Response of nutrient cycles of an old-growth montane forest in Ecuador to experimental low-level nutrient amendments

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List of abbreviations

AAS	atomic absorption spectrometry
BD*i*	bulk deposition of an element *i*
BS	base saturation
CB	canopy budget
CFA	continuous flow analysis
dbh	diameter at breast height
DD*i*	dry deposition of an element *i*
ECEC	effective cation exchange capacity
ENSO	El Niño Southern Oscillation
FDR	frequency domain reflectometry
LA	leaf area
LAI	leaf area index
LM	leaching losses from the mineral soil
LO	leaching losses from the organic layer
NUMEX	nutrient manipulation experiment
RBSF	Reserva Biológica San Francisco
SD	standard deviation
SLA	specific leaf area
TD	total deposition
TFD*i*	throughfall deposition of an element *i*
Th	throughfall
TOC	total organic carbon in solution
Tr	transpiration
ΔS_O	change in water storage of the organic layer
ΔS_M	change in water storage of the mineral soil

Summary

Atmospheric nitrogen (N) and phosphorus (P) depositions are expected to increase in the tropics as a consequence of increasing human activities in the next decades. Furthermore, a possible shortened El Niño Southern Oscillation cycle might come along with more frequent calcium (Ca) depositions on the eastern slope of the Ecuadorian Andes originating from Saharan dust. It is crucial to understand the response of the old-growth montane forest in Ecuador to increased nutrient deposition to predict the further development of this megadiverse ecosystem.

I studied experimental additions of N, P, N+P and Ca to the forest and an untreated control, all in a fourfold replicated randomized block design. These experiments were conducted in the framework of a collaborative research effort, the NUtrient Manipulation EXperiment (NUMEX). I collected litter leachate, mineral soil solution (0.15 and 0.30 m depths), throughfall and fine litterfall samples and determined N, P and Ca concentrations and fluxes. This approach also allowed me to assess whether N, P and/or Ca are limiting nutrients for forest growth. Furthermore, I evaluated the response of fine root biomass, leaf area index, leaf area and specific leaf area, tree diameter growth and basal area increment contributed from a cooperating group in the Ca applied and control treatments.

During the observation period of 16 months after the first fertilizer application, less than 10, 1 and 5% of the applied N, P and Ca, respectively, leached below the organic layer which contained almost all roots but no significant leaching losses occurred to the deeper mineral soil. Deposited N, P and Ca from the atmosphere in dry and wet form were, on balance, retained in the canopy in the control treatment. Retention of N, P and Ca in the canopy in their respective treatments was reduced resulting in higher concentrations and fluxes of N, P and Ca in throughfall and litterfall. Up to 2.5% of the applied N and 2% of the applied P and Ca were recycled to the soil with throughfall. Fluxes of N, P and Ca in throughfall+litterfall were higher in the fertilized treatments than in the control; up to 20, 5 and 25% of the applied N, P and Ca, respectively, were recycled to the soil with throughfall+litterfall.

In the Ca-applied plots, fine root biomass decreased significantly. Also the leaf area of the four most common tree species tended to decrease and the specific leaf area increased significantly in *Graffenrieda emarginata* Triana, the most common tree species in the study area. These changes are known plant responses to reduced nutrient stress. Reduced aluminium (Al) toxicity as an explanation of the Ca effect was unlikely, because of almost complete organo-complexation of Al and molar Ca:Al concentration ratios in solution above the toxicity threshold.

The results suggest that N, P and Ca co-limit the forest ecosystem functioning in the northern Andean montane forests in line with recent assumptions in which different ecosystem compartments and even different phenological stages may show different nutrient limitations (Kaspari et al. 2008). I conclude that (1) the expected elevated N and P deposition will be retained in the ecosystem, at least in the short term and hence, quality of river water will not be endangered and (2) increased Ca input will reduce nutrient stress of the forest.

Zusammenfassung

Aufgrund zunehmender Wirtschaftsaktivitäten wird in den Tropen in den nächsten Jahrzehnten ein Anstieg der Deposition von Stickstoff (N) und Phosphor (P) aus der Atmosphäre erwartet. Zudem könnte eine mögliche Verkürzung des südlichen El Niño Oszillations-Zykluses einhergehen mit häufigerer Deposition kalzium(Ca)-reichen Saharasandes auf den östlichen Hängen der ecuadorianischen Anden. Es ist wichtig, die Reaktion des Bergregenwaldes in Ecuador auf vermehrten Nährstoffeintrag zu verstehen, um die weitere Entwicklung dieses megadiversen Ökosystems voraussagen zu können.

Im Rahmen des gemeinschaftlichen Forschungsvorhabens „NUtrient Manipulation EXperiment“ (NUMEX) wird die Reaktion des Waldes auf die Applikation von N, P, N+P, Ca und Kontrolle untersucht. Das Experiment wurde in einem vierfach wiederholten randomisierten Blockdesign durchgeführt. In Streuperkolat, Bodenlösung aus dem Mineralboden (0.15 und 0.30 m Tiefe), Bestandesniederschlag und Streufall wurden die N-, P- und Ca-Konzentrationen bzw. -gehalte gemessen und anschließend die jeweiligen Elementflüsse berechnet. Diese Methode ermöglicht zu bestimmen, ob N, P und/oder Ca limitierende Nährstoffe für das Waldwachstum sind. In Zusammenarbeit mit einer anderen Gruppe evaluierte ich die Reaktionen von Feinwurzelbiomasse, Blattflächenindex, Blattgröße und spezifischer Blattgröße, Baumdurchmesserwachstum und Grundflächenwachstum in den Ca- und Kontroll-Plots.

Jeweils weniger als 10, 1, und 5% der applizierten N, P und Ca-Massen passierte im Beobachtungszeitraum von 16 Monaten nach der ersten Düngung die organische Auflage, welche fast alle Wurzeln enthält. Im Mineralboden wurden dagegen kaum Auswaschungsverluste beobachtet. Aus der Atmosphäre nass oder trocken eingetragenes N, P und Ca wurde in der Kontrollbehandlung im Kronenraum zurückgehalten. Die Aufnahme von N, P und Ca im Kronenraum wurde durch die Düngung jedes einzelnen Elementes reduziert, wodurch die Konzentrationen von N, P und Ca in Bestandesniederschlag und Streufall anstiegen. Bis zu 2.5% des applizierten N und 2% des applizierten P und Ca wurden über den Bestandesniederschlag wieder zum Boden recycelt. Die Flüsse von N, P und Ca in Bestandesniederschlag + Streufall waren in den gedüngten Behandlungen höher als in den Kontrollen und umfassten 20% der gedüngten Elementmasse für N, 5% für P und 25% für Ca.

Die Behandlung mit Ca führte zu einer signifikanten Abnahme der Feinwurzelbiomasse. Auch die Blattfläche der vier häufigsten Baumarten neigte dazu abzunehmen und bei *Graffenrieda emarginata* Triana, der häufigsten Baumart im Untersuchungsgebiet, nahm die spezifische Blattfläche deutlich zu. Diese Veränderungen sind bekannte Reaktionen der Pflanzen auf verringerten Stress durch Nährstofflimitierung. Eine reduzierte Aluminium (Al)-Toxizität kann als Erklärung für den Ca-Effekt ausgeschlossen werden, da Al fast vollständig organisch komplexiert war und die molaren Ca:Al-Verhältnisse in der Bodenlösung oberhalb der Toxizitäts-Grenze lagen.

Die Ergebnisse deuten auf eine Co-Limitierung des Bergwaldökosystems in den nördlichen Anden durch N, P und Ca. Dies ist in Übereinstimmung mit aktuellen Annahmen in der verschiedene Ökosystembestandteile oder verschiedene phänologische Stadien verschiedene Nährstofflimitierungen aufweisen können (Kaspari et al. 2008). Ich folgere daraus, dass (1) die erwarteten erhöhten N- und P-Depositionen zunächst zurückgehalten werden und somit, wenigstens kurzfristig, die Qualität des Flusswassers nicht gefährdet ist, und (2) vermehrte Ca-Einträge den Nährstoffstress des Waldes mindern.

Resumen

Se predice que en las siguientes décadas los depósitos atmosféricos de nitrogeno (N) y fósforo (P) se incrementarán en los trópicos como consecuencia de la actividad humana. Una reducción en las oscilaciones del El Niño del Sur pudieran combinarse con depósitos más frecuentes de calcio (Ca) en la ladera occidental de los Andes Ecuatorianos, originarios de masas de polvo provenientes del Sahara. Por eso, es de crucial importancia entender la respuesta del bosque viejo montañoso de Ecuador a incrementos en los depósitos de nutrientes para así poder predecir el desarrollo futuro de éste ecosistema megabiodiverso.

En éste estudio yo analizé adiciones experimentales de N, P, N+P y Ca en el bosque y en un tratamiento control, todos en un cuarteto de réplicas con un diseño azaroso. Estos experimentos fueron parte del marco de un proyecto colaborativo llamado Experimento de Manipulaciones de Nutrientes (NUMEX). Yo recolecté el agua que atraviesa la capa orgánica, el agua que atraviesa el suelo mineral (0.15 y 0.30 m de profundidad), el agua que atraviesa el dosel y la hojarazca para así determinar las concentraciones y flujos de N, P, y Ca. Este acercamiento me permitió adicionalmente evaluar si es que N, P y/o Ca son nutrientes que limitan el crecimiento del bosque. Además en colaboración con otro grupo, se evaluó la respuesta de la biomasa de raíces primarias, índice de área de las hojas, área de las hojas y área especifica de las hojas, diámetro de crecimiento de los árboles e incremento del área basal en los tratamientos de Ca y el control.

Durante un período de observación de 16 meses, de los nutrientes adicionados, menos de un 10, 1 y 5% de N, P y Ca, respectivamente, se percolaron debajo de la capa orgánica la cual contiene casi todas las raíces, pero la percolación en la parte mineral profunda del suelo no fue estadistícamente significativa. Los depósitos atmosféricos de N, P y Ca en forma húmeda y seca estuvieron en balance, siendo retenidos en el dosel en el tratamiento control. La retención de N, P y Ca en el dosel en sus grupos respectivos disminuyó significativamente, resultando en concentraciones y flujos más altos de N, P y Ca en el agua que atraviesa el dosel y en la hojarazca. Más de un 2.5% del N y 2% de cada uno del P y de Ca adicionados fueron reciclados al suelo por medio del agua que atraviesa el dosel. Además, encontré incrementos en los flujos de N, P y Ca en el agua que atraviesa el dosel + hojarazca; un 20, 5 y 25% del N, P y Ca adicionado, respectivamente, fue reciclado al suelo con el agua que atraviesa el dosel + hojarazca.

En los tratamientos donde se adicionó Ca, la biomasa de raíces finas disminuyó significativamente. El tamaño de las hojas de las cuatro especies de árboles más comunes tendió a disminuir y el área de hoja especifica incrementó significativamente en *Graffenrieda emarginata* Triana, la especie de árbol más común en el área. Es bien sabido que las plantas responden con estos cambios bajo situaciones estrezantes en disminución de nutrientes. Una reducción de la toxicicidad por aluminio (Al) como explicación del efecto de la adición del Ca es poco probable, debido a que el Al es casi completamente complejado por matería orgánica y a que las concentraciones molares Ca:Al superan el límite de toxicidad en solución.

Estos resultados sugieren que N, P y Ca co-limitan el ecosistema en el bosque de montaña del norte de los Andes, así como se ha asumido recientemente en el cual distintas unidades del ecosistema e incluso distintas fases fenológicas, pueden presentar limitaciones de nutrientes (Kaspari et al. 2008). Yo concluyo que (1) los niveles elevados de depósicion de N y P en los trópicos serán retenidos en el ecosistema, al menos a corto plazo y por lo tanto, la calidad del agua de río no estará en peligro y (2) un incremento en las aportaciones de Ca reduce el estrés nutritivo del bosque.

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Prescribed by the regulations of the internet publisher (ArchiMed) this section had to be deleted for data protection reasons. I am still very grateful for all the help and discussion offered by the persons still listed in the print-version.

A Summarizing overview

1. Introduction

Among the most widespread anthropogenically caused environmental changes is changed nutrient deposition, which belongs to the important drivers of global biodiversity (Sala et al. 2000, Thuiller 2007). The expected increased nutrient deposition in the tropics (Galloway et al. 2004, Phoenix et al. 2006) will therefore likely have detrimental effects on the high biodiversity of many of the tropical ecosystems. Understanding the response of the north Andean tropical montane forest, one of the biodiversity hotspots of the world (Barthlott et al. 2007), requires experimental approaches involving nutrient amendments in the range of the expected changed deposition and a whole-ecosystem consideration.

Atmospheric N enters ecosystems as reactive N, i.e. inorganic oxidized and reduced forms and organic compounds or by abiotic and biotic N fixation via lightning or biological N_2 fixation (Galloway et al. 2004, 2008). Human activities can alter the N supply in forest soils mainly via elevated N concentrations in rainfall. The main reasons for increasing N deposition in the tropics are increasing biomass burning (Da Rocha et al. 2005, Fabian et al. 2005), a higher consumption of N fertilizers in agriculture and a higher fossil fuel consumption (Galloway et al. 2004, 2008). Increasing N deposition from the atmosphere causes soil acidification and consequently reduced availability of P and base metals (Matson et al. 1999), NH_4^+ and NO_3^- leaching, emissions of NO (which regulates the production of tropospheric ozone and is a precursor of nitric acid) and N_2O (which is a greenhouse gas, Matson et al. 1999, Koehler et al. 2009).

Phosphorus deposition from the atmosphere into ecosystems may result from biomass burning or has biogenic origin (e.g., pollen and spores, Mahowald et al. 2005), but is dominated by mineral aerosol dust deposition (Pett-Ridge 2009), which account for 5, 12 and 82%, respectively, of the total P deposition at the global scale (Mahowald et al. 2008). Anthropogenic disturbance (slash-and-burn with subsequent wind erosion) in Amazonia can lead to a total P loss of 36 kg ha^{-1} (Kauffman et al. 1993). More frequent forest fires are expected in Amazonia because of intensified land clearing (Cochrane and Laurance 2008), which might be further enhanced by climate change (reduced precipitation and a coupled forest dieback) (Cochrane and Barber 2009). The emitted P is transported with the trade winds to the Equatorial Andes.

