes by a forest soil under laboratory-controlled moisture and temperature conditions. *Soil Biology and Biochemistry 30*: 1591–1597.

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 Biogeochemical Cycles 22*: GB4011.

Bradford MA, Ineson P, Wookey PA, Lappin-Scott HM (2001) Role of CH_4 oxidation, production and transport in forest soil CH_4 flux. *Soil Biology and Biochemistry 33*: 1625–1631.

Bubb P, May I, Miles L, Sayer J (2004) *Cloud forest agenda*. UNEP-WCMC, Cambridge, UK. Online: http://www.unep-wcmc.org/resources/publications/UNEP_WCMC_bio_series/20.htm

Carillo JH, Hastings MG, Sigman DM, Huebert BJ (2002) Atmospheric deposition of inorganic and organic nitrogen and base cations in Hawaii. *Global Biogeochemical Cycles 16*: 24-1 – 24-16.

Cleveland CC, Townsend AR, Schmidt SK (2002) Phosphorus limitation of microbial processes in moist tropical forests: evidence from short-term laboratory incubations and field studies. *Ecosystems 5*: 680–691.

Conrad R (1989) Control of methane production in terrestrial ecosystems. In: Andreae, M.O. and Schimel D.S. (eds). *Exchange of trace gases between terrestrial ecosystems and the atmosphere.* Wiley, Chichester, UK, pp. 39–58.

Crawley MJ (2007) *The R book*. John Wiley and Sons Ltd, Chichester, UK.

Denman KL, Brasseur G, Chidthaisong A, et al. (2007) Couplings between changes in the climate system and biogeochemistry. In: Solomon S, Qin D, Manning M, et al. (eds) *Climate change 2007: The physical science basis, contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change*. Cambridge University Press, Cambridge, UK and New York, USA. pp. 499-587.

Dörr H, Katruff L, Levin I (1993) Soil texture parameterization of the methane uptake in aerated soils. *Chemosphere 26*: 697-713.

Dutaur L, Verchot LV (2007) A global inventory of the soil CH_4 sink. *Global Biogeochemical Cycles 21*: GB4013.

Emck P (2007) *A climatology of south Ecuador with special focus on the major Andean ridge as Atlantic–Pacific climate divide*. PhD thesis, Friedrich-Alexander-Universität Erlangen-Nürnberg.

Etheridge D, Steele L, Francey R, Langenfelds R (1998) Atmospheric methane between 1000 A.D. and present: evidence of anthropogenic emissions and climatic variability. *Journal of Geophysical Research 103*: 15979-15993.

FAO (1993) *Forest resources assessment 1990 - Tropical countries*. FAO Forestry Paper number. 112, Rome. Food and Agriculture Organization of the United Nations. Available: http://www.fao.org/docrep/007/t0830e/t0830e00.htm

Galloway JN, Townsend AR, Erisman JW, et al. (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science 320*: 889-892.

Glatzel S, Well R (2008) Evaluation of septum-capped vials for storage of gas samples during air transport. *Environmental Monitoring and Assessment 136*: 307-311.

Hanson RS, Hanson TE (1996) Methanotrophic bacteria. *Microbiological Reviews 60*: 439-471.

Harpole WS, Ngai JT, Cleland EE, et al. (2011) Nutrient co-limitation of primary producer communities. *Ecology Letters 14*: 852–862.

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, Werner FA, Gradstein SR, Breckle S-W, Richter M (2008) Potential vegetation and floristic composition of Andean forests in south Ecuador, with a focus on the RBSF. In: Beck E, Bendix J, Kottke I, Makeschin F, Mosandl R (eds) *Ecological Studies, Vol. 198, Gradients in a tropical mountain ecosystem of Ecuador*. Springer Verlag Berlin Heidelberg. pp. 87–100.

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. *PLoS ONE 7*: e47128.

Homeier J, Leuschner C, Bräuning A, et al. (2013) Effects of nutrient addition on the productivity of montane forests and implications for the carbon cycle. In: Bendix J, Beck E, Bräuning A, Makeschin F, Mosandl R, Scheu S, Wilcke W (eds). *Ecological Studies, Vol. 221, Ecosystem services, biodiversity and environmental change in a tropical mountain ecosystem of south Ecuador*. Springer Verlag, Berlin Heidelberg. pp. 315–329.

Kaspari M, Garcia MN, Harms KE, Santana M, Wright SJ, Yavitt JB (2008) Multiple nutrients limit litterfall and decomposition in a tropical forest. *Ecology Letters 11*: 35-43.

Kirschke S, Bousquet P, Ciais P, et al. (2013) Three decades of global methane sources and sinks. *Nature Geoscience 6*: 813-823.

Klüber HD, Conrad R (1998) Inhibitory effects of nitrate, nitrite, NO and N_2O on methanogenesis by *Methanosarcina barkeri* and *Methanobacterium bryantii*. *FEMS Microbiology Ecology 25*: 331–339.

Larmola T, Leppänen SM, Tuittila E-S, Aarva M, Merilä P, Fritze H, Tiirola M (2014) Methanotrophy induces nitrogen fixation during peatland development. *PNAS 111*: 734-739.

Le Mer J, Roger P (2001) Production, oxidation, emission and consumption of methane by soils: A review. *European Journal of Soil Biology 37*: 25–50.

Litherland M, Aspen JA, Jemielita RA (1994) *The metamorphic belts of Ecuador*. Overseas Memoir 11, British Geological Survey, Nottingham, UK.

Loftfield N, Flessa H, Augustin J, Beese F (1997) Automated gas chromatographic system for rapid analysis of the atmospheric trace gases methane, carbon dioxide, and nitrous oxide. *Journal of Environmental Quality 26*: 560-564.

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.

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.

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.

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

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.

Piepho HP, Büchse A, Richter C (2004) A mixed modelling approach for randomized experiments with repeated measures. *Journal of Agronomy and Crop Science 190*: 230–247.

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. *Global Biogeochemical Cycles 20*: GB3010.

R Development Core Team (2012) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

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.

Schnell S, King GM (1994) Mechanistic analysis of ammonium inhibition of atmospheric methane consumption in forest soils. *Applied and Environmental Microbiology 60*: 3514–3521.

Schnell S, King GM (1996) Responses of methanotrophic activity in soils and cultures to water stress. *Applied and Environmental Microbiology 62*: 3203–2309.

Sousa Neto E, Carmo JB, Keller M, et al. (2011) Soil-atmosphere exchange of nitrous oxide, methane and carbon dioxide in a gradient of elevation in the coastal Brazilian Atlantic forest. *Biogeosciences 8*: 733-742.

Tanner EVJ, Vitousek PM, Cuevas E (1998) Experimental investigation of nutrient limitation of forest growth on wet tropical mountains. *Ecology 79*: 10-22.