A significant source of Ca for transatlantic ecosystems is Saharan mineral dust (Kauffman et al. 1993, Boy and Wilcke 2008, Pett-Ridge et al. 2009b). Pett-Ridge et al. (2009a) reported that 83% of the atmospheric Ca input in a watershed in Puerto Rico originated from Saharan dust. In south Ecuador, Boy and Wilcke (2008) demonstrated that during a strong La Niña event in

1999/2000, Ca and magnesium (Mg) were deposited from the atmosphere to the east exposed slope of the eastern Andean cordillera which originated from the Sahara. The Saharan dust was transported across the Amazon basin during short spells of dry weather conditions in an overall wetter La Niña period along the wind trajectories. Boy and Wilcke (2008) therefore hypothesized that the Ca supply by Saharan dust deposition is linked with the El Niño Southern Oscillation (ENSO). The amplitude or frequency of ENSO might change as a consequence of the global climate change possibly resulting in a shortening of the currently approximately 7-yr cycle (Timmermann et al. 1999, Richardson et al. 2009). A consequence could be increased Ca deposition via Saharan dust to the east-exposed slopes of the north Andes.

It is frequently assumed that N limits plant production on young soils since N is accumulated from the atmosphere and P limits plant production on old soils, since P gradually becomes unavailable during soil development (Walker and Syers 1976). The model of Walker and Syers (1976) was tested and confirmed in a fertilization experiment on the Hawaiian Islands (Vitousek et al. 1993, Herbert and Fownes 1995, Vitousek and Farrington 1997, Harrington et al. 2001). Since tropical lowland forests mainly grow on old soils and tropical montane forests on young soils, Tanner et al. (1998) speculated that lowland forests are P-limited while montane forest are N-limited. However, tropical montane forests responded to N (Tanner et al. 1990, Tanner et al. 1992, Cavelier et al. 2000) and P fertilization (Tanner et al. 1990, Cavelier et al. 2000) with increased growth. In contrast, stem growth in tropical lowland forest of Borneo did neither respond to N nor to P addition (Mirmanto et al. 1999). In summary, the type of nutrient limitation in tropical forests remains unclear.

Recently, Kaspari et al. (2008) showed that tropical forests can be limited by different nutrients, whereby Liebig´s Law of the Minimum postulated that the same single element limits the whole ecosystem does not hold. In a tropical lowland forest in Panama, N influenced tree reproduction, P and K influenced the decomposition rate of cellulose and P and at least one micronutrient influenced the rate of leaf litter decomposition. Similarly, Hedin et al. (2009) suggested that individual compartments of tropical forests like the canopy of the organic layer are N-limited while other compartments are not. There are also indirect indications that Ca can be a limiting element for forest growth (McLaughlin and Wimmer 1999, Huggett et al. 2007, Paoli and Curran 2007, Boy and Wilcke 2008) since Ca plays an essential role in regulating numerous physiological processes in plants like cell division, synthesis and function of membranes and cell walls, stomatal regulation, activation of the enzyme system involved in the plant response to

environmental stimuli like low temperature, carbohydrate metabolism, disease resistance and wound repair (McLaughlin and Wimmer 1999).

In acid mineral soils, Ca deficiency and Al toxicity can appear simultaneously (Marschner 1993) which makes it difficult to distinguish between both growth limiting factors. While the free Al^{3+} ion is phytotoxic and the $Al(OH)^{2+}$ and $Al(OH)_2^{+}$ ions are potentially toxic, organically complexed Al is not toxic (Alva et al. 1986, Savory and Wills 1991, Cronan and Grigal 1995). In the thick organic layers of tropical montane forest soils, where most roots are located (Soethe et al. 2006), Al is most likely almost entirely organically complexed.

In the response of transatlantic forest ecosystems to Sahara dust-derived Ca depositions, the potential Ca effect cannot be separated from the simultaneously occurring pH effect (Boy and Wilcke 2008). An increased pH in the soil solution likely stimulates organic matter turnover and thus the release of organically bound nutrients such as N. Soethe et al. (2006) observed a non-significant increase in fine root growth in an Ecuadorian tropical montane forest after the addition of lime to an in-growth core. Again, it was not possible to separate the effects of Ca and pH. To study the Ca effect alone, a pH-neutral salt such as $CaCl_2$ needs to be added experimentally.

My thesis reports changes in the N, P and Ca fluxes in an Ecuadorian montane forest growing on Palaeozoic bedrock in response to low-level amendments of these nutrients spread over several application dates. The overall goal was to assess the impact of elevated N, P and Ca depositions on N, P and Ca cycling in the Andean tropical montane forest. I tested four hypotheses:

1. The forest is N-limited and therefore added N remains in the aboveground cycle between soil organic layer and vegetation.
2. Added P is mainly abiotically retained in the mineral soil and little recycled in the ecosystem.
3. Calcium reduces plant nutrient stress because Ca is at least co-limiting and alleviates Al toxicity.
4. Calcium remains in the ecosystem as indicated by Ca retention in the forest canopy and low Ca leaching to greater soil depths.

I tested these hypotheses by applying N, P and Ca to the forest soil. My study was conducted in the framework of the interdisciplinary NUtrient Manipulation EXperiment (NUMEX) and I could also make use of results of cooperating working groups in my evaluation.

2. Materials and methods

2.1. Study area

The studied forest is located in south Ecuador on the eastern slope of the Cordillera Real of the Andes (i.e. the eastern cordillera) at an altitude between 2020 and 2120 m a.s.l. (3°59´S, 79°05´W), in the Reserva Biológica San Francisco (RBSF) in the deeply incised valley of the Rio San Francisco draining to the Amazon (Fig. A-1). The vegetation at the study site can be classified as "evergreen lower montane forest" according to Homeier et al. (2008). In the study area, more than 280 tree species have been identified so far with Lauraceae, Melastomataceae and Rubiaceae as the most abundant plant families (Homeier and Werner 2007). *Graffenrieda emarginata* Triana (Melastomaceae) is the most common tree species.

The 4-yr mean annual rainfall (2004-2008) ranged between 2527 ± 400 and 2611 ± 397 mm (standard deviation; SD) at two rainfall gauging stations (Wullaert et al. 2009). Rainfall has an unimodal distribution with a maximum between April and September and without a pronounced dry season (Fleischbein et al. 2005, 2006). Annual bulk N, P and Ca depositions with rainfall were 9.5-10, 0.64-1.1 and 4.4-29 kg ha^{-1} yr^{-1}, respectively, between 1998 and 2003 (Boy and Wilcke 2008, Boy et al. 2008a). Mean annual temperature at 1950 m a.s.l. is 15.2°C. The coldest months are June and July with a mean temperature of 14.4 °C; the warmest month is November with a mean temperature of 16.1 °C (Bendix et al. 2008). The soil is a Stagnic Cambisol (Hyperdystric, Chromic) (IUSS Working Group WRB 2007) developed from Palaeozoic phyllites, quartzites and metasandstones.

2.2. Experimental design

The study site was located on the upper slope near a major ridge and has an average inclination of 51% (range: 25% to 84%). The experiment consists of N, P, N+P and Ca addition and unfertilized control treatments. Each treatment was fourfold replicated in a randomized block design with the restriction that the unfertilized control plots were the uppermost located plots and the combined treatment of N+P addition the lowermost located plots in each block to avoid nutrient leaching from fertilized to control plots and of N, P and Ca to a plot fertilized with the other element. The location of each treatment plot was selected in a way that the vegetation was representative for the area and similar on all plots. Each plot had an area of 400 m² (20 m x 20 m) and the distance between the plots was at least 10 m (Fig. A-2).

Fig. A-1: Location of the research plots (within red circle), seen from the research station.

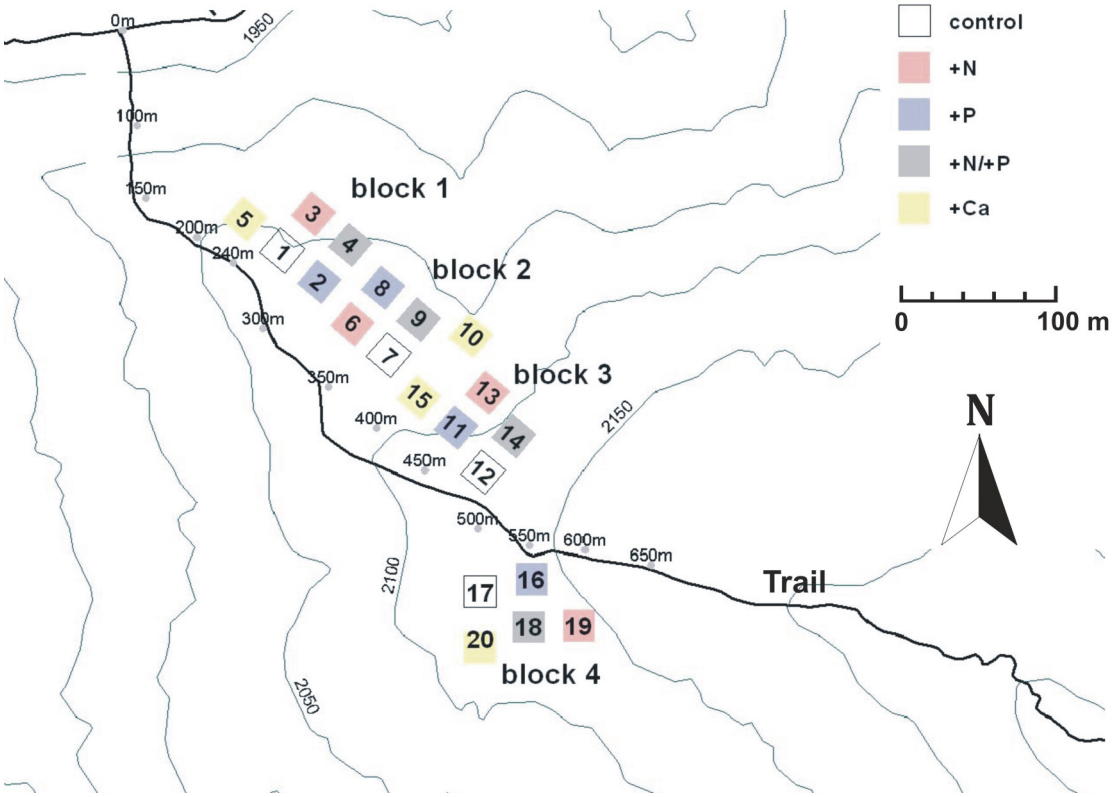

Fig. A-2: Experimental design of the collaborative NUtrient Manipulation EXperiment (NUMEX).

The N, P, N+P and Ca addition plots received 50 kg ha^{-1} yr^{-1} N, 10 kg ha^{-1} yr^{-1} P, 50 + 10 kg ha^{-1} yr^{-1} N and P, and 10 kg ha^{-1} yr^{-1} Ca, respectively, split in two applications per year. For security and availability reasons, N was added in the form of commercially available urea. Phosphorus was applied as $NaH_2PO_4 \cdot 2H_2O$ and Ca as $CaCl_2 \cdot 2H_2O$, both in *pro analysi* quality. Nutrient additions started in January 2008, followed by two further nutrient applications in August 2008 and February 2009. Nutrients were applied manually. The research equipment (except throughfall collectors) was installed in subplots of 2 m x 2 m within the 20 m x 20 m plots. Subplots were located along two perpendicular random transects and had at least 2 m distance to the border of the plot to avoid edge effects.

2.3. Sampling

2.3.1. Soil and fine root sampling

All soil horizons of each plot were sampled before (in August 2007) and after two nutrient applications (in October 2008). Furthermore, the horizons of the organic layer were sampled in a representative way per plot after the third fertilizer application between February and May 2009 for total P analyses. Fine roots were sampled in six subplots per experimental plot with a soil corer (3.5 cm in diameter) by D. Hertel of the University of Göttingen.

2.3.2. Litterfall and leaf sampling

Litterfall was collected once per month from six litterfall traps (0.60 m x 0.60 m) per plot from October 2007 until March 2008. From April 2008 on, sub-samples were taken from only two randomly-chosen litterfall traps per plot whereby the litterfall remained only seven days in the field. Samples of the pre-treatment months were bulked to result in one sample per plot. After the first nutrient application, samples from subplots were bulked per plot and per month for further analyses.

Leaf samples from sun-exposed branches of the four most common tree species (*Alchornea grandiflora* Müll. Arg., *G. emarginata*, *Hieronyma moritziana* Pax & K.Hoffm. and *Myrcia* sp.) from 4-5 individual trees per treatment and species were collected in January 2009 by the working group of Homeier et al. to quantify changes in leaf morphology and foliar nutrient concentrations one year after the first fertilization. Ten to 20 fully developed leaves were sampled without visible damages from two different branches of each tree.

2.3.3. Water sampling

Throughfall was collected with 20 fixed-positioned funnel gauges on each plot. Each plot had two transects – parallel to the plot border – and each transect contained 10 throughfall collectors randomly distributed along the transect in a way that the collectors were at least 2 m away from the plot border to avoid edge effects. The funnel gauges consisted of a 2-l polyethylene sampling bottle and a polyethylene funnel with 115 mm diameter. The rims of the funnels were 0.3 m above the soil surface to avoid splash-in effects from the forest floor. The funnel edges were vertical to avoid splash-out. To reduce evaporation, a table-tennis ball was used in the funnel and the collecting bottle was wrapped with aluminium foil. Throughfall volumes of the 20 collectors per plot were measured with a graduated cylinder in the field and were volume-weighted bulked to result in one sample per plot per collecting date.

Per plot, litter leachate was collected using zero-tension lysimeters, which consisted of plastic boxes with a collecting surface area of 0.15 m x 0.15 m. Zero-tension lysimeters were connected with a plastic tube to a collecting bottle wrapped in aluminium foil to reduce the impact of radiation. The plastic box was covered with a polyethylene net (0.5 mm mesh width) and installed below the organic layer. The organic layer remained intact during and after installation. Per plot, three zero-tension lysimeters were installed. The collected litter leachate samples from one plot were bulked to one individual sample per plot per collecting date.

Furthermore, from each plot, mineral soil solution was collected using suction lysimeters (ceramic suction cups with 1 µm pore size) at 0.15 and 0.30 m depths. Per depth, soil solution was a mixture from three suction lysimeters with a distance of 0.40 m between each other to produce spatially representative samples, so installed that bulking of the soil solution per soil depth occurred *in situ*. Since the majority of the tree roots were located in the organic layer, the collected soil solution can be considered as from below the rooting zone. The collecting bottles of the soil solution were brown-coloured and were placed in a closed bucket to reduce the radiation impact. The lysimeters did not collect the soil solution quantitatively. To calculate water fluxes in the mineral soil, tensiometers were installed in each plot close to the suction lysimeters and electronic FDR and tensiometer sensors were installed in the middle of the whole research site.

Fortnightly, throughfall, litter leachate and mineral soil solution were sampled. After sampling the mineral soil solution, a vacuum was applied to the suction lysimeters in order to collect sufficient sample for the next sampling period. There was a five-months measurement

period prior to the first nutrient addition. Sampling of throughfall, litter leachate and soil solution were collected from August 2007 until April 2009.

2.4. Physical and chemical analyses

Bulk density was measured by the soil core method (Blake and Hartge 1986) at four soil profiles.

Per leaf sample, more than 10 fresh leaves were scanned by the working group of Homeier et al. using a flat bed scanner (CanonScan LIDE 30, Canon). Subsequently, images were analysed with the software WinFolia 2001a (Regent Instruments Inc., Canada) for calculation of the average single leaf area (LA). Leaves were then dried at 60 °C to constant mass and specific leaf area (SLA) was calculated as the ratio of LA to leaf dry weight.

To assess fine root biomass, the soil samples were transferred to plastic bags and transported to the laboratory, where processing of the stored samples (4 °C) took place within six weeks by the working group of Hertel et al. In the lab, the samples were soaked in water and cleaned from soil residues using a sieve with a mesh size of 0.25 mm. Only fine roots (roots <2mm in diameter) of trees were considered for analysis. Live fine roots (biomass) were separated from dead rootlets (necromass) under the stereomicroscope based on colour, root elasticity and the degree of cohesion of cortex, periderm and stele. A dark cortex and stele, or a white, but non-turgid cortex, or the complete loss of the stele and cortex with only the periderm being present, were used as indicators of root death (Persson 1978, Leuschner et al. 2001). The fine root biomass of each sample was dried at 70 °C for 48 h and weighed. The data were expressed as fine root abundance (g m^{-2}).

Soil and litterfall samples were dried at 40 °C to constant mass after field collection. Dried litterfall samples and samples of the organic layer were ground with a ballmill at 650 rounds per minute for 10 min. Dried mineral soil samples were sieved (<2 mm) before further analysis.

I determined total C and N concentrations of soil and litterfall samples with an Elemental Analyzer (vario EL III, Elementar Analysensysteme, Hanau, Germany). Soil pH was measured in a soil:distilled water mixture (ratio of 1:10 and 1:2.5 for the organic horizons and mineral soil, respectively). Mineral soil texture was determined with the pipette method after DIN ISO 11277:2002-08 standards. To measure the effective cation-exchange capacity (ECEC) of mineral soil samples, 5 g of the samples were shaken in 100 ml unbuffered 1 mol l^{-1} NH_4NO_3 for one h,

then filtered and analysed for Al, Ca, K, Mg and Na concentrations. Base saturation (BS) was calculated as the percentage of the sum of charge equivalents of the base metal cations of the ECEC.

Leaf samples (by the working group of Homeier et al.) and litterfall were digested with 65% HNO_3 under pressure in a microwave system (Mars Xpress, CEM GmbH, Kamp-Lintfort, Germany) to determine the total P, Ca and Al concentrations.

After collection in the field, litter leachate and soil solution samples were transported to a field laboratory where pH (Sentix HWS, WTW GmbH, Weilheim, Germany) was immediately measured in an aliquot of each sample. Another aliquot was filtered (ashless filters with pore size 4-7 µm, folded filter type 389; Munktell & Filtrak GmbH, Bärenstein, Germany) and frozen until transport to Germany for further analysis. During transport to Germany, samples were stored cool at <4 °C and transport did not take longer than one week.

Water samples were analysed for concentrations of total dissolved N (further referred to as total N), total dissolved P (further referred to as total P) and dissolved Cl^- using continuous flow analysis (CFA, Bran+Luebbe GmbH, Norderstedt, Germany). Total P concentrations in soil and litter extracts were also determined with CFA. Concentrations of Ca, K, Mg and Na in water samples and in soil extracts, and plant digests also Al were determined with flame atomic absorption spectrometry (AAS, Varian AA240FS, Thermo Fisher, Darmstadt, Germany). Elemental concentrations of Ca and Al in digests of fresh leaves were determined with an Inductively Coupled Plasma Analyzer (Optima 5300DV ICP-OES, Perkin Elmer, Rodgau, Germany) by the working group of Homeier et al. Total organic carbon in solution (TOC) was measured with a high temperature TOC analyzer (high TOC *II*, Elementar Analysensysteme, Hanau, Germany).

To assess the precision of the analysis of N, P and Ca, I analysed the N, P and Ca concentration of an internal reference standard (a selected water sample from the field site in Ecuador) in every laboratory run of about 100 samples. The relative standard deviations of the reference standard were 11, 22 and 11% for the measurements of total N, total P and Ca concentrations, respectively.

2.5. Stem diameter growth and plot basal area growth

Stem diameter growth of all 384 trees with a diameter at breast height (dbh) ≥10 cm on the Ca and control treatments was 6-weekly monitored with permanent girth-increment tapes

(D1 dendrometer, UMS, Munich, Germany) at breast height (1.3 m) by the working group of Homeier et al. The increase of plot basal area was calculated as the sum of all individual tree increments from 15.02.2008 (after the first fertilization) until 25.04.2009.

2.6. Leaf area index

The leaf area index (LAI) was quantified with two LAI-2000 plant canopy analyzers (LI-COR Inc., Lincoln, NE, USA) by the working group of Homeier et al. The LAI measurements were conducted in the remote mode, i.e. by synchronous readings below the canopy at 2 m height above the forest floor and in a nearby open area ("above-canopy" reading) using two devices. One measurement was done above each litter trap and a second at the same time outside the forest. The LAI measurements were realized in January 2008 (before first fertilization) and in January 2009 (one year after the first fertilization). All measurements were conducted during periods of overcast sky. To avoid reductions in the sky sector seen by the LAI-2000 fish-eye lens (by high mountains or trees at the horizon), only data of the three inner rings was analysed (0-43° from zenith).

2.7. Speciation of aluminium

The speciation of Al in litter leachate was calculated with Visual MINTEQ (Version 2.61). I used pH values and TOC concentrations from own measurements at the NUMEX site to run the programme. I did not measure Al concentrations in the soil solutions of the study site. Instead, I inferred that the concentrations of Al and Ca, Mg, Na, K, SO_4^{2-}, Cl^- and NO_3^- at the study site were similar as at an adjacent forest site between 1900 and 2010 m a.s.l. for which a 5-yr data set was available (Wilcke et al. 2001, Boy et al. 2008b). I used the 5-yr mean concentrations of Ca, Mg, Na, K, SO_4^{2-}, Cl^- and NO_3^- reported in Boy et al. (2008b) to calculate the ionic strength of the litter leachate.

2.8. Hydrological calculations

For the calculation of reference evapotranspiration, I used meteorological data obtained from the automatic meteorological station on a clear-cut area in ridge-top position at 1950 m a.s.l. run by the cooperating working groups of Richter of the University of Erlangen-Nürnberg and of Bendix at the University of Marburg. Air temperature was measured at a height of 2 m above soil surface with an electronic sensor (Pt100). Relative air humidity was measured with a hygrometer

at 2 m above soil surface. Wind velocity was measured with a four-cup anemometer at 2.5 m above soil surface. Total radiation was measured with a pyranometer at 2 m above soil surface with a sensor after WMO and ISO 9060 standards. Rainfall was measured with a tipping-bucket funnel gauge. All meteorological data were recorded with measuring intervals of 1 h and stored with a data logger (Logger DL 15).

Leaching losses from the organic layer were calculated using a one-dimensional soil water balance model (DVWK 1996) as throughfall minus transpiration minus change in water storage (t_2-t_1) in the organic layer. Reference evapotranspiration was calculated with REF-ET (University of Idaho and Dr. R.G. Allen; version 2.0) using the ASCE Penman-Monteith method based on daily mean wind speed, air humidity and irradiation, daily precipitation sum and daily minimum and maximum temperature. Transpiration losses were determined as the difference between reference evapotranspiration and interception losses. For the calculation of transpiration, I assumed direct evaporation from the soil as negligible. Interception losses were directly measured as the difference between incident rainfall and throughfall. I used frequency domain reflectometry (FDR) probes to calculate change in water storage in the organic layer and the mineral soil. FDR probes were installed at 0.10, 0.20, 0.30 and 0.40 m mineral soil depth and one in the organic layer. No upward flux from the mineral soil to the organic layer was allowed in the calculations. Leaching losses from the organic layer were used as an input for the calculation of the leaching losses of the mineral soil. The latter was calculated as the difference between leaching losses from the organic layer and change in water storage (t_2-t_1). I assumed that water uptake from the mineral soil by tree roots was negligible because of the almost complete lack of roots in the mineral soil. From earlier results in our research area, Boy et al. (2008b) calculated that <10% of the total water flux contributed to lateral flow. I therefore assumed it reasonable to use a one-dimensional soil water balance model to calculate water fluxes in the mineral soil. Nutrient fluxes were calculated as the product of fortnightly fluxes and nutrient concentrations of the respective flux type.

2.9. Canopy budget

To set up the canopy budget (CB) of an element *i*, I used the model of Ulrich (1983), where CB is calculated as the difference between throughfall deposition and total deposition. I did not include stemflow in the experimental setup since in most tropical forests including the north Andean montane forest, stemflow does not exceed 2% of rainfall (Lloyd and Marques

1988, Wilcke et al. 2001, Fleischbein et al. 2006). Positive values of CB indicate leaching, negative ones uptake of an element *i* by the canopy. The estimate of CB includes an unknown contribution of dry gaseous deposition of N. If CB is negative, then N uptake by the canopy is underestimated by the unaccounted gaseous deposition. If CB is positive, leaching is overestimated by the unaccounted gaseous deposition.

Total deposition of an element *i*, was calculated as the sum of bulk rainfall deposition and dry deposition. Dry deposition of an element *i* (DD_i), was calculated according to Eq. A-1,

$$DD_i = [(TFD_{Cl} / BD_{Cl}) \times BD_i] - BD_i \qquad \text{(A-1)}$$

whereby TFD_{Cl} represents the throughfall deposition and BD_{Cl} the bulk deposition of Cl^-. The bulk deposition of element *i* is represented by BD_i. It is assumed that Cl^- is a non-reactive tracer which was confirmed in earlier work at the same study site (Boy et al. 2008a, Boy and Wilcke 2008, Wilcke et al. 2009). Furthermore, it is assumed that the DD/BD ratio is similar for all elements. Chloride concentrations were volume-weighted averaged for the periods between the first and the second, the second and the third and after the third fertilization to reduce the influence of measurement uncertainty of Cl^- on the canopy budget calculations.

2.10. Statistical analyses

To test differences in fine root biomass between the Ca and control treatments, subplots were considered as independent replicates because the distance between the sample locations was large enough. Statistical analyses on fine root biomass were performed by D. Hertel with SAS software, (version 8.2; SAS Institute, Cary, NC, USA) using a one-factorial non-parametric Kruskal-Wallis test, followed by a post-hoc paired comparison (Wilcoxon-U-test).

For the time period before the first nutrient application, I tested if there were *a priori* differences of the later fertilized plots and the unfertilized control plots with respect to nutrient concentrations in soil, litter leachate, soil solution, throughfall and litterfall. The period after the first fertilization was analysed separately from the period before the first fertilization. Differences in volume-weighted mean nutrient concentrations and fluxes in litter leachate, soil solutions, throughfall and litterfall between the various fertilized and the unfertilized control treatments were analysed by ANOVA using linear mixed effects models. This type of model allowed us to analyse the data set even when several data were missing because of e.g., a lack of sufficient amount of sample to analyse. The treatment was defined as fixed effects and the four plot repetitions as random effects. To evaluate leaching or retention of the different soil nutrients,

attributed to nutrient application, the fertilized treatments were compared with the unfertilized control treatment.

To test differences between mean soil properties, a one-way ANOVA was used with a Dunnett post-hoc test. To test differences between mean LAI, tree diameter growth, leaf morphology and foliar Ca and Al concentrations between the Ca and control treatments, I used a *t*-test for independent data sets. Significance was set at $P \leq 0.05$. All analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA), except statistics of root biomass data (SAS).

3. Results and discussion

3.1. Soil solid phase

Physical (soil texture) and chemical (C:N ratio, total N and total P concentrations, ECEC, BS and pH) parameters did not differ significantly among the treatments prior to the first nutrient application.

Samples of the Oi and Oe horizons, collected two months after the second nutrient application, tended to contain more N in the N and N+P treatments than in the unfertilized control treatment. After three nutrient applications, the P concentrations in the Oe horizon had significantly increased in the P and N+P treatments compared to the unfertilized control treatment, but no significant differences among all treatments existed in the Oi and Oa horizons. The Ca concentrations in the organic layer increased in the Ca treatment after three Ca applications in the Oi and Oe horizons but not (yet) in the Oa horizon. After two nutrient applications, the pH in the forest floor and mineral soil as well as the ECEC and BS in the mineral soil did not differ significantly among the different fertilization and control treatments. The BS in the mineral soil of the Ca treatment did not differ from the control treatment since the leached Ca from the organic layer only accounted for 0.8% of the exchangeable base metal pool in the A horizon.

One factor limiting forest growth might be Al toxicity. Cronan and Grigal (1995) reported in their review several threshold conditions to detect Al stress in forests including (i) soil BS <15% of ECEC, (ii) molar Ca:Al concentration ratio <1 in soil solution and (iii) a molar foliar tissue Ca:Al concentration ratio ≤12.5. After two Ca applications at our study plots, the BS in the A horizon of the Ca treatment was 2.3 ± 1.1%, i.e. clearly <15%. The fact that the majority of the tree roots are located in the thick organic layer might therefore be an adaptation in order to avoid Al toxicity in the mineral soil. Chemical speciation modelling revealed that in the organic layer

almost no free Al^{3+} occurred in solution. The maximum modelled free Al^{3+} concentrations was calculated to be 0.04% of the total Al representing 0.01 μmol l^{-1} Al^{3+}, which is far below the reported toxic Al^{3+} concentration of 1 μmol l^{-1} (Wheeler et al. 1992). The remaining 99.96% of the total Al concentration in litter leachate were organically complexed. According to the speciation calculation neither $Al(OH)^{2+}$ nor $Al(OH)_2^{+}$, which may potentially also be phytotoxic (Marschner 1993), occurred in litter leachate under any of the assumed conditions. At the adjacent study sites between 2000-2003, the mean molar Ca:Al ratio was 50 ± 73, 10 ± 17, 12 ± 17 in litter leachate, soil solution at 0.15 m and soil solution at 0.30 m mineral soil depth, respectively (Boy et al. 2008b), i.e. well above the toxicity threshold value. From the studied most common trees, there were no indications of Al toxicity based on the foliar molar Ca:Al concentration except for *G. emarginata* which had a mean foliar molar Ca:Al concentration ratio of 0.3, i.e. below the threshold value of ≤12.5, due to higher foliar Al concentrations compared to the other analysed species. *Graffenrieda emarginata* belongs to the plant family of Melastomataceae which are known to contain several species that accumulate Al (Cuenca et al. 1990). These results demonstrate that there is little indication of Al toxicity in the organic layer.