Teh YA, Diem T, Jones S, et al. (2014) Methane and nitrous oxide fluxes across an elevation gradient in the tropical Peruvian Andes. *Biogeosciences 11*: 2325–2339.

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

White RE (2006) *Principles and Practice of Soil Science: The soil as a Natural Resource*. Fourth edition, Blackwell Publishing.

Wolf K, Flessa H, Veldkamp E (2012) Atmospheric methane uptake by tropical montane forest soils and the contribution of organic layers. *Biogeochemistry 111*: 469–483.

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.

Zhang W, Mo J, Zhou G, et al. (2008) Methane uptake responses to nitrogen deposition in three tropical forests in southern China. *Journal of Geophysical Research: Atmospheres (1984-2012) 113*: D11116.

Zhang T, Zhu W, Mo J, Liu L, Dong S (2011) Increased phosphorus availability mitigates the inhibition of nitrogen deposition on CH_4 uptake in an old-growth tropical forest, southern China. *Biogeosciences 8*: 2805-2813.

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) *Mixed effects models and extensions in ecology with R*. Springer, New York, USA.

CHAPTER 5

Synthesis

5.1 Net soil global warming potential of tropical montane forests

5.1.1 Net soil global warming potential along elevation gradients

The GWP is a parameter that quantifies the radiative forcing of a GHG relative to that of CO_2 over a certain time and helps to evaluate the combined impact of different GHGs on global temperatures (IPCC 2013). We calculated the net GWP of our soils in 'CO_2 equivalents' (CO_2 eq.) over a 100 year time period, including CO_2, N_2O and CH_4 fluxes. We used the conversion factors: 1 for CO_2, 298 for N_2O and 34 for CH_4 (Table 1.1). Annual net soil GWPs in our study forests decreased from 32.8 to 19.9 to 8.7 Mg CO_2 eq. ha^{-1} yr^{-1} along the 1000 m to 3000 m elevation gradient (Table 5.1). These are all much smaller than the calculated average net soil GWP of 47.2 Mg CO_2 eq. ha^{-1} yr^{-1} for tropical forests (Dutaur and Verchot 2007; Raich and Schlesinger 1992; Werner et al. 2007) and slightly smaller than values from other TMFs at comparable elevations (Table 5.1). The calculated net soil GWP at 3000 m is even smaller than 11.8 Mg CO_2 eq. ha^{-1} yr^{-1}, the calculated average of boreal forests (Dalal and Allen, 2008; Dutaur and Verchot 2007; Raich and Schlesinger 1992). However, only four other studies have reported annual fluxes of all three GHGs, and one of these studies was in our study area. Compiled data from this small dataset of TMFs suggest that there is a linear decrease in net soil GWP with increasing elevation (Figure 5.1). This relationship between GWP and elevation is mainly attributed to decreasing soil CO_2 emissions with increasing elevation, since the net soil GWP of TMFs is dominated by the large soil GWP of CO_2 (Table 5.1; notice unit differences). N_2O and CH_4, despite their relatively larger GWP, play only a minor role in the net soil GWP of TMFs, due to small absolute fluxes.

Table 5.1 Compilation of soil global warming potentials (GWP) from published greenhouse gas fluxes of old-growth tropical montane forest soils, sorted by elevation*

Country	Elevation (m asl)	Annual mean Rainfall (mm)	Annual mean Temp (°C)	Soil type	Thickness Organic layer (cm)	Mean annual soil GWP (CO_2 eq. ha^{-1} yr^{-1}) CO_2 (Mg)	N_2O (kg)	CH_4 (kg)	Net soil GWP** (Mg CO_2 eq. ha^{-1} yr^{-1})	Reference
Brazil	400	3050	~22.3	Inceptisol	0	50.05	899.11	-210.80	50.74	Sousa Neto et al. 2011
Peru	600-1200	5318	23.4	-	-	-	505.75	-6.35	-	Teh et al. 2014
Hawaii	760	6000	19	Hydrudand	> 0	32.63	-	-	-	Raich 1998
China	870	2198	19.7	Lateritic yellow soil	-	61.34	-	-	-	Zhou et al. 2013
Ecuador	990-1100	2230	19.4	Cambisol	0	32.45	449.55	-101.09	32.80	Martinson et al. 2013; present study
Ecuador	990-1200	2230	19.4	Cambisol	4	37.14	262.24	-253.87	37.15	Wolf et al. 2011, 2012
Brazil	1000	2300	~22.3	Inceptisol	0	47.23	899.11	-173.63	47.95	Sousa Neto et al. 2011
Peru	1000	3090	21.3	Gleysol	3	47.34	-	-	-	Zimmermann et al. 2010
Indonesia	1050	2901	20.6	Nitisol	4	42.90	-	-	-	Van Straaten et al. 2011
Indonesia	1190	1590	22.5	Fluvisol, Entisol	0	44.33	1039.59	-111.07	45.26	Purbopuspito et al. 2006
Venezuelan Guyana	1200	2200	20.6	Acrohumox	9	38.65	-	-	-	Priess and Fölster 2001
Panama	1200-1300	5532	20.1	Andosol	~ 8	34.36	1058.33	-16.77	35.40	Corre et al. 2014; Koehler et al. 2009a,b; Veldkamp et al. 2013
Peru	1200-2200	2631	18.8	-	-	-	468.29	-31.28	-	Teh et al. 2014

Country	Elevation (m asl)	Annual mean Rainfall (mm)	Annual mean Temp (°C)	Soil type	Thickness Organic layer (cm)	Mean annual soil GWP (CO_2 eq. ha^{-1} yr^{-1}) CO_2 (Mg)	N_2O (kg)	CH_4 (kg)	Net soil GWP** (Mg CO_2 eq. ha^{-1} yr^{-1})	Reference
Peru	1500	2630	18.3	Gleysol	7	49.28	-	-	-	Zimmermann et al. 2010
Hawaii	1660	2600	13	Histosol	> 10	26.22	-	-	-	Raich 1998
Indonesia	1800	n.a.	18.3	Inceptisol	20	29.08	271.61	-150.51	29.20	Purbopuspito et al. 2006
Ecuador	1800-2100	1950	15.7	Cambisol, Planosol	13	28.23	533.85	-140.53	28.63	Wolf et al. 2011, 2012
Ecuador	1950-2100	1950	15.7	Cambisol	20	19.91	149.85	-125.57	19.93	Martinson et al. 2013; present study
Peru	2200-3200	1706	12.5	-	-	-	122.39	-36.27	-	Teh et al. 2014
Indonesia	2470	n.a.	14.6	Inceptisols	15	27.43	945.94	-65.73	28.31	Purbopuspito et al. 2006
Ecuador	2800-3000	4500	9.4	Cambisol, Planosol	14	19.32	65.56	-48.51	19.34	Wolf et al. 2011, 2012
Ecuador	2900-3050	4500	9.4	Histosol	25	8.84	-47.93	-65.73	8.70	Martinson et al. 2013; present study
Peru	3030	1710	12.5	Lithosol	17	39.01	-	-	-	Zimmermann et al. 2010