3.2. Plants in the Ca and control treatments

For the evaluation of the Ca effects, I could additionally to my own data make use of plant data obtained from the group of Homeier et al. After two Ca fertilization periods, mean living fine root biomass was significantly lower (16.5%) in the Ca than the control treatment. At the same time, mean dead fine root biomass tended to be lower in the Ca treatment but was not statistically significant from the control. This suggests that the trees invested less C (and energy) in fine root mass because the additional Ca improved the growth conditions. The reason for improved growth conditions may be related with the alleviation of Ca deficiency. Decreased fine root necromass is attributable to the relative high fine root turnover (Graefe et al. 2008a, b). Mean root longevity at 1890 m a.s.l. in the study area is 467 ± 67 days (Graefe et al. 2008a).

Graffenrieda emarginata was the most common tree species contributing 28.3% of all trees in the Ca and control treatments having a dbh ≥10 cm. After three Ca applications, 15 months after the first Ca application, the mean cumulative tree diameter increment tended to increase slightly in all species, including three of the four most common tree species (*A. grandiflora, G. emarginata, H. moritziana*). In contrast, the mean cumulative tree diameter increment of *Myrcia* sp. was less in the Ca than the control treatments.

Leaves of the most common tree specie, *G. emarginata*, became significantly thinner since the SLA in the Ca treatment was significantly higher than in the unfertilized control treatment. Thinner leaves are a morphological adaptation to less stress or higher nutrient availabilities (McLaughlin and Wimmer 1999). I interprete this as an indication of improved nutrient availability. As at the same time the living fine root biomass was reduced by Ca addition, it is unlikely that the higher nutrient availability to the plants is associated with a more extensive exploration of the soil. Therefore, it is reasonable to assume that the added Ca improved the nutrient availability which is an indication of Ca deficiency.

The average LA of the four most common tree species (*A. grandiflora, G. emarginata, H. moritziana* and *Myrcia* sp.) was smaller one year after the first Ca application in the Ca than the control treatments but changes were not significant. In the Ca treatment, LA slightly decreased in the four most common tree species indicating that the trees not only reduced their investments in root but also in leaf biomass. Reduced LA might indicate that less water needs to be transpired to satisfy the nutrient demand of the leaves because of the improved nutrient availability. Consequently, more C can be invested in tree growth.

3.3. Litter leachate

I installed zero-tension lysimeters just below the organic layer to determine how much of the applied nutrients would leach from the forest floor. In the litter leachate after three nutrient applications, higher fluxes of total N occurred in the N (significant) and N+P treatments (non-significant after correction for the *a priori* differences), higher fluxes of total P in both the P and N+P treatments (significant) and higher fluxes of Ca in the Ca treatment (non-significant) relative to the unfertilized control treatment. From the applied N, $10.0 \pm 2.9\%$ and $8.0 \pm 2.5\%$ were leached to below the organic layer in the N and N+P treatments, respectively. Only $0.84 \pm 0.62\%$ and $0.84 \pm 0.53\%$ of the applied P leached to below the organic layer in the P and N+P treatments, respectively, and $4.0 \pm 5.1\%$ of the applied Ca leached to below the organic layer in the Ca treatment (Fig. A-3).

Almost all tree roots are located in the organic layer and it is therefore reasonable to assume that elements leaching from the organic layer are no longer plant-available. A maximum of 10% of the applied N, P and Ca leached to below the organic layer and hence, ≥90% was retained in the organic layer and aboveground biomass by adsorption, microbial immobilization

and plant uptake followed by storage in below or aboveground biomass. The most likely explanation for the retention of fertilized P in the organic layer is microbial immobilization, because the C:P ratio in the organic layer was with 1117 ± 146 far above the value of 100 which is considered as initiating strong P immobilization (White 2000). This suggests P limitation of the microorganisms which will be affected by nutrient limitation prior to trees (Kaspari et al. 2008, Kaspari and Yanoviak 2008). Alternatively, P might be precipitated as aluminium phosphate because the organic layer contained a roughly estimated 10% of mineral soil material and stored 768 ± 199 kg ha^{-1} Al although most of the Al will be organically complexed and hence, not available for precipitation as aluminium phosphate. The fact that only 4% of the applied Ca was leached from the organic layer into the mineral soil in spite of the very acid soil reaction indicates that Ca is taken up by the plants and soil organisms and only adsorbed to a small extent in the organic layer.

3.4. Soil solution

Total N fluxes in the soil solutions of the N and N+P treatments did not differ from the control treatment. The P concentrations in mineral soil solution were below the detection limit, probably because of the precipitation of aluminium phosphates and some adsorption to Fe oxides in the studied acid soils. Calcium fluxes in the soil solution did not differ between Ca and control treatments (Fig. A-3).

Nitrogen and Ca leached from the organic layer but not from the mineral soil. Total N stock and exchangeable Ca in the A horizon did not differ in their respective treatments compared to the control treatment. This can be explained by the fact that the total amount of leached N was small compared to the total N stock in the A horizon. Leached N from the organic layer only accounted for 2.0 and 1.8% of the N stock of the A horizon in N and N+P treatments, respectively. The leached Ca from the organic layer even only accounted for 0.8% of the exchangeable base metal pool in the A horizon.

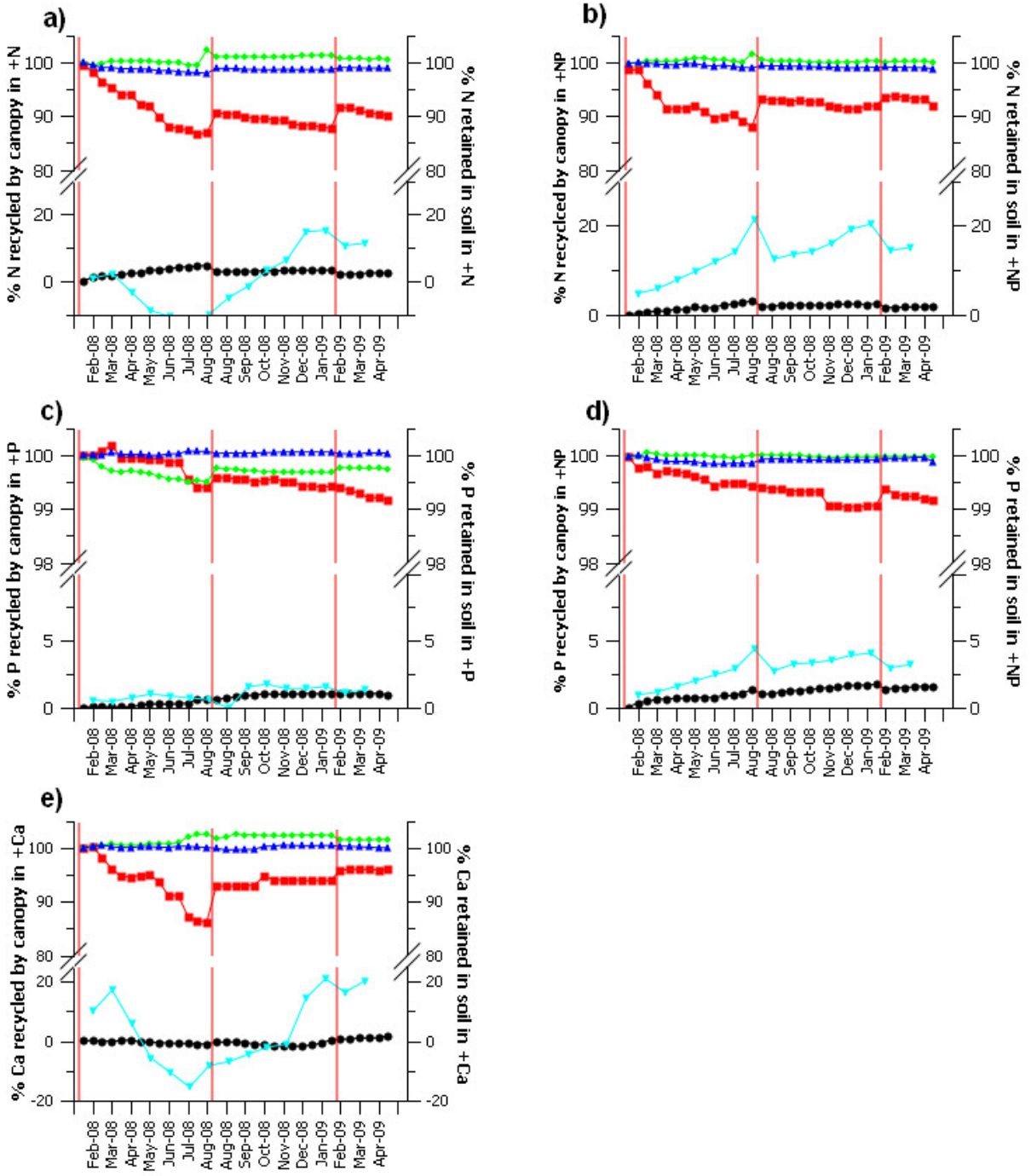

Fig. A- 3: Cumulative losses of N, P and Ca from the canopy with throughfall (●) and litterfall (▼) and cumulative retention of applied N and P in soil; litter leachate (■), soil solution at 0.15 m (♦), soil solution at 0.30 m (▲). (a) N in N treatment, (b) N in N+P treatment, (c) P in P treatment, (d) P in N+P treatment and (e) Ca in Ca treatment. The shown values were calculated from differences between the mean N, P or Ca fluxes in their respective treatments and the mean N, P and Ca fluxes in the unfertilized controls. Fertilizer application dates are indicated by vertical red lines.

3.5. Canopy budget

Nutrient retention in the canopy can be interpreted as an indication of a scarcity of the considered nutrient (Ulrich 1983). To determine if N, P and Ca deposited from the atmosphere were taken up by the canopy, I set up a canopy budget using the model of Ulrich (1983).

Nitrogen deposited from the atmosphere was retained by the canopy. In the unfertilized control treatment, 37; 57 and 38% of total deposited N was retained by the canopy in the first, second and third fertilization periods, respectively. In the N treatment, 31, 38 and 34% of total deposited N were retained by the canopy in the first, second and third fertilization periods, respectively, while this was 35, 56 and 44%, respectively, in the N+P treatment. Differences between N and control treatments were significant for the whole study period, but not between the N+P and control treatments (Fig. A-4a). Nitrogen uptake might partly be attributed to the abundant epiphytes (Fleischbein et al. 2005) that are known to be able to absorb N from deposition (Chuyong et al. 2004). Retention of N deposited from the atmosphere is frequently observed in N-limited forests (Clark et al. 1998, Laclau et al. 2003, Oyarzun et al. 2004).

In all treatments, P was also retained in the canopy. In the unfertilized control treatment, 77, 59 and 49% of total atmospheric deposition was retained by the canopy in the first, second and third fertilization periods, respectively. In the P treatment, 70, 11 and 21% of total deposited P were retained by the canopy in the first, second and third fertilization periods, respectively, while this was 62, 1 and 11% respectively in the N+P treatment (Fig. A-4b).

Similarly as N and P, also Ca was retained from total deposition by the canopy. In the unfertilized control treatment, 42; 61 and 28% of deposited Ca was retained by the canopy in the first, second and third fertilization periods, respectively. In the Ca treatment, 49, 43 and 17% of total deposited Ca were retained by the canopy in the first, second and third fertilization periods, respectively. Overall, there was less Ca uptake (i.e. higher Ca flux in throughfall) in the Ca than the control treatment (Fig. A-4c).

The addition of N, P and Ca to the soil changed the canopy budget. Nitrogen, P and Ca were generally retained in the canopy suggesting a demand of the plants. However, the addition of N, P and Ca to the soil decreased the uptake of these macronutrients by the canopy from total deposition. Obviously, N, P and Ca application to the soil reduced the deficiency of these nutrients because the plants took up more N, P and Ca, respectively, from the soil.