* Only studies of in-situ year-round measurements, comprising at least one year of data are included. This listing is not meant to be a complete summary of all studies that have been done, but to give an overview of the range of published data

**Net soil GWP over a 100 year time period, calculated from annual mean fluxes, based on conversion factors of 1 for CO_2, 298 for N_2O and 34 for CH_4 (IPCC, 2013)

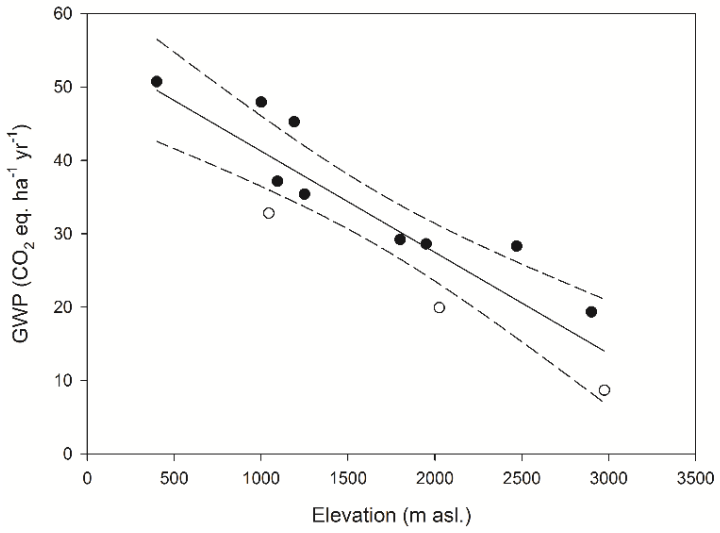


Figure 5.1 The relationship between the elevation and net soil GWP of TMFs in the present study (○) (including data of Martinson et al. 2013) and from literature (●) (Corre et al. 2014; Koehler et al. 2009a,b; Purbopuspito et al. 2006; Sousa Neto et al. 2011; Veldkamp et al. 2013; Wolf et al. 2011, 2012). The line shows the best-fit regression through all points ± 95% confidence interval (GWP = 55.01-0.01x, R^2 = 0.81, P < 0.005, n = 12, where x = elevation)

To assess the global impact of soil GHG fluxes from TMFs, there are a number of aspects that need to be considered before upscaling measured data. First, decreasing net soil GWPs with increasing elevation highlight the importance of incorporating elevation models into upscaling approaches. In addition, the dominance of steep slopes, recently estimated to be 75% of the planimetric (horizontal) area of TMFs (Spracklen and Righelato 2014), may not only underestimate the global surface area of global TMFs (Spracklen and Righelato 2014) but also complicate upscaling since soil GHG fluxes can be significantly affected by landscape position (Teh et al. 2014; Wolf 2010; Wolf et al. 2011, 2012). Finally, high spatial heterogeneity of soil GHG fluxes in tropical forests (e.g. Breuer et al. 2000; Ishizuka et al. 2005; van Haren et al. 2010), also shown in the standard errors of soil GHG fluxes in our study forests (Chapters 2 to 4), stress the importance of selecting datasets that are spatially representative of their study area.

Although, soils have positive net GWPs that reinforce global warming, net ecosystem GWPs of natural terrestrial ecosystems are, on average, negative (Dalal and Allen, 2008), since the CO_2 uptake capacity of vegetation via photosynthesis induces a negative GWP that exceeds the positive net GWP of soils. However, although tropical forests are estimated to have the highest CO_2 uptake rates of all terrestrial ecosystems, they have the highest (i.e. the least negative) annual net-ecosystem GWP (-0.03 ± 0.44 Mg CO_2 eq. ha^{-1} yr^{-1}), owing to their high N_2O fluxes. Boreal forests, on the other hand, have the lowest (i.e. the most negative) annual net ecosystem GWP (-1.18 ± 0.44 Mg CO_2 eq. ha^{-1} yr^{-1}) (Dalal and Allen, 2008), despite their lower CO_2 uptake rates, due to negligible N_2O emissions. Since TMFs have low net soil GWPs, especially at high elevations (Table 5.1) and usually have continuous year-round CO_2 uptake capacity due to the absence of seasonality, they might be particularly important ecosystems, acting to counteract global warming.

5.1.2 Nutrient effects on the net soil global warming potential

Effects of nutrient addition on the net soil GWP in our study forests over 5 years (Table 5.2) roughly reflect changes observed for soil CO_2 fluxes (Chapter 2), with some influence of high N_2O fluxes during the first two years with N and N+P addition (Martinson et al. 2013). Nutrient effects on soil CH_4 fluxes were significant (Chapter 4) but minor in terms of their soil GWPs, relative to the large net soil GWP. Differential effects of nutrient addition on the net soil GWP along our elevation gradient ranged from -12% with P addition at 1000 m elevation to +71% with N addition at 3000 m elevation (Table 5.2). This illustrates how important it is to investigate nutrient effects on net soil GWP not only at one elevation, but also along elevation gradients.

Table 5.2 Mean (± SE, *n* = 3) soil global warming potential (GWP) from montane forest soils along a 1000- to 3000-m elevation gradient and mean soil GWP across the elevation gradient of TMFs in southern Ecuador over the first five years (2008-2012) of nutrient manipulation

Elevation (m)	Treatment	soil GWP (Mg CO_2 eq. ha^{-1} yr^{-1})†					Relative difference (%)	
		CO_2	N_2O	CH_4	Net*	Area-weighted net#	Net	Area-weighted net#
1000	Control	34.4 ± 1.1	0.39 ± 0.16	-0.11 ± 0.02	34.7 ± 1.2^{A,a}	-	-	-
	Nitrogen (N)	34.1 ± 1.1	0.80 ± 0.21	-0.13 ± 0.04	34.7 ± 1.2^{a}	-	0	-
	Phosphorus (P)	30.4 ± 0.7	0.40 ± 0.04	-0.14 ± 0.04	30.7 ± 0.7^{c}	-	-12	-
	N + P	31.9 ± 2.3	0.91 ± 0.10	-0.14 ± 0.05	32.7 ± 2.3^{b}	-	-6	-
2000	Control	21.1 ± 1.7	0.14 ± 0.04	-0.12 ± 0.01	21.2 ± 1.8^{B,b}	-	-	-
	N	21.3 ± 0.9	0.36 ± 0.03	-0.13 ± 0.01	21.5 ± 0.8^{b}	-	2	-
	P	21.9 ± 0.4	0.10 ± 0.06	-0.14 ± 0.01	21.9 ± 0.3^{ab}	-	3	-
	N + P	22.8 ± 1.2	0.45 ± 0.05	-0.15 ± 0.01	23.1 ± 1.2^{a}	-	9	-
3000	Control	9.4 ± 1.7	-0.02 ± 0.02	-0.07 ± 0.02	9.3 ± 1.7^{C,c}	-	-	-
	N	15.9 ± 3.5	0.11 ± 0.03	-0.09 ± 0.03	15.9 ± 3.4^{a}	-	71	-
	P	12.0 ± 3.2	0.02 ± 0.08	-0.07 ± 0.02	11.9 ± 3.3^{b}	-	28	-
	N + P	10.0 ± 1.3	0.20 ± 0.01	-0.06 ± 0.01	10.1 ± 1.3^{c}	-	8	-
1000-3000	Control	21.7	0.17	-0.10	21.7	26.9	-	-
	N	23.8	0.42	-0.12	24.1	28.0	11	4
	P	21.4	0.17	-0.12	21.5	25.2	-1	-6
	N + P	21.6	0.52	-0.12	22.0	26.4	1	-2

† Means are calculated from the mean across 5 years of measurements and do not take into account seasonality or inter-annual variability. Measurements within three weeks after fertilization and dates without CH_4 measurements (June 2009 to July 2009 and May 2011 to July 2011) were excluded

* Means at 1000, 2000 and 3000 m followed by superscript capital letters indicate significant difference across the elevation gradient for control plots, and means followed by superscript small letters indicate significant differences among treatments within each elevation (linear mixed effects model at $P \leq 0.05$)

Estimate of area weighted net soil GWP based on areal extent reported by Körner et al. (2006); conversion factors: 0.56 at 1000 m, 0.28 at 2000 m and 0.16 at 3000 m

But how does the net soil GWP of our TMFs change, on average, with nutrient addition, considering effects across the entire elevation gradient? To answer this question, we calculated not only the average net soil GWP across the 1000- to 3000-m elevation gradient, but also made a rough area-weighted estimate of net soil GWP, based on the size of elevation bands reported for moist tropical mountain forests (1000-1500 m asl: 545,700 km²; 1500-2500 m asl: 277,000 km²; > 2500 m asl: 1,580 km²; Körner et al. 2006). Our results show that the area-weighted net soil GWP across all elevations for 5 years of nutrient addition increased with N addition by 4%, while it decreased with P and N+P addition by 6% and 2% respectively (Table 5.2). This indicates that with N deposition, our soils could contribute to reinforce global warming, while P and N+P addition could slightly counteract this process. Although N addition increased average-weighted net soil GWP to 28.0 Mg CO_2 eq. ha^{-1} yr^{-1}, which is higher than the estimated averages of 24.6 Mg CO_2 eq. ha^{-1} yr^{-1} for temperate forests (Dalal and Allen 2008; Dutaur and Verchot 2007; Raich and Schlesinger 1992), it is still > 40% smaller than the estimated average net soil GWP of tropical forests (47.2 Mg CO_2 eq. ha^{-1} yr^{-1}; Dutaur and Verchot 2007; Raich and Schlesinger 1992; Werner et al. 2007). Thus, even with increasing N deposition, TMFs are in the mid-range of estimated net soil GWPs of forest ecosystems. However, it is interesting to notice that the increase of area-weighted net soil GWP with N addition across the 1000 m to 3000 m elevation gradient is relatively small, considering the large increase in net soil GWP observed with N addition at 3000 m. This was the result of the combined effects of relatively small GHG fluxes and the small areal extent of high elevations (Körner et al. 2006) and highlights once more, how important it is to consider nutrient effects at different elevations and include elevation gradients in the upscaling processes.

Although N addition can increase the net soil GWP, its effect on entire forest ecosystems, including vegetation, can differ; as shown in temperate forests (Magnani et al.

2007; Quinn Thomas et al. 2010), it has the potential to increase forest productivity and thus CO_2 uptake capacity, consequently reducing the net ecosystem GWP. Since NPP of TMFs seems to be N+P co-limited (Homeier et al. 2012; Tanner et al. 1998), however, we speculate that input of N+P to TMFs, but not single input of N or P, could reduce the net ecosystem GWP; even more than results from our soils suggest. However, the general assumption of N+P co-limitation of TMFs is questionable, since nutrient availability in our study area varied considerably along the elevation gradient (Table S2.1; Schrumpf et al. 2001; Wolf, 2010). Furthermore, responses of tree growth to nutrient addition tended to be specific not only for each elevation (Homeier et al. 2013) but also for single tree species (Homeier et al. 2012), and some positive effects on tree growth were observed with single nutrient additions. Thus, increasing tree growth with N addition at only one elevation or within only one species could still partly compensate increases in net soil GWP. However, we do not expect that effects with N input were as large as those reported from temperate forests, where effects on soils were completely neutralized by vegetation (Janssens et al. 2010).

Overall, our results suggest that N deposition to TMF soils has the potential to reinforce global warming, while P and N+P depositions could counteract global warming, potentially quite significantly if nutrient addition increases forest productivity.

5.1.3 Implications for chronic nutrient addition on the net soil global warming potential

During 5 years of nutrient addition, nutrient effects on the net soil GWP also changed between years (not shown), since we observed differential nutrient effects on all three GHG fluxes over time. While CO_2 fluxes were apparently a function of the duration of nutrient addition, with some uncertainties added by the differential effects of soil CO_2 sources (Chapter 2), nutrient effects (of N and N+P) on soil N_2O fluxes displayed large inter-annual variability, which did not seem to be a function of nutrient addition but depended on soil

moisture conditions (Chapter 3). Nutrient effects on soil CH_4 fluxes (Chapter 4), although negligible in terms of the net soil GWP (Table 5.2), also failed to follow a predictable pattern over time, due to a 2-year lag-phase where no nutrient effects were detectable. These findings illustrate the importance of long-term nutrient manipulation studies, which are so far limited to a small number (Bowden et al. 1998; Corre et al. 2014). Further, they show that we need to better understand interactions of soil GHG fluxes with both ecosystem components and environmental conditions, in order to identify 'critical loads' and predict future changes of soil GHG fluxes with atmospheric nutrient deposition.

We reported observations made within 5 years of nutrient addition, but how will chronic nutrient addition change soil GHG fluxes and the net soil GWP in the long term? N-addition effects on soil GHG fluxes have been studied frequently in many ecosystems and include not only short-term, but also some long-term (> 10 years) manipulation studies (Bowden et al. 2010; Corre et al. 2014). In contrast to N, effects of P and N+P addition are far less investigated and understood. Studies from tropical forests and plantations indicate, however, that P effects on soil GHG fluxes can be a result of complex interactions with vegetation in these ecosystems (Fisher et al. 2012; Mori et al. 2013; Zhang et al. 2011). What makes predictions on the effect of chronic nutrient effects even more difficult is, that many studies (including the present study) have shown that nutrient addition effects on soil GHG fuxes often react in a non-linear fashion to duration and amounts of nutrient addition (Aber et al. 1998; Hall and Matson, 2003; Liu and Graever 2009), owing to the presence of threshold values and occurrence of unpredictable 'hot-spots' and/or 'hot-moments' (Hagedorn and Bellamy 2011).