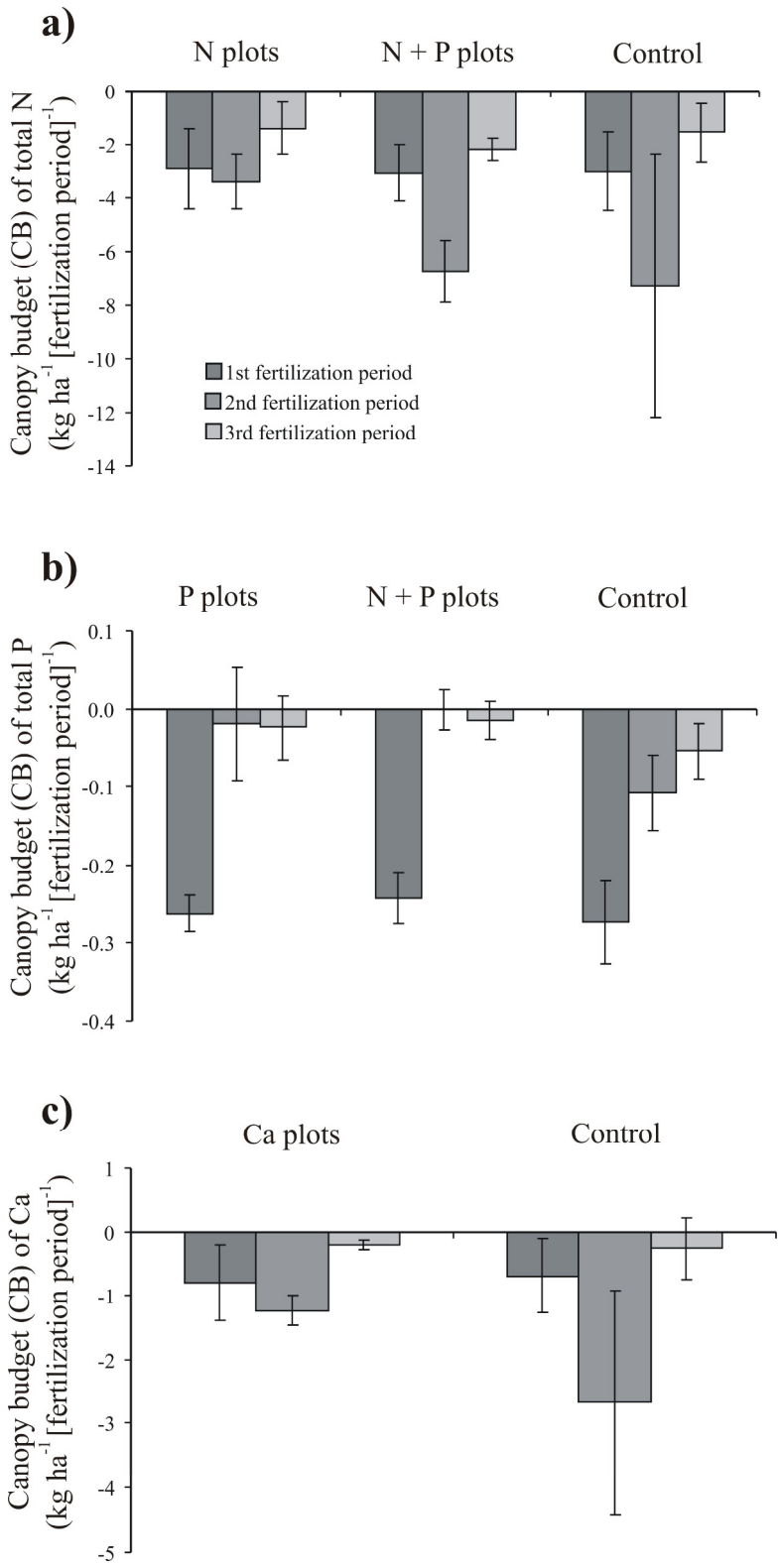

Fig. A- 4: Canopy budget of total N in N, N+P and control treatments (a), total P in P, N+P and control treatments (b) and Ca in Ca and control treatments (c) in the first, second and third fertilization periods.

3.6. Throughfall and litterfall

Not canopy leaching but reduced canopy uptake of total deposited N, P and Ca – in response to fertilizer application – tended to increased N, P and Ca fluxes in throughfall and litterfall in their respective treatments, although total fine litterfall production did not differ among the treatments. This further indicates that trees took up part of the applied N, P and Ca.

After three fertilization periods, the reduced canopy uptake of N resulted in a cumulative extra N flux to the soil with throughfall of 2.5 ± 1.5 and 2.0 ± 1.5% of the applied N in the N and N+P treatments, respectively, compared to the control treatment. An even higher percentage of the applied N (11 ± SD 13 and 15 ± 10%) was returned to the soil with litterfall in the N and N+P treatments, respectively (Fig. A-5a). Increased N fluxes with litterfall were also reported by Campo et al. (2007) after N application.

Reduced canopy uptake of P resulted in a cumulative extra P flux to the soil with throughfall of 1.0 ± 0.4 and 1.6 ± 0.4% of the applied P in the P and N+P treatments, respectively, compared to the control treatment. An even higher percentage of the applied P (1.3 ± SD 2.4 and 3.2 ± 2.2%) was returned to the soil with litterfall in the P and N+P treatments, respectively (Fig. A-5b). A similar observation was reported from a tropical rain forest in Borneo (Mirmanto et al. 1999) and from a P-limited forest in Hawaii (Harrington et al. 2001) where the addition of P and N+P also resulted in increased P fluxes.

Similarly, reduced canopy uptake of Ca resulted in a cumulative extra Ca flux to the soil with throughfall of 1.7 ± 3.5% of the applied Ca in the Ca compared to the control treatment. A total of 20 ± 27% of the applied Ca returned to the organic layer by litterfall (Fig. A-5c).

Since deposited N, P and Ca from the atmosphere were taken up by the canopy, and applied N, P and Ca to the soil were taken up via the roots one might expect elevated concentrations in plant tissues, including leaves. Although, I do not avail direct data of nutrient concentrations in the leaves, elevated N, P and Ca concentrations and fluxes in litterfall support this assumption. The extent of the increase in N, P and Ca concentrations and fluxes in litterfall depends on the nutrient retranslocation before leaf senescence. In line with the review from Parker et al. (1983), litterfall transported higher N, P and Ca fluxes from the canopy to the forest floor than throughfall, which can however, not directly be seen in Fig. A-5 because of the different scalings of the Y axes depicting nutrient fluxes with throughfall and litterfall, respectively.

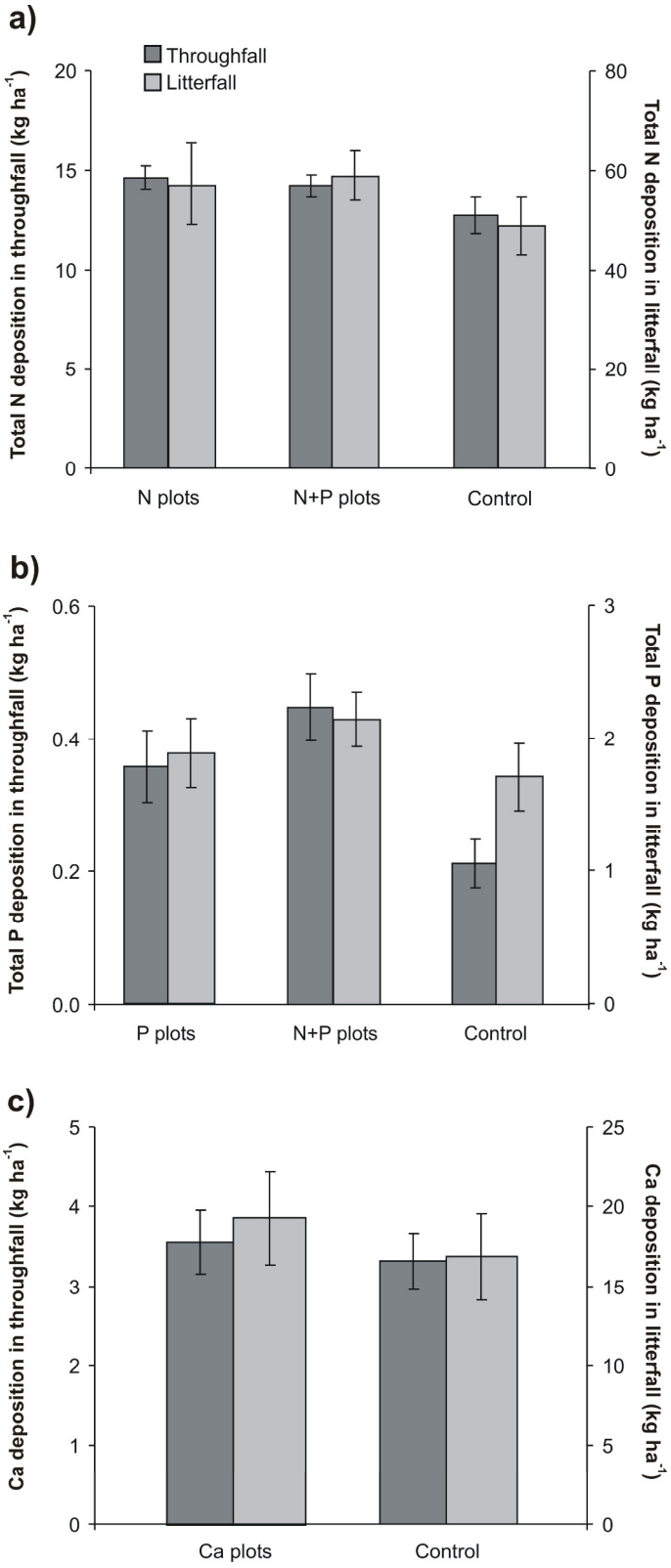

Fig. A- 5: Cumulative flux of total N in N, N+P and control treatments (a), total P in P, N+P and control treatments (b) and Ca in Ca and control treatments (c) to the soil with throughfall and litterfall after three fertilizer applications.

3.7. Error discussion

Field studies always occur under less controllable conditions compared to laboratory experiments. Therefore, the largest part of uncertainties is related to activities in the field. One source of error is that the nutrients were applied based on the plot area in the field (20 x 20m) and not the horizontally projected area. If the horizontally projected area of the research plots were taken into account for the amount of applied nutrients, then one might have more realistic results to predict the effects of changed deposition from the atmosphere in future. On the other hand, if the same fertilizer amount per horizontally projected area was used, each individual plot (which all had a different inclination) would have received different fertilizer amounts. This would have led to more variability among the replicate plots of the same treatment. Furthermore, the fertilizer application based on the horizontally projected area was practically not feasible because of the difficult access and orientation in the plots.

To cope with spatial variability regarding nutrient concentrations on plot scale, I installed as much research equipment as possible per plot including 20 throughfall collectors, three zero-tension lysimeters and three suction lysimeters. The chemical composition of the soil solution extracted with ceramic suction cups could have been influenced by adsorption or desorption of chemical elements to the ceramic cups. According to the supplier (ecoTech Umweltmesssysteme GmbH, Bonn, Germany), suction cups of glass or plastic might have been more suitable for research on phosphate and inorganic components. To avoid adsorption/desorption effects on the chemical composition of the collected soil solution, I applied a continuous vacuum right after installation of the suction cups in the forest to extract soil solution (around five to seven litres) which was discarded during the whole first month. Sampling interval of the solutions was two weeks. I did not add chemicals in toxic concentrations to the sampling collectors to avoid bacterial growth and hence, conversion of N species might have occurred. I therefore only presented concentrations and fluxes of total N and total P. To reduce microbial growth, the samples were kept frozen until transport to Germany for further analysis after collection in the field. During transport to Germany, samples were stored cool at <4 °C and transport did not take longer than one week. In Germany, samples were also kept frozen until analysis. I tried to reduce algae growth as much as possible in field samples by covering every collecting bottle with aluminium foil to avoid light entry. Collectors for soil solution were brown coloured and placed in buckets.

In Appendix A, I assessed the variability of throughfall volume. Since this investigation was related but had a different objective, I included the manuscript as an appendix. In conclusion, to estimate the error in spatial variation, I calculated in appendix A that the spatial variability of throughfall in the studied humid Andean tropical montane forest is high, requiring 20 to 27 throughfall samplers for measurement of annual throughfall volumes with an accepted error of <10% of the annual mean and with a 95% confidence interval in a forest where improvement fellings took place and an undisturbed forest respectively (Wullaert et al. 2009). Twenty samplers in each plot resulted in 400 throughfall collectors in my experiment; more collectors would not have been manageable in such a difficultly accessible forest. Assuming similar throughfall heterogeneity in the NUMEX site compared to the forest where improvement fellings took place and the undisturbed forest, then the error of my throughfall measurement on each plot is likely between 10 and 15% with a 95% confidence interval. Spatial variability of the concentrations of the studied elements in the solutions are likely even higher (Kostelnik et al. 1989, Pucket 1991, Lawrence and Fernandez 1993, Houle et al. 1999, Zimmermann et al. 2007), but were not assessed.

To avoid evaporation and to obtain throughfall volumes as accurate as possible, I placed a table-tennis ball in each funnel of the throughfall collectors and the collecting bottle was wrapped with aluminium foil. From earlier results in the research area, Boy et al. (2008b) calculated that <10% of the total water flux contributed to lateral flow. I therefore assumed it reasonable to use a one-dimensional soil water balance model to calculate water fluxes in the mineral soil. I furthermore assumed water uptake by tree roots from the mineral soil as negligible because of the almost complete lack of roots in the mineral soil. To calculate changes in soil water storage, I had to convert matrix potential into volumetric water content. I therefore determined a soil moisture retention curve with the use of electronic FDR sensors and electronic tensiometers which were installed at only one location (in the centre of the research area). I applied the same soil moisture retention curve across the studied area. Soil water content in each plot was then calculated using this soil moisture retention curve and the matrix potential from a tensiometer in each plot. Before I established the soil moisture retention curve, I calibrated the electronic FDR sensors with soil samples collected from within five meter distance of the FDR sensors and of which I determined the volumetric water content by drying in an oven. This calibration showed that soil water content in the manually collected samples was more variable in space and time than the registered values from the FDR sensors.

The concentrations of the analysed elements were partly low in the solutions. The detection limits of the measured elements are summarized in Table A-1. To assess the precision of my laboratory measurements, I analysed in every laboratory run of about 100 samples an internal reference standard – a throughfall sample – and determined the relative standard deviation (Table A-1). As a consequence of the low concentrations of the studied elements in most solutions, the relative standard deviations of N and Ca concentrations were around 10% and those of P and Cl^- around 20%.

Table A - 1: Detection limits and relative standard deviations of the repeated measurements of an internal standard of the respective elements (CFA: Continuous Flow Analyzer, AAS: Atom Absorption Spectroscopy).

Element	**Analytical device**	**Detection limit ($mg\ l^{-1}$)**	**Relative standard deviation (%)**	**Mean concentration of the internal reference standard ($mg\ l^{-1}$)**
Total N	CFA	0.08	11	3.0
Total P	CFA	0.012	22	0.16
Cl^-	CFA	0.10	21	2.6
Ca	AAS	0.015	11	3.2
K	AAS	0.009	3	19.2
Na	AAS	0.009	27	0.19
Mg	AAS	0.006	5	1.9
TOC	TOC analyzer	0.50	7	11.3

Total N and total P concentrations were determined by oxidation with $K_2S_2O_8$ and UV radiation in the continuous flow analysis. This method may underestimate total N and total P concentrations, particularly if recalcitrant N- or P-containing organic compounds that are not easily oxidizable were present in the solution (Kaiser et al. 2003). However, it can be assumed that the error is similar at least for the same type of samples, so that the comparisons among different treatments are unbiased.

I furthermore tested the purity of the applied fertilizers to make sure not to contaminate the plots with unwanted chemical elements (Table A-2). The P addition with N and Ca fertilizers was higher than the current input by incident rainfall but about 10 times lower than in the P treatment. Thus, even the N and Ca treatments received a low amendment of P – but already higher than the current deposition – which was unavoidable because it was not possible to purchase less contaminated chemicals.

Table A - 2: Yearly input of N, P and Ca with incident rainfall during the measurement period and by addition of fertilizers.