Nevertheless, we assume that with chronic N and N+P inputs, the biological demand of TMFs will eventually become N saturated, although it is unclear how long it will take for this change to happen (Aber et al. 1998). Some studies (e.g. Gundersen et al. 1998; Hall and

Matson 2003) indicate that compared to N-rich and/or P-limited forests, it will take longer for N-limited forests to reach N saturation, due to conservative N cycling, low N availability and high N retention capacity of soils and vegetation, which were the conditions we found in our study forests (Chapter 3; Baldos 2014). We can further assume that increased plant N uptake with N+P addition to TMFs (co-limited by N and P) will delay the occurrence of N saturation as compared to TMFs with the addition of N alone. However, a study from Panama indicates, that N retention of TMFs may not only depend on the nutrient limitation of NPP (Adamek et al. 2009) but also on the N retention capacity of soils and presence/absence of an organic layer (Corre et al. 2010; Koehler et al. 2009a). However, once an ecosystem is N saturated, continuous N input can decrease CO_2 fluxes due to decreasing heterotrophic and potentially autotrophic respiration, as indicated by findings in temperate forest stands, where 13 years of N addition decreased not only microbial respiration but also altered forest productivity, even causing substantial tree mortality (Bowden et al. 2004). On the other hand, a study from a lowland forest in Panama with 9-11 years of N addition found no effect on soil CO_2 fluxes (Koehler et al. 2009b). A trend towards decreasing soil CO_2 fluxes with N addition at our lower elevations, already detectable within 5 years of N addition (Chapter 2), suggests that these sites are likely to have decreasing CO_2 fluxes with increasing N addition, but differential effects of N and N+P addition with elevation and component of soil respiration do not allow further specification. Predicting changes is especially complex for CO_2 fluxes, since nutrient effects might be characterized by the relative contributions of different components of soil CO_2 fluxes, which have been shown to vary with elevation and were estimated in TMFs at: 20-40% from fresh litter respiration (van Straaten et al. 2011; Zhou et al. 2013; Zimmermann et al. 2009), 25-60% from SOM respiration (van Straaten et al. 2011; Zimmermann et al. 2010) and 30-65% from root respiration (Fisher et al. 2013; Girardin et al. 2014; Huaraca Huasco et al. 2014; van Straaten et al. 2011). Quantifying the contribution of

components of soil CO_2 fluxes within our study area would help to foresee chronic N effects, since, for example, the dominance of SOM respiration from organic layers and the known hampering effect of N on SOM respiration indicates that soil CO_2 fluxes will decrease in the future. N_2O fluxes will likely increase with increasing N availability in our TMFs, as found in many ecosystems (e.g. Butterbach-Bahl et al. 2002; Corre et al. 2014; Liu and Graever 2009), provided that soil moisture conditions are conducive to denitrification, and that denitrification remains the main source of N_2O (Chapter 3; Corre et al. 2014). It is probable that observed increases in soil CH_4 uptake with N and N+P addition will not only stagnate but decrease in the long term, as indicated by several laboratory and field studies from different ecosystems (e.g. Butterbach-Bahl et al. 2002; Liu and Graever 2009; Saari et al. 1997). Considering that changes in soil CH_4 fluxes play only a minor role for the net soil GWP, counteracting effects of chronic N input on soil GWP of CO_2 and N_2O could lead to everything from decreasing (dominance of CO_2) to increasing (dominance of N_2O) net soil GWPs.

As previously mentioned, predictions of the effect of chronic P input on soil GHG fluxes and the net soil GWP are highly speculative. Nevertheless, we assume that excessive P, above levels where plant and microbial demand is fulfilled, has no large effects on soil GHG formation, since so far no mechanisms of direct influence (restraining or promoting) have been described. Results from our study (which, to our knowledge, is the only study that has investigated the effect of P addition on all three GHG fluxes for 5 years), point towards a new steady state, with lower CO_2 fluxes at lower elevations and higher CO_2 fluxes at higher elevations (Chapter 2). However, since plants in our study still seem to be limited by nutrients, 5 years of addition are insufficient to answer questions about chronic addition. For N_2O fluxes we can, however, speculate from our results (Chapter 3), that P addition will either have no effect on N_2O fluxes or they may decrease, where plant uptake is restricted by

P and/or NP. Similarly, our results indicate that CH_4 fluxes will either be unchanged or increase compared to the current state (Chapter 4), the latter presumably due to alleviation of microbial P demand. However, the fact that we can only speculate, rather than provide detailed predictions about changes in soil GHG fluxes and the net soil GWP with chronic P addition clearly shows that there is a lack of studies investigating P effects on soil GHG fluxes, especially in the long term. Considering the increasing use and deposition of P in ecosystems, further research on both mechanisms behind GHG response to P addition and actual *in situ* long-term responses, is clearly required.

We reported potential effects of increasing nutrient deposition on soil GHG fluxes and radiative forcing (net soil and net ecosystem GWP) in a single manipulation experiment. However, in a changing world, not only one factor (e.g. nutrient deposition), but several factors (e.g. temperature, rainfall patterns, atmospheric CO_2 concentrations) will change, partly due to the complex feedback mechanisms mentioned in Chapter 1, all of which leave their unique 'fingerprint' (Lewis et al. 2004).

We will probably never be able to completely predict soil GHG fluxes due to the high level of complexity and uncertainty of global changes, but using integrated approaches, where not only multiple ecosystem components are connected on larger scale, but also different (new) methodological approaches are combined (e.g. eddy covariance measurement and ground based gas chamber measurements), will be useful in the future. However, to feed complex predictions models, data from field measurements and a better mechanistic understanding of the interaction of controlling factors on soil GHG fluxes are essential.

5.2 Closing the N cycle – measurements of soil N_2 fluxes

Although soil N_2O fluxes in our study area were lower than in other TMFs, N_2O fluxes from the top 5 cm were predominantly derived from denitrification (Chapter 3). Denitrification is the stepwise reduction of NO_3^- to N_2, with other forms of N, such as nitrite (NO_2^-), nitric oxide (NO) and N_2O, involved as intermediates (Robertson and Groffman, 2007). It is, apart from anaerobic ammonium oxidation, the only pathway through which reactive forms of N in terrestrial ecosystems re-enter the atmosphere as inert and climate-neutral N_2 gas (Galloway et al. 2004; Robertson and Groffman, 2007) - in this way 'closing' the N cycle and being important to primary production, water quality and the atmosphere.

Nonetheless, this important process is one of the least quantified processes in the N cycle. *In situ* measurements of N_2 face several methodological difficulties, with the fundamental problem being the quantification of a relatively small N_2 flux against the high background N_2 concentration in the atmosphere (Groffman et al. 2006). Globally, continental N_2 fluxes to the atmosphere via denitrification are an estimated 109 Tg N_2-N yr^{-1} and thus six times larger than the total natural and anthropogenic N_2O sources together (17.9 Tg N_2O-N yr^{-1}; IPCC, 2013). Moreover, there are indications from Hawaiian TMFs, that under moist conditions, N_2 losses from these ecosystems might be substantial (Houlton et al. 2006). In the Hawaiian study, N_2 losses were ~10 times higher than N_2O+NO losses, indicating their importance for the calculation of total ecosystem N budgets (Houlton et al. 2006).

In general, conditions promoting high N_2/N_2O ratios include high soil moisture (representative of low oxygen contents) and high carbon contents, while high NO_3-concentrations inhibit the conversion of N_2O to N_2, reducing N_2/N_2O ratios (Robertson and Groffman, 2007; Saggar et al. 2013; Weier et al. 1993). In our study, especially in the organic layers at 2000 m and 3000 m, soil conditions were in favor of large N_2/N_2O ratios (Chapter 3). Therefore, we can speculate that N losses via N_2 in our soils might have been

substantial, despite the low N_2O fluxes that we measured. In combination with the low absolute N_2O and negligible NO fluxes found in our study area (Wolf et al. 2011), this indicates that moist TMFs might be of global importance in returning reactive N in terrestrial ecosystems to unreactive atmospheric N_2. This may not be the case in drier and seasonal tropical forests, however, since the relatively larger NO fluxes compared to N_2O fluxes indicate the importance of nitrification processes and therefore less significant N_2 fluxes (Butterbach-Bahl et al. 2004; Holtgrieve et al. 2006).

Although N addition did not change the relative contribution of nitrification and denitrifcation to N_2O fluxes in our study area, increasing nutrient deposition might affect denitrification potential and thus N_2 fluxes from TMFs. Direct N effects on net N-cycling rates and N_2O fluxes of our soils (Martinson et al. 2013), as well as indirect P effects on mineral N concentrations and N_2O fluxes via plant feedback in our study area (Chapter 3), suggest that absolute amounts of N_2 fluxes will be affected by nutrient inputs. Higher N loads might increase NO_3- concentrations, which can increase absolute N_2 fluxes due to increased substrate availability for denitrification but can also reduce N_2/N_2O ratios through NO_3^- inhibition (Saggar et al. 2013; Weier et al. 1993). Furthermore, nutrient addition might induce indirect changes in denitrification due to shifts in microbial communities and changes in soil moisture and nutrient status caused by plant feedbacks (Corre et al. 2014; Parton et al. 1996).

Thus, we suggest that future research should focus on methodological techniques to quantify soil N_2 fluxes in-situ. This will then help to improve our understanding of N budgets, reducing current uncertainties and allowing us to evaluate the potential of ecosystems to turn reactive forms of N into climate-neutral N_2.

5.3 References

Aber J, McDowell W, Nadelhoffer K, et al. (1998) Nitrogen saturation in temperate forest ecosystems: hypotheses revisited. *BioScience 48:* 921-934.

Adamek M, Corre MD, Hölscher D (2009) Early effect of elevated nitrogen input on above-ground net primary production of a lower montane rain forests, Panama. *Journal of Tropical Ecology 25*: 637-647.

Baldos AP (2014) *Soil nitrogen cycling and fate of nitrogen in montane forests along a 1000- to 3000-m elevation gradient in the Ecuadorian Andes.* PhD thesis, Georg August Universität, Göttingen.

Bowden RD, Newkirk KM, Rullo GM (1998) Carbon dioxide and methane fluxes by a forest soil under laboratory-controlled moisture and temperature conditions. *Soil Biology and Biochemistry 30*: 1591-1597.

Bowden RD, Davidson E, Savage K, Arabia C, Steudler P (2004) Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the Harvard Forest. *Forest. Ecology and Management, 196*: 43-56.

Breuer L, Papen H, Butterbach-Bahl K (2000) N2O emission from tropical forest soils of Australia. *Journal of Geophysical Research 105*: 26,353-26,367.

Butterbach-Bahl K, Breuer L, Gasche R, Willibald G, Papen H (2002) Exchange of trace gases between soils and the atmosphere in Scots pine forest ecosystems of the northeastern German lowlands. 1. Fluxes of N2O, NO/NO2 and CH4 at forest sites with different N-deposition. *Forest Ecology and Management 167*: 123-134.

Butterbach-Bahl K, Kock M, Willibald G, Hewett B, Buhagiar S, Papen H, Kiese R (2004) Temporal variations of fluxes of NO, NO_2, N_2O, CO_2 and CH_4 in a tropical rain forest ecosystem. *Global Biogeochemical Cycles 18*: GB3012.

Corre MD, Veldkamp E, Arnold A, 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.

Dalal RC, Allen DE (2008) Greenhouse gas fluxes from natural ecosystems. *Australian Journal of Botany 56*: 369-407.

Dutaur L, Verchot LV (2007) A global inventory of the soil CH_4 sink. *Global Biogeochemical Cycles 21*: GB4013.

Fisher JB, Malhi Y, Torres IC, et al. (2012) Nutrient limitation in rainforests and cloud forests along a 3,000-m elevation gradient in the Peruvian Andes. *Oecologia 172*: 889-902.

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

Girardin CAJ, Silva Espejob JE, Doughty CE, et al. (2014) Productivity and carbon allocation in a tropical montane cloud forest in the Peruvian Andes. *Plant Ecology and Diversity 7*: 107-123.

Groffman PM, Altabet MA, Böhlke JK, et al. (2006) Methods for measuring denitrification: diverse approaches to a difficult problem. *Ecological Applications 16*: 2091-2122.

Gundersen P, Emmett BA, Kjønaas OJ, Koopmans CJ, Tietema A (1998) Impact of nitrogen deposition on nitrogen cycling in a forest: a synthesis of NITREX data. *Forest Ecology and Management 101*: 37-55.

Hagedorn F, Bellamy P (2011) Hot spots and hot moments for greenhouse gas emissions from soils. In: Jandl R, Rodeghiero M, Ollson M (eds) Soil carbon in sensitive European ecosystems: from science to land management, John Wiley and Sons, Chichester pp. 13-32.

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

Holtgrieve GW, Jewett PK, Matson PA (2006) Variations in soil N cycling and trace gas emissions in wet tropical forests. *Oecologia 146*: 584-594.

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. *PLoS ONE 7*: e47128.

Homeier J, Leuschner C, Bräuning A, et al. (2013) Effects of nutrient addition on the productivity of montane forests and implications for the carbon cycle. 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, Springer, Heidelberg, pp 315-329.

Houlton BZ, Sigman DM, Hedin, LO (2006) Isotopic evidence for large gaseous nitrogen losses from tropical rainforests. *PNAS 103*(23): 8745-8750.