	Total N	**Total P**	**Ca**
		kg ha^{-1} yr^{-1}	
Incident rainfall	15.2	0.18	3.5
N fertilizer: Urea	50	1.13	0.08
P fertilizer: $NaH_2PO_4 \cdot 2H_2O$	0.013	10	<0.001
Ca fertilizer: $CaCl_2 \cdot 2H_2O$	0.038	0.40	10

I used the model of Ulrich (1983) to calculate leaching or retention of chemical elements by the canopy. With this model, dry deposition was estimated using Cl^- as a non-reactive tracer which was confirmed in earlier work at the same study site (Boy et al. 2008a, Boy and Wilcke 2008, Wilcke et al. 2009). For two reasons this estimation can be criticized: (1) the deposition of other elements and compounds might depend on other factors than those controlling Cl^- deposition, and (2) Cl^- ions might be taken up by or leached from the canopy.

4. General conclusions

The forest responded with reduced N retention in the canopy and added N remained in the aboveground part of the studied ecosystem. This can be interpreted as an indication of N limitation. However, there were also strong indications that both other applied nutrients – P and Ca – were in short supply. The added P also was retained in the vegetation and organic layer, probably because of microbial immobilization of P in the organic layer as suggested by the C:P ratio >1000 and plant uptake as illustrated by increased P cycling with throughfall and litterfall in the P and N+P treatments.

Calcium addition resulted in morphological plant changes suggesting reduced nutrient stress which is in support of the hypothesis that Ca was co-limiting plant performance. As the roots are concentrated in the organic layer, where Al toxicity was unlikely because of almost complete organo-complexation, the positive effect of Ca was not related to alleviated Al toxicity. A biological Ca requirement is further supported by the finding that Ca was little leached to below the organic layer in spite of the strongly acid soil reaction which should favour Ca leaching.

The results demonstrate that N, P and Ca are all deficient in the north Andean montane forests resulting in the retention of these nutrients in the ecosystem and for Ca in positive responses of the plant performance. A possible explanation for the simultaneous positive responses of the north Andean montane forest to the addition of all studied nutrients would be the assumption that different ecosystem compartments and even different phenological stages may show different nutrient limitations in line with suggestions of Kaspari et al. (2008) and Hedin et al. (2009). I conclude that (1) the expected elevated N and P deposition in the tropics will be retained in the ecosystem, at least in the short term and hence, quality of river water will not be endangered and (2) increased Ca input will reduce nutrient stress of the forest.

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B Response of the N and P cycles of an old-growth montane forest in Ecuador to experimental low-level N and P amendments *

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1. Abstract

Atmospheric nitrogen (N) and phosphorus (P) depositions are expected to increase in the tropics as a consequence of increasing human activity in the next decades. In the literature, it is frequently assumed that tropical montane forests are N-limited, while tropical lowland forests are P-limited. In a low-level N and P addition experiment, we determined the short-term response of N and P concentrations and fluxes within a north Andean montane forest on Palaeozoic shists and metasandstones at an elevation of 2100 m a.s.l. to increased N and P inputs. We evaluated experimental N, P and N+P additions (50 kg ha^{-1} yr^{-1} of N, 10 kg ha^{-1} yr^{-1} of P and 50 kg + 10 kg ha^{-1} yr^{-1} of N and P, respectively) and an untreated control in a fourfold replicated randomized block design. We collected litter leachate, mineral soil solution (0.15 and 0.30 m depths), throughfall and litterfall before the treatment began and 16 months after the first nutrient applications. Less than 10 and 1% of the applied N and P, respectively, leached below the organic layer which contained almost all roots and no significant leaching losses of N and P occurred to below 0.15 m mineral soil depth. Deposited N and P from the atmosphere in dry and wet form were retained in the canopy of the control treatment using a canopy budget model. Nitrogen and P retention by the canopy were reduced and N and P fluxes in throughfall and litterfall increased in their respective treatments. The increase in N and P fluxes in throughfall after fertilization was equivalent to 2.5% of the applied N and 2% of the applied P. The fluxes of N and P in litterfall were up to 15% and 3%, respectively, higher in the N and N+P than in the control treatments. We conclude that the expected elevated N and P deposition in the tropics will be retained in the ecosystem, at least in the short term and hence, quality of river water will not be endangered. Our results suggest that N and P co-limit the studied tropical montane forest ecosystem on Palaeozoic bedrock.

2. Introduction

Atmospheric nitrogen (N) enters ecosystems in dissolved, particulate or gaseous inorganic and organic forms with rainfall. Furthermore, lightning and biological N_2 fixation convert atmospheric N_2 to reactive N species introduced into the ecosystems (Galloway et al. 2004, 2008). Human activities can alter the N supply to forest soils mainly via elevated N concentrations in rainfall. The greatest future increase in N deposition from the atmosphere to land surface is expected in tropical areas (Galloway et al. 2004, Phoenix et al. 2006). The main reasons for increased N deposition are increasing biomass burning (Da Rocha et al. 2005, Fabian et al. 2005), increasing use of N fertilizers in agriculture and a higher fossil fuel consumption (Galloway et al. 2004, 2008). Increasing N deposition from the atmosphere can lead to soil acidification and consequently reduced availability of phosphorus (P) and base metals (Matson et al. 1999), NH_4^+ and NO_3^- leaching, emissions of NO (which regulates the production of tropospheric ozone and is a precursor of nitric acid) and N_2O (which is a greenhouse gas, Matson et al. 1999, Koehler et al. 2009).

Phosphorus is deposited to ecosystems predominantly (82%) in the form of mineral aerosol dust (Pett-Ridge 2009), but also includes aerosol P from biomass burning (5%) and biogenic materials such as pollen and spores (12%; Mahowald et al. 2005, 2008). Anthropogenic disturbance like slash-and-burn activities can lead to a total P loss of 36 kg ha^{-1} in Amazonia, through combustion and wind erosion (Kauffman et al. 1993). More frequent forest fires are expected in Amazonia because of intensified land clearing (Cochrane and Laurance 2008), which might be further enhanced by climate change (reduced precipitation with subsequent forest dieback, Cochrane and Barber 2009). The emitted P is transported with the trade winds to the Equatorial Andes.

It is frequently assumed that N limits plant production on young soils since N is accumulated from the atmosphere and P limits plant production on old soils, since P gradually becomes unavailable during soil genesis (Walker and Syers 1976). Since tropical lowland forests mainly grow on old soils and tropical montane forests on young soils, Tanner et al. (1998) speculated that lowland forests are P-limited while montane forests are N-limited. Vitousek (1982, 1984) reported that tropical lowland forests – which mainly occur on “old” soils – showed a low N use efficiency whereas the P and calcium (Ca) use efficiencies were high. In contrast, tropical montane forests were frequently characterized by higher N use efficiency. Nutrient use efficiency for trees is defined as the amount of biomass produced per unit of assimilated nutrient.

For practical measurements, this can be approximated as the amount of biomass lost per unit of nutrient lost.

The model of Walker and Syers (1976) was tested and confirmed in fertilization experiments in Hawaii on differently aged soils (Vitousek et al. 1993, Herbert and Fownes 1995, Vitousek and Farrington 1997, Harrington et al. 2001). However, there are a number of studies showing that tropical montane forests on young soils respond to N (Tanner et al. 1990, Tanner et al. 1992, Cavelier et al. 2000) and P fertilization (Tanner et al. 1990, Cavelier et al. 2000) with increased growth. Similar results were reported for a dry tropical lowland forest in Yucatan, Mexico (Campo and Vazquez-Yanes, 2004). In contrast, stem growth in tropical lowland forest of Borneo on old soils did neither respond to N nor to P addition (Mirmanto et al. 1999).

Besides response of growth to fertilizer application, also the response of a number of other biogeochemical processes was reported in the literature. In early- and late-succession dry tropical lowland forests in Mexico, Solis and Campo (2004) observed increased N mineralisation and nitrification after N, P and N+P fertilization. Furthermore, N and P fluxes with litterfall increased, while in the early-succession forest, 15 and 30% of added N and P, respectively, were microbially immobilized (Campo et al. 2007). Campo et al. (2007) concluded that in dry tropical forests, fertilization with N and/or P reduced N and P limitation since they measured increased N and P fluxes to the soil by litterfall after two years in plots treated with N and P, respectively. In a 6 yr-old secondary forest in Amazonia growing on degraded pastures where repeated fires and other N losses occurred, plants took up 20% of 100 kg ha^{-1} applied N and 10% of 50 kg ha^{-1} applied P which was determined as the fraction not recovered in the soil. No changes were detected in soil inorganic N and soil microbial biomass N concentrations, or litter decomposition rates but more than twice as many arthropod individuals (excluding ants) were encountered in P and N+P plots than in the control plots (Davidson et al. 2004).

In summary, the type of nutrient limitation in tropical forests remains unclear and the biogeochemical responses to fertilizer applications are complex. Furthermore, it is possible that different compartments of the forest such as the canopy, the soil organic layer or the mineral soil show different nutrient limitations (Kaspari et al. 2008, Hedin et al. 2009). Recently, Kaspari et al. (2008) showed that in a tropical lowland forest in Panama, N influenced tree reproduction, P and K the decomposition rate of cellulose and P and at least one micronutrient the rate of leaf litter decomposition. In the same forest, Barron et al. (2009) reported that Mo, a cofactor in the N_2-fixing enzyme nitrogenase, limited N_2 fixation by free-living heterotrophic bacteria in soils. In

the Andean tropical montane forest in Ecuador, an incubation experiment showed that Mn, Zn and Ca were immobilized after adding a specific nutrient solution to soils. The mineralisation of S was found to be too slow to satisfy plants needs. The results therefore, suggested that S, Mn, Zn and Ca might contribute to limit plant growth (Wilcke et al. 2002). Boy et al. (2008a) showed that Amazonian forest fires cause the deposition of N and Mn to the Andean tropical montane forest in Ecuador which is retained in the canopy suggesting that both, N and Mn are in short supply to the plants. Furthermore, additional Ca and Mg input into the Andean tropical montane forest by Sahara dust surprisingly changed the Ca and Mg budgets from loss to retention, suggesting that the additionally available Ca and Mg was used by the ecosystem possibly as the consequence of the input of another unknown limiting element which enhanced nutrient uptake in general (Boy and Wilcke 2008). The differential nutrient requirements of the complex mixture of taxa and even ecosystem strata together with the temporal variation in nutrient requirements e.g., during different phenological stages of vascular plants might therefore result in a positive response of tropical forest to the amendment of any essential mineral nutrient.

Our overall goal was to assess short-term effects of elevated N and P depositions on N and P cycling in the Andean tropical montane forest by adding N and P at low levels to an old-growth forest. We hypothesized that (i) the forest is N-limited, that therefore (ii) added N remains in the aboveground cycle between soil organic layer and vegetation while (iii) added P is mainly abiotically retained by adsorption to soil minerals and little recycled in the ecosystem.

3. Materials and methods

3.1. Study area

The studied forest belongs to the Reserva Biologica San Francisco (RBSF) and is located in southern Ecuador on the eastern slope of the Cordillera Real of the Andes (i.e. the eastern cordillera) at an altitude between 2020 and 2120 m above sea level (3°59´S, 79°05´W), in the deeply incised valley of the Rio San Francisco draining to the Amazon. The vegetation at the study site can be classified as “evergreen lower montane forest” according to Homeier et al. (2008). Mean canopy height at the study site is about 12-14 m. The estimated crown radius for canopy trees is between 2-4 m, some of the biggest trees may reach 5-6 m. In the study area, more than 280 tree species have been identified so far with Lauraceae, Melastomataceae and Rubiaceae as the most abundant plant families (Homeier and Werner 2007). *Graffenrieda emarginata* Triana (Melastomaceae) is the most abundant tree species with a diameter at breast

height ≥10 cm. The 4-yr mean annual rainfall (2004-2008) ranged between 2527 ± standard deviation, SD 400 and 2611 ± 397 mm at two rainfall gauging stations (Wullaert et al. 2009). Rainfall has a unimodal distribution with a maximum between April and September and without a pronounced dry season (Fleischbein et al. 2005, 2006). Annual bulk N and P depositions with rainfall were 9.5-10 and 0.64-1.1 kg ha^{-1} yr^{-1} respectively between 1998 and 2003 (Boy et al. 2008a). Mean annual temperature at 1950 m is 15.2 °C. The coldest months are June and July with a mean temperature of 14.4 °C; the warmest month is November with a mean temperature of 16.1 °C (Bendix et al. 2008). The soil is a Stagnic Cambisol (Hyperdystric, Chromic) (IUSS Working Group WRB 2007) developed from Palaeozoic phyllites, quartzites and metasandstones.

3.2. Experimental design

Our study was conducted in the framework of the interdisciplinary NUtrient Manipulation EXperiment (NUMEX). The study site was located on the upper slope near a major ridge and has an average slope of 51% (range: 25% to 84%). The experiment consists of N, P and N+P addition and unfertilized control treatments. Each treatment was fourfold replicated in a randomized block design with the restriction that the unfertilized control treatments were the uppermost located plots and the combined treatment of N+P addition the lowermost located plots in each block to avoid nutrient leaching from fertilized to control plots and of N or P to a plot fertilized with the other element. Three blocks had a north aspect and the fourth block a south-southwest aspect. The location of each plot was selected in a way that the vegetation was representative of the area and similar on all plots. Each plot was 400 m² (20 m x 20 m) large and the distance between the plots was at least 10 m (Fig. B-1). There is no data available about the root radius of canopy trees. Therefore, we cannot fully rule out that roots of trees growing close to the edge of a plot reach another plot.

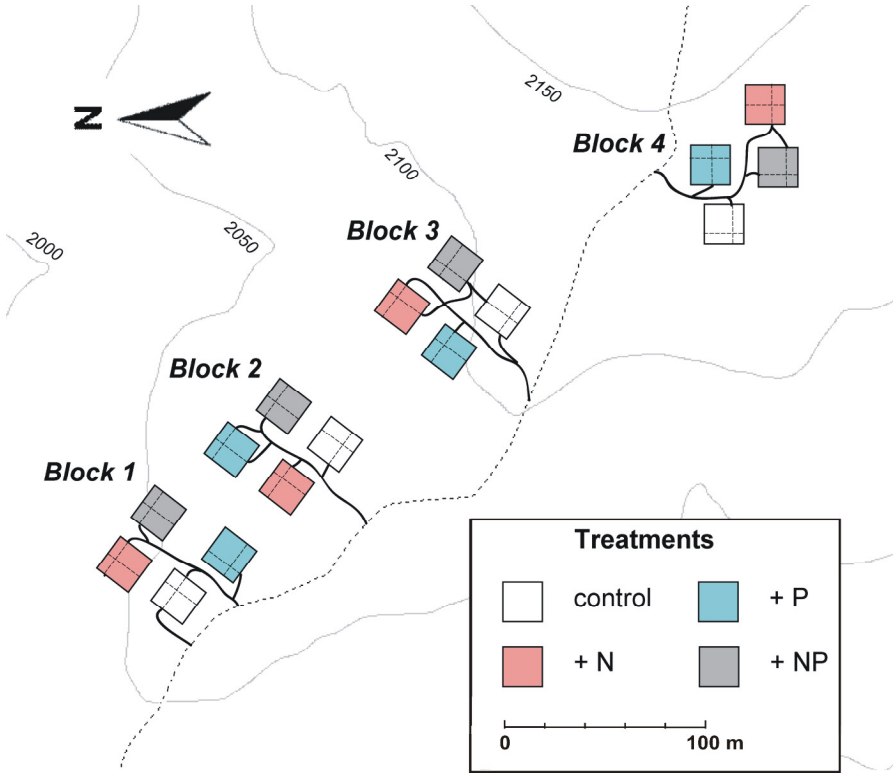

Fig. B- 1: Location of the study sites. Dotted lines within plots represent transects and continuous lines between the plots represent the access roads.

The N, P and N+P addition plots received 50 kg ha^{-1} yr^{-1} of N, 10 kg ha^{-1} yr^{-1} of P and 50 + 10 kg ha^{-1} yr^{-1} of N and P, respectively, split in two applications per year. For security and availability reasons, N was added in the form of commercially available urea. Phosphorus was applied as $NaH_2PO_4 \cdot 2H_2O$ in *pro analysi* quality. Nutrient additions started in January 2008, followed by two further nutrient applications in August 2008 and February 2009. Nitrogen was applied in granular form and P as a salt. The fertilizers were applied manually by four persons walking parallel in strips of 5 m. Throughfall collectors and collecting bottles of litter percolate were covered with plastic bags during nutrient application. The research equipment (except throughfall collectors) was installed in subplots of 2 m x 2 m within the 20 m x 20 m plots. Subplots were located along two perpendicular random transects which were at least 4 m from the plot border so that the subplots were located at minimum 2 m from the plot border to avoid edge effects (Fig. B-1).

3.3. Sampling

3.3.1. Water sampling

Throughfall was collected with 20 fixed-positioned funnel gauges on each plot. Each plot had two transects – parallel to the plot border – and each transect contained 10 throughfall collectors randomly distributed along the transect in a way that the collectors were at least 2 m away from the plot border to avoid edge effects. The funnel gauges consisted of a 2-l polyethylene sampling bottle and a polyethylene funnel with 115 mm diameter. The rims of the funnels were 0.3 m above the soil surface to avoid splash-in effects from the forest floor. The funnel edges were vertical to avoid splash-out (around 0.1 m vertical edge). To reduce evaporation, a table-tennis ball was used in the funnel and the collecting bottle was wrapped with aluminium foil. Throughfall volumes of the 20 collectors per plot were measured with a graduated cylinder in the field and were volume-weighted bulked to result in one sample per plot per collecting date.

Zero-tension lysimeters were used to collect leachate from the organic layer. They were made of plastic boxes with a 0.15 m x 0.15 m collection area and covered with a polyethylene net with a mesh of 0.5 mm. The organic layer remained intact during and after installation. A tube connected the lysimeter to a collection bottle which was wrapped in aluminium foil to reduce the impact of radiation. Three replicates were installed per plot but at each sampling the leachates were bulked to one sample per plot.

Soil solution at 0.15 and 0.30 m mineral depth was collected with suction lysimeters which had a ceramic cup with 1 µm pore size. To produce spatially representative samples, three suction lysimeters were installed per plot at a distance of 0.40 m from each other. The set of three suction lysimeters per plot were connected with tubes to the same collecting bottle so that the collected soil solution was bulked *in situ.* Based on our visual inspection, we estimate that the total fine root fraction in the organic layer is >95% so that the collected soil solution can be considered as from below the rooting zone. The collecting bottles for the soil solution were brown-coloured and were placed in a closed bucket to reduce the radiation impact.

Throughfall, litter leachate and mineral soil solution were sampled fortnightly. After each sampling, we applied a vacuum of -0.6 bar to the suction lysimeters in order to collect sufficient sample for the next sampling period. Sampling of throughfall, litter percolate and soil solution took place from August 2007 until April 2009 of which the first five months were before the first nutrient addition.

3.3.2. Soil and litterfall sampling

Before the first nutrient application in August 2007, we sampled the Oa, A and B horizon of each plot from hand-dug profiles (where we later installed the zero-tension lysimeters) within 2 weeks. After two nutrient applications (October 2008), the Oi, Oe, Oa, A and B horizons were re-sampled with an Edelman auger. At both sampling dates we sampled to a depth of about 0.5 m in the mineral soil. Furthermore, the horizons of the organic layer were sampled in a representative way per plot after the third fertilizer application between February and May 2009 for total P analyses. The soil sampled after the second or third nutrient application was collected from a location within the subplots which we considered as not disturbed with a soil auger.

Litterfall was collected once per month from six litterfall traps (0.60 m x 0.60 m) per plot from October 2007 until March 2008. From April 2008 on, only two out of the six litterfall traps were sampled where the litterfall remained only seven days in the field. The two litterfall traps were randomly chosen. Samples of the pre-treatment months were bulked to one sample per plot. After the first nutrient application, samples from subplots were bulked per plot and per month for further analyses.

3.4. Physical and chemical analyses

Bulk density was measured by the soil core method (Blake and Hartge 1986) at four soil profiles. After field collection, throughfall, litter leachate, and soil solution samples were transported to our field laboratory where pH (Sentix HWS, WTW GmbH, Weilheim, Germany) was immediately measured in an aliquot of each sample. Another aliquot was filtered (ashless filters with pore size 4-7 µm, folded filter type 389; Munktell & Filtrak GmbH, Bärenstein, Germany) and frozen until transport to Germany for further analysis. During transport to Germany, samples were stored cool at <4 °C and transport did not take longer than one week. Water samples were analysed for concentrations of total dissolved N (the sum of NO_3^-, NH_4^+ and organic N in solution and is further referred to as total N), total dissolved P (the sum of PO_4^{3-} and organic P in solution and is further referred to as total P) and dissolved Cl^- using continuous flow analysis (CFA, Bran+Luebbe GmbH, Norderstedt, Germany).

Soil and litterfall samples were dried at 40 °C to constant mass after field collection. Dried litterfall samples and samples of the organic layer were ground with a ballmill at 650 rpm for 10 min. Dried mineral soil samples were sieved (<2 mm) before further analysis. We determined total C and N concentrations in an aliquot of soil and litterfall samples with an

Elemental Analyzer (vario EL III, Elementar Analysensysteme, Hanau, Germany). Another aliquot of litterfall samples were digested with 65% HNO_3 under pressure in a microwave system (Mars Xpress, CEM, Kamp-Lintfort, Germany) to determine the total P concentrations. Soil pH was measured in a soil:deionized water mixture (ratios of 1:10 and 1:2.5 for the organic horizons and mineral soil, respectively). Mineral soil texture was determined with the pipet method (DIN 19683, 1973). Total P concentrations in soil and litter extracts were determined with CFA.

3.5. Quality control of chemical analyses

To assess the precision of the analysis of N and P, we analysed the N and P concentration of an internal reference standard (a throughfall sample from our field site in Ecuador) in every laboratory run of about 100 samples. The relative standard deviations of the reference standard were 9 and 20% for the measurements of total N and P concentrations respectively. The relative standard deviations of P were relatively high because the P concentration in the reference solution – similar to many other samples – was close to the detection limit of 0.012 mg l^{-1}.

We furthermore tested the purity of the applied chemicals to make sure that we did not contaminate the plots with unwanted chemical elements (Table B-1). The N concentrations in the P fertilizer were more than 1000 times lower than the input by incident rainfall or the input due to the addition of the N fertilizer. Total P input via urea was in the same range as the input by incident rainfall but was about 10 times lower than in the P treatment and therefore does not interfere with our experimental setup.

Table B - 1: Annual input of N and P by incident rainfall and input of N and P due to addition of fertilizers.

	Total N	Total P
	kg ha^{-1} yr^{-1}	
Incident rainfall (kg ha^{-1} yr^{-1} , this study)		
Bulk deposition	15.2	0.18
Dry deposition	1.7	0.0
Incident rainfall (kg ha^{-1} yr^{-1}) [a]		
Bulk deposition	9.5 – 10	0.64 - 1.1
Dry deposition	2.3 – 8.4	0.17 – 0.94
Dry deposition during biomass burning events in the Amazon	11-19	0.73 – 2.9
Urea	50	1.13
$NaH_2PO_4 \cdot 2H_2O$	0.01	10

[a] Boy et al. (2008a); same research area, period 1998-2003.

3.6. Meteorological data

For the calculation of reference evapotranspiration, we used meteorological data obtained from the automatic meteorological station on a clear-cut area in ridge-top position at 1950 m a.s.l. Air temperature was measured at a height of 2 m above soil surface with an electronic sensor (Pt100). Relative air humidity was measured with a hygrometer at 2 m above soil surface. Wind velocity was measured with a four-cup anemometer at 2.5 m above soil surface. Total radiation was measured with a pyranometer at 2 m above soil surface with a sensor after WMO and ISO 9060 standards. Rainfall was measured with a tipping-bucket funnel gauge. All meteorological data were recorded with measuring intervals of 1 h and stored with a data logger (Logger DL 15).

3.7. Hydrological calculations

Leaching losses from the organic layer (LO) were calculated using a one-dimensional soil water balance model from throughfall (Th), transpiration (Tr) and change in water storage of the organic layer (ΔS_O; t_2-t_1) (Eq. B-1).

$$LO = Th - Tr - \Delta S_O \quad \text{(B-1)}$$

More details are described in DVWK (1996) and Oelmann et al. (2007). Transpiration losses were determined as the difference between reference evapotranspiration and interception losses. For the calculation of transpiration, we assumed direct evaporation from the soil as negligible. Reference evapotranspiration was calculated with REF-ET (University of Idaho and Dr. R.G. Allen; version 2.0) using the ASCE Penman-Monteith method based on daily mean wind speed, air humidity and irradiation, daily precipitation sum and daily minimum and maximum temperature. Interception losses were directly measured as the difference between incident rainfall and throughfall. We used frequency domain reflectometry (FDR) probes to calculate change in water storage in the organic layer and the mineral soil. FDR probes were installed at 0.10, 0.20, 0.30 and 0.40 m mineral soil depth and one in the organic layer. No upward flux from the mineral soil to the organic layer was allowed (Eq. B-2) and hence,

$$Tr > Th - \Delta S_O; \text{ then } LO = 0 \quad \text{(B-2)}$$

leaching losses from the organic layer were used as an input for the calculation of the leaching losses of the mineral soil (LM) (Eq. B-3)

$$LM = LO - \Delta S_{M0.15 \text{ or } 0.30} \quad \text{(B-3)}$$

where $\Delta S_{M0.15 \text{ or } 0.30}$ is the change in water storage of the mineral soil (0-0.15 or 0.15-0.30 m depth). To determine the soil water content per plot we used data of tensiometers which were installed in each plot close to the suction lysimeters and data of electronic FDR and electronic tensiometer sensors which were installed in the middle of the whole research site.

We assumed that water uptake from the mineral soil was negligible because of the almost complete lack of roots in the mineral soil. From earlier results in our research area, Boy et al. (2008b) calculated that <10% of the total water flux contributed to lateral flow. We therefore considered it reasonable to use a one-dimensional soil water balance model to calculate water fluxes in the mineral soil. Nutrient fluxes were calculated as the product of fortnightly water fluxes and nutrient concentrations of the respective flux type.

3.8. Canopy budget

To set up the canopy budget (CB) of an element *i*, we used the model of Ulrich (1983), where CB is calculated as the difference between throughfall deposition (TFD) and total deposition (TD, Eq. B-4).

$$CBi = TFDi - TDi \quad \text{(B-4)}$$

We did not include stemflow in our experimental setup since in most tropical forests including the north Andean montane forest, stemflow does not exceed 2% of rainfall (Lloyd and Marques 1988, Wilcke et al. 2001, Lilienfein and Wilcke 2004, Fleischbein et al. 2006). Positive values of CB indicate leaching, negative ones uptake of an element *i* by the canopy. Our estimate of CB includes an unknown contribution of dry gaseous deposition of N. If N is taken up by the canopy, then uptake is underestimated by the unaccounted gaseous deposition. Total deposition (TD) of an element *i*, was calculated with Eq. B-5:

$$TDi = BDi + DDi \quad \text{(B-5)}$$

where bulk deposition (BD) refers to bulk rainfall deposition. Dry deposition (DD) was estimated with Eq. B-6.

$$DDi = [(TFD_{Cl} / BD_{Cl}) \times BDi\ ] - BDi \quad \text{(B-6)}$$

In Eq. B-6, TFD_{Cl} represents the throughfall deposition and BD_{Cl} the bulk deposition of Cl^-. It is assumed that Cl^- is a non-reactive tracer which was confirmed in earlier work at the same study site (Boy et al. 2008a, Boy and Wilcke 2008, Wilcke et al. 2009). Chloride concentrations were volume-weighted averaged for the periods between the first and the second, the second and the third and after the third fertilization application to reduce the influence of measurement uncertainty of Cl^- on the canopy budget calculations.

3.9. Statistical evaluation

For the time period before the first nutrient application we tested if there were *a priori* differences of the later fertilized plots and the unfertilized control plots with respect to nutrient concentrations in throughfall, litterfall, litter leachate, soil solution and soil. The period after the first nutrient application was analysed separately from the period before the first nutrient application. Differences in volume-weighted mean nutrient concentrations and fluxes in throughfall, litterfall, litter leachate and soil solutions between the various fertilized and the unfertilized control treatments were analysed by ANOVA using linear mixed effects models. This type of model allowed us to analyse the data set even when several data were missing because of

e.g., a lack of sufficient amount of sample to analyse. The treatment was defined as fixed effects and the four plot repetitions as random effects. To evaluate leaching or retention of the different soil nutrients, attributed to nutrient application, the fertilized treatments were compared to the unfertilized control treatments. The Kolmogorov-Smirnov test was used to test normality of our datasets. To test differences between mean soil properties, a one-way ANOVA was used with a Dunnett post-hoc test. Significance was set at $P \leq 0.05$. All analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

4. Results

4.1. Soil solid phase

The thickness of the whole organic layer averaged 0.30 m (range: 0.15 – 0.60 m) at all study plots before the start of fertilizing to which the Oi and Oe horizons had a minor contribution (Table B-2). The thickness of the A horizon averaged 0.16 m (range: 0.05 – 0.37 m) at all study plots. Physical (soil texture) and chemical (C:N ratio, total N and P concentration, ECEC, BS and pH) parameters did not differ significantly among the treatments prior to the first nutrient application.