Huaraca Huasco W, Girardin CAJ, Doughty CE, et al. (2014) Seasonal production, allocation and cycling of carbon in two mid-elevation tropical montane forest plots in the Peruvian Andes. *Plant Ecology and Diversity 7*: 125-142.

IPCC (2013) *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Stocker TF, Qin D, Plattner G-K, et al. (eds). Cambridge University Press, Cambridge, United Kindom and New York.

Ishizuka S, Iswandi A, Nakajima Y, Yonemura S, Sudo S, Tsuruta H, Muriyarso D (2005) Spatial patterns of greenhouse gas emission in a tropical rainforest in Indonesia. *Nutrient Cycling in Agroecosystems 71*: 55-62.

Janssens IA, Dieleman W, Luyssaert S, et al. (2010) Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience 3*: 315-322.

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

Koehler N, Corre MC, Veldkamp E, Sueta JP (2009b) Chronic nitrogen addition causes a reduction in soil carbon dioxide efflux during the high stem-growth period in a tropical montane forest but no response from a tropical lowland forest on a decadal time scale. *Biogeosciences 6*: 2973-1983.

Körner C, Ohsawa M, Spehn E, et al. (2006) Mountain systems. In: Hassan R, Scholes R, Ash N (eds) *Ecosystem and human well-being: current state and trends*. Millenium Ecosystem Assessment, Island Press, Washington, pp. 681-716.

Lewis SL, Malhi Y, Phillips OL (2004) Fingerprinting the impacts of global change on tropical forests. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 359*: 437-462.

Liu L, Graever TL (2009) A review of nitrogen enrichment effects on three biogenic GHGs: the CO_2 sink may be largely offset by stimulated N_2O and CH_4 emissions. *Ecology Letters 12*: 1103-1117.

Magnani F, Mencuccini M, Borghetti M, et al. (2007) The human footprint in the carbon cycle of temperate and boreal forests. *Nature 447*: 849-851.

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.

Mori T, Ohta S, Ishizuka S, et al. (2013) Soil greenhouse gas fluxes and C stocks as affected by phosphorus addition in a newly established *Acacia mangium* plantation in Indonesia. *Forest Ecology and Management 310*: 643-651.

Parton WJ, Mosier AR, Ojima DS, Valentine DW, Schimel DS, Weier K, Kulmala AE (1996) Generalized model for N_2 and N_2O production from nitrification and denitrification. *Global Biogeochemical Cycles 10*: 401-412.

Priess JA, Fölster H (2001) Microbial properties and soil respiration in submontane forests of Venezuelian Guyana: characteristics and response to fertilizer treatments. *Soil Biology and Biochemistry 33*: 503-509.

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. *Global Biogeochemical Cycles 20*: GB3010.

Quinn Thomas R, Canham CD, Weathers KC, Goodale CL (2010) Increased tree carbon storage in response to nitrogen deposition in the US. *Nature Geoscience 3*: 13-17.

Raich JW, Schlesinger WH (1992) The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus 44B*: 81-99.

Raich JW (1998) Aboveground productivity and soil respiration in three Hawaiian rain forests. *Forest Ecology and Management 107*: 309-318.

Robertson GP, Groffman PM (2007) Nitrogen transformation. In: Paul EA (ed) *Soil microbiology, biochemistry, and ecology*. Springer New York, New York, pp 341-364.

Saari A, Martikainen PJ, Ferm A, Ruuskanen J, De Boer W, Troelstra SR, Laanbroek HJ (1997) Methane oxidation in soil profiles of Dutch and Finnish coniferous forests with different soil texture and atmospheric nitrogen deposition. *Soil Biology and Biochemistry 29*: 1625-1632.

Saggar S, Jha N, Deslippe J, et al. (2013) Denitrification and N_2O:N_2 production in temperate grasslands: processes, measurements, modelling and mitigating negative impacts. *Science of the Total Environment 465*: 173-195.

Schrumpf M, Guggenberger G, Valarezo C, Zech W (2001) Tropical montane rainforest soils. Development and nutrient status along an altitudinal gradient in the south Ecuadorian Andes. *Die Erde 132*: 43-59.

Sousa Neto E, Carmo JB, Keller M, et al. (2011) Soil-atmosphere Exchange of nitrous oxide, methane and carbon dioxide in a gradient of elevation in the coastal Brazilian Atlantic forest. *Biogeosciences 8*: 733-742.

Spracklen DV, Righelato R (2014) Tropical montane forests are a larger than expected global carbon store. *Biogeosciences 11*: 2741-2754.

Tanner EVJ, Vitousek PM, Cuevas E (1998) Experimental investigation of nutrient limitation of forest growth on wet tropical mountains. *Ecology 79*: 10-22.

Teh YA, Diem T, Jones S, et al. (2014) Methane and nitrous oxide fluxes across an elevation gradient in the tropical Peruvian Andes. *Biogeosciences 11*: 2325-2339.

Van Haren JLM, de Oliveira Jr RC, Restrepo-Coupe N, Hutyra L, de Camargo PB, Keller M, Saleska SR (2010) Do plant species influence soil CO2 and N2O fluxes in a diverse tropical forests? *Journal of Geophysical Research 115*: G03010.

Van Straaten O, Veldkamp E, Corre MD (2011) Simulated drought reduces soil CO_2 efflux and production in a tropical forest in Sulawesi, Indonesia. *Ecosphere 2*: art119.

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

Weier KL, Doran JW, Power JF, Walters DT (1993) Denitrification and dinitrogen/nitrous oxide ratio as affected by soil water, available carbon, and nitrate. *Soil Science Society of America Journal 57*: 66-72.

Werner C, Butterbach-Bahl K, Haas E, Hickler T, Kiese R (2007) A global inventory of N_2O emissions from tropical rainforest soils using a detailed biogeochemical model. *Global Biogeochemical Cycles 21*: GB3010.

Wolf K (2010) Trace gas fluxes and belowground carbon allocation in tropical montane forest soils of Southern Ecuador. PhD thesis, Georg-August-Universität Göttingen, Göttingen.

Wolf K, Veldkamp E, Homeier J, Martinson GO (2011) Nitrogen availability links forest productivity, soil nitrous oxide and nitric oxide fluxes of a tropical montane forest in southern Ecuador. *Global Biogeochemical Cycles 25*: GB4009.

Wolf K, Flessa H, Veldkamp E (2012) Atmospheric methane uptake by tropical montane forest soils and the contribution of organic layers. *Biogeochemistry 111*: 469–483.

Zhang T, Zhu W, Mo J, Liu L, Dong S (2011) Responses of CH_4 uptake to the experimental N and P additions in an old-growth tropical forest, southern China. *Biogeosciences 8*: 2805-2813

Zhou Z, Jiang L, Du E, Hu H, Li Y, Chen D, Fang J (2013) Temperature and substrate availability regulate soil respiration in the tropical mountain rainforests, Hainan Island, China. *Journal of Plant Ecology 6*: 325-334.