Samples of the Oi and Oe horizons, collected two months after the second nutrient application, tended to contain more N in the N and N+P plots than in the unfertilized controls ($P = 0.05$ and 0.09, respectively, in the Oi and 0.13 and 0.11, respectively, in the Oe horizon). The C concentrations of all soil horizons in fertilized treatments remained similar to those in the unfertilized control treatment. Consequently, the C:N ratio were smaller in the N and N+P than in the unfertilized control treatments ($P = 0.09$ and 0.10 in the N and N+P treatment respectively, for the Oi horizon and 0.05 and 0.03 in the N and N+P treatment, respectively, for the Oe horizon). In contrast to the organic layer, there was no change in the C:N ratio of the mineral soil after the second nutrient application. The P concentrations in the Oe horizon increased significantly ($P < 0.001$ for both treatments) after three nutrient applications in the P and N+P treatments compared to the unfertilized control treatments. The Oi and Oa horizons however did not show significant differences among all treatments.

Table B - 2: Selected soil properties of the different treatments (means ± SD, *n* = 4) determined in October 2008, two months after the second nutrient application unless otherwise stated.

Characteristic	Unfertilized control plots	N addition plots	P addition plots	N + P addition plots
		Organic layer		
		Oi horizon		
Thickness (m)	0.019 ± 0.006	0.016 ± 0.002	0.018 ± 0.008	0.022 ± 0.004
pH range (1:10 H_2O)	4.52 – 5.07	4.83 – 5.18	4.80 – 4.85	4.82 – 5.42
Total C (mg g^{-1})	475 ± 4	475 ± 3	477 ± 7	465 ± 23
Total N (mg g^{-1})	14.1 ± 1.4	16.8 ± 0.8 *	13.7 ± 1.9	16.5 ± 1.5
C:N ratio	34.0 ± 3.2	28.3 ± 1.4	35.3 ± 4.7	28.4 ± 2.8
Total P (mg g^{-1}) [a]	0.27 ± 0.04	0.24 ± 0.03	0.34 ± 0.07	0.32 ± 0.06
		Oe horizon		
Thickness (m)	0.036 ± 0.012	0.046 ± 0.010	0.041 ± 0.010	0.050 ± 0.024
pH range (1:10 H_2O)	3.92 – 4.48	4.21 – 4.63	4.02 – 4.52	4.21 – 4.94
Total C (mg g^{-1})	475 ± 4	474 ± 11	434 ± 45	471 ± 6
Total N (mg g^{-1})	17.0 ± 1.3	19.2 ± 1.3	16.0 ± 2.4	19.3 ± 0.5
C:N ratio	28.0 ± 2.1	24.8 ± 1.7 *	27.4 ± 2.3	24.4 ± 0.9 *
Total P (mg g^{-1}) [a]	0.41 ± 0.06	0.47 ± 0.04	0.62 ± 0.05 *	0.65 ± 0.06 *
		Oa horizon		
Thickness (m)	0.22 ± 0.06	0.20 ± 0.02	0.23 ± 0.06	0.26 ± 0.04
pH range (1:10 H_2O)	3.35 – 3.91	3.53 – 3.66	3.40 – 3.65	3.46 – 3.70
Total C (mg g^{-1})	462 ± 17	461 ± 8	473 ± 4	457 ± 18
Total N (mg g^{-1})	19.6 ± 1.5	20.5 ± 1.0	20.5 ± 2.0	21.7 ± 1.9
C:N ratio	23.6 ± 1.3	22.5 ± 0.8	23.2 ± 2.4	21.2 ± 1.8
Total P (mg g^{-1}) [a]	0.44 ± 0.03	0.48 ± 0.05	0.46 ± 0.01	0.51 ± 0.07

Table B - 2: Continued.

		Mineral soil		
		A horizon		
Thickness (m)	0.17 ± 0.06	0.15 ± 0.04	0.14 ± 0.06	0.17 ± 0.04
pH range (1:2.5 H_2O)	3.67 – 3.81	3.69 – 3.82	3.68 – 3.85	3.69 – 3.81
Total C (mg g^{-1})	14.1 ± 0.3	16.8 ± 2.5	19.5 ± 3.8	16.8 ± 2.7
Total N (mg g^{-1})	0.98 ± 0.08	1.20 ± 0.15	1.26 ± 0.22	1.17 ± 0.05
C:N ratio	14.4 ± 1.2	13.9 ± 0.9	15.4 ± 0.8	14.3 ± 2.4
Coarse sand (%) [b]	0.77 ± 0.27	0.76 ± 0.13	1.03 ± 0.87	0.68 ± 0.45
Medium sand (%) [b]	8.0 ± 1.9	6.9 ± 2.0	6.4 ± 2.6	5.8 ± 2.8
Fine sand (%) [b]	27.0 ± 3.1	23.0 ± 3.3	24.7 ± 8.2	21.2 ± 7.7
Silt (%) [b]	49.3 ± 3.0	51.9 ± 2.0	48.9 ± 11.3	53.6 ± 7.8
Clay (%) [b]	14.9 ± 3.3	17.4 ± 3.3	18.9 ± 2.2	17.5 ± 3.4
		B horizon		
pH range (1:2.5 H_2O)	4.01 – 4.20	3.95 – 4.13	4.10 – 4.17	4.08 – 4.25
Total C (mg g^{-1})	7.42 ± 2.46	7.18 ± 2.16	7.62 ± 1.40	7.64 ± 2.98
Total N (mg g^{-1})	0.67 ± 0.18	0.75 ± 0.18	0.70 ± 0.1	0.67 ± 0.32
C:N ratio	11.0 ± 1.6	9.6 ± 0.8	10.9 ± 1.5	11.7 ± 1.8
Coarse sand (%) [b]	0.79 ± 0.72	1.30 ± 1.21	0.27 ± 0.16	0.50 ± 0.33
Medium sand (%) [b]	6.3 ± 1.6	5.5 ± 2.5	5.1 ± 4.3	4.5 ± 2.8
Fine sand (%) [b]	22.5 ± 4.7	17.0 ± 7.0	20.4 ± 10.4	15.0 ± 6.8
Silt (%) [b]	55.2 ± 6.1	57.2 ± 7.9	55.9 ± 11.9	61.0 ± 4.4
Clay (%) [b]	15.3 ± 1.6	18.9 ± 2.3	18.4 ± 5.6	18.9 ± 5.4

[a] Determined after three nutrient applications.

[b] Determined on samples before first nutrient application.

***** Means differed significantly from unfertilized control treatment (Dunnett-T post-hoc test; $P \leq 0.05$)

4.2. Water fluxes

There were no significant differences in water fluxes among the different treatments for the whole observation period. We also did not observe any differences in water fluxes when the 5-month period before the first fertilization and each period after the three fertilizer applications were evaluated separately (results not shown). Figure B-2 summarizes the mean water fluxes of all study plots.

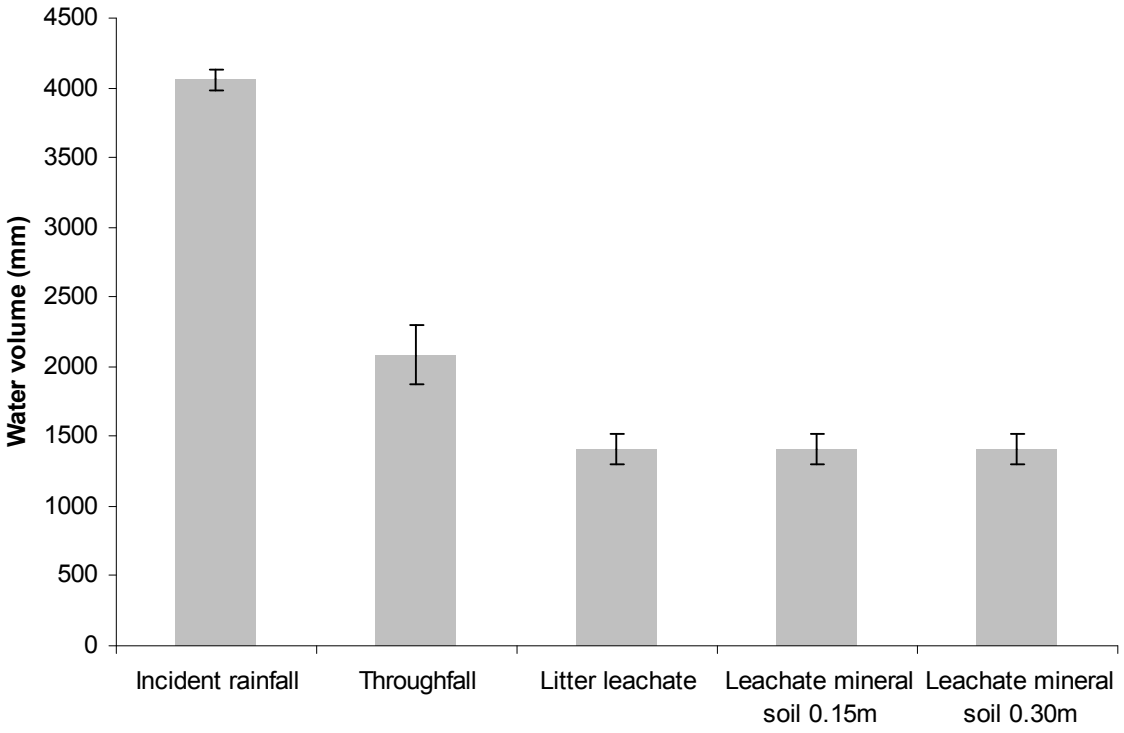

Fig. B- 2: Water budget during the study period (19/08/2007 – 25/04/2009). Error bars represent standard deviation.

4.3. Effect of N addition on N concentrations and fluxes

For most weeks, the N flux in litter leachate from the control plots was greater than the N flux in throughfall from same control plots. In general, N flux in the soil solution at the 0.15 and 0.30 m depths was similar and smaller than in throughfall and litter leachate. We did not detect seasonal trends in the N fluxes in the control treatment (Fig. B-3). Before nutrient application, N concentrations and fluxes in throughfall, litter leachate, soil solution and litterfall did not differ among the different fertilized and the control treatments except for total N concentrations in the soil solution at 0.30 m soil depth in the N plots and total N fluxes in litter leachate in N+P plots. To account for these *a priori* differences we multiplied the respective N concentration or flux

with the ratio of the mean N concentration or flux of the control to that of the fertilized treatments. After three nutrient applications, significantly higher total N concentrations occurred in the litter leachate of the N ($P < 0.001$) and the N+P treatments ($P < 0.001$). This resulted in significantly higher total N fluxes in the N treatments ($P = 0.005$), while total N fluxes in the N+P treatments were not significantly different ($P = 0.54$) from the control treatment after correction for the *a priori* differences. Consequently, 10 ± SD 2.9% and 8.0 ± 2.5% of the applied N were leached from the organic layer in the N and N+P treatments, respectively, implying a loss from the rooting zone.

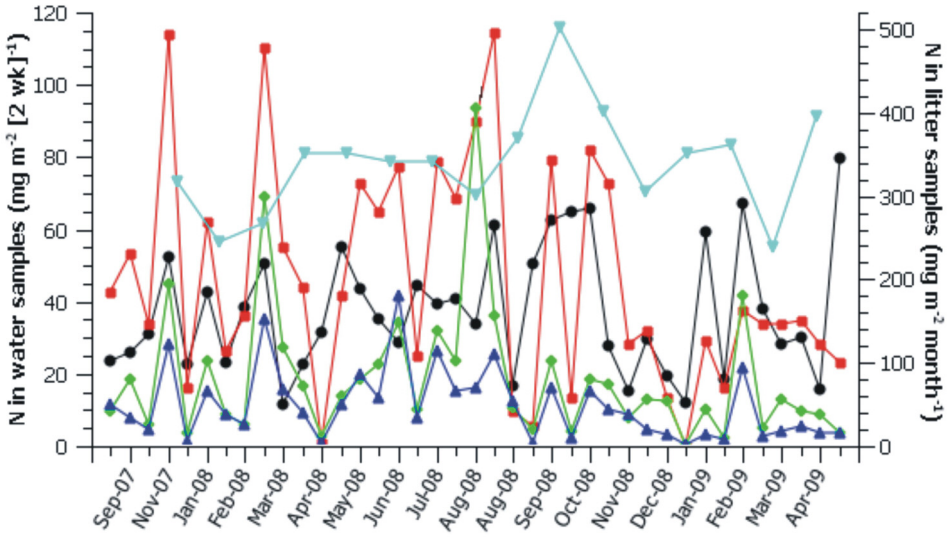

Fig. B- 3: Total N fluxes with throughfall (●), litter leachate (■), soil solution at 0.15m (♦), soil solution at 0.30m (▲) and litterfall (▼) in the control treatment.

The N concentrations in soil solution at 0.30 m mineral soil depth of the N+P treatments were significantly higher ($P = 0.043$) and in the N treatments significantly lower ($P = 0.036$) than in the unfertilized control treatments. Total N fluxes in the soil solutions did not differ significantly between the N and the unfertilized control treatment or between the N+P and the unfertilized control treatment after correction for the *a priori* differences.

In N and N+P treatments, N deposited from the atmosphere (TD_N, i.e. measured bulk deposition + estimated dry deposition) was retained by the canopy (i.e. the canopy budget CB_N was negative). In the unfertilized control treatment, 37, 57 and 38% of TD_N were retained by the

canopy in the first, second and third fertilization periods, respectively. In the N treatment, 31, 38 and 34% of TD_N were retained by the canopy in the first, second and third fertilization periods, respectively, while this was 35, 56 and 44%, respectively, in the N+P treatment. This indicates that the canopy uptake of N was reduced in N applied plots (Fig. B-4). The differences between N treatment and control treatment for the whole study period was significant ($P = 0.039$), but that between N+P treatment and control treatment was not ($P = 0.9$). This illustrates that N application tended to increase net througfall (i.e. decrease N uptake in the canopy) in N and N+P treatments (Fig. B-5). After three fertilization periods, the reduced canopy uptake of N resulted in a cumulative extra N flux to the soil with throughfall of 1.9 ± SD