Zimmermann M, Meir P, Bird M, Malhi Y, Ccahuana AJQ (2009) Litter contribution to diurnal and annual soil respiration in a tropical montane cloud forest. *Soil Biology and Biochemistry 41*: 1338-1340.

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 Biogeochemical Cycles 24*: GB4012.

ACKNOWLEDGEMENTS

I want to thank Edzo Veldkamp and Marife Corre for their supervision of this PhD thesis.

Dank geht an die Deutschen Forschungsgemeinschaft, für die Finanzierung des Projektes im Rahmen der Forschereinheit RU816 ‘Biodiversity and Sustainable Management of a Megadiverse Mountain Ecosystem in South Ecuador‘.

Gracias por la cooperación y ayuda del personal del la UTPL, especialmente a Juan Ignacio Burneo Valdivieso, Omar Malagón Avilés y Edwin Daniel Capa Mora.

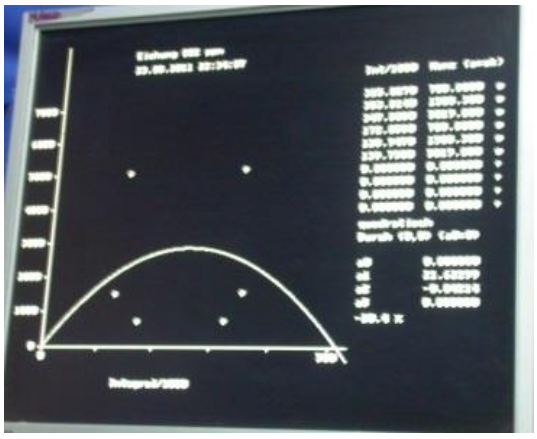
Gracias por mis asistentes del campo y laboratorio para ayudarme: Patricio Salas, Pablo Ramirez, Fabián Cuenca y Richard Samaniego.

Dank dem Labor-Team des Büsgen-Instituts und dem Kompetenzzentrum Stabile Isotope der Universität Göttingen für ihre Hilfe bei der Bearbeitung meiner unzähligen Proben.

Dank gilt all den großartigen Leuten die ich in den letzten Jahren in Ecuador und Göttingen kennenlernen durfte. Danke für eure Unterstützung und Freundschaft! Da es viel zu viele waren, um sie alle einzelnen aufzuzählen, möchte ich ein paar wenige benennen: Guntars Martinson - der mich nicht nur mit einer Menge Daten versorgt hat, sondern mir hilfreich unter die Arme gegriffen hat. Amanda Matson, Angelica Baldos and Daisy Carate - without you it would not have been half as much fun and I’ll always remember our Science Saturdays! Marc Adams, Jürgen Homeier, Daniel Kübler und David Windhorst – unschätzbare Mithäftlinge und Langzeitinsassen, die den Stationsaufenthalt geprägt haben. Zuletzt geht mein spezieller Dank an all die “Packesel“, die einige Tonnen an Probenmaterial von und nach Ecuador transportiert haben!

Ich danke all meinen Freunde und meine Familie die mich motiviert haben alles zu geben.

At the end, I have to mention Maud and Murphy who gave us a hard time in Ecuador but contributed to the mad number-song we invented during one of our sleepless lab-nights!



Maud is having a bad day

DECLARATION OF ORIGINALITY AND CERTIFICATE OF AUTHORSHIP

I, Anke K. Müller, hereby declare that I am the sole author of this dissertation entitled '**Soil greenhouse gas fluxes under elevated nutrient input along an elevation gradient of tropical montane forests in southern Ecuador'**. All references and data sources that were used in the dissertation have been appropriately acknowledged. I furthermore declare that this work has not been submitted elsewhere in any form as part of another dissertation procedure. I certify that the manuscripts presented in Chapters 2, 3 and 4 have been written by me as first author.

Chapters 2 and 4: Guntars O. Martinson provided data on soil CO_2 and CH_4 fluxes.

Göttingen, August 2014 _____

(Anke K. Müller)

Curriculum Vitae

Personal Information

Name	Anke K. Müller
Nationality	German
Languages	German, English (fluent), Spanish (basic), Latin (Latin proficiency certificate)

Career

Since 05/2010	**Ph.D. Program: Biological Diversity and Ecology** Georg-August University of Göttingen, Center for Biodiversity and Ecology
05/2010 – 05/2013	**Research Assistant at the Büsgen Institute** Georg-August University of Göttingen, Soil Science of Tropical and Subtropical Ecosystems
04/2005 – 05/2010	**Diploma in Agricultural Biology** University of Hohenheim Major: Biology of Agricultural Plants Subjects: Agricultural Ecology in the Tropics and Subtropics Biology of Special Crops Plant Protection

Publications

Müller AK, Corre MD, Veldkamp E. Soil N_2O fluxes along a 1000- to 3000-m elevation gradient of tropical montane forests with five years of nitrogen and phosphorus inputs. *Under Review with Biogeochemistry (submitted September 2014)*

Müller AK, Corre MD, Martinson GO, Burneo Valdivieso JI, Veldkamp E. Differential responses of soil CO_2 fluxes to nutrient inputs along an elevation gradient of Andean tropical montane forests. *Under Review with Journal of Geophysical Research – Biogeosciences (submitted July 2014)*

Baldos AP, **Müller AK** (2013) Nitrogen additions affects N cycling but not N_2O surface fluxes. In: *Tropical Mountain Forest (TMF) Newsletter No. 19*, DFG Research Unit 816, doi:10.5678/lcrs/for816.cit.1233.

Conferences

2013 Annual Conference of the Society for Tropical Ecology, Vienna, Austria
Response of soil trace gas fluxes to elevated nutrient inputs in tropical montane forests (Müller AK, Martinson GO, Veldkamp E, Corre MD) – *Oral presentation*

2012 BES Annual Meeting 2012, Birmingham, UK
Effect of nutrient deposition on gas emissions of tropical montane forest soils in southern Ecuador (Müller AK, Martinson GO, Veldkamp E, Corre MD) – *Poster presentation*

2011 FOR 816 - Status Symposium, Loja, Ecuador
Gas emissions and nitrogen cycling in a tropical montane rainforest in southern Ecuador at 2000 m elevation (Baldos AP, Matson AL, Müller AK, Corre MD, Veldkamp E) – *Poster presentation*

2010 FOR 816 - Status Symposium, Loja, Ecuador
Effect of nutrient addition on N_2O fluxes and N cycling in montane tropical forest soils (Müller AK, Martinson GO, Veldkamp E, Corre MD, Flessa H) – *Poster presentation*

2009 Tropentag, Hamburg, Germany
Developing an improved strip-intercropping system for maize and Chinese cabbage in the North China Plain (Müller A, Feike T, Chen Q, Gräff-Hönninger S, Pfenning J, Claupein W) – *Oral presentation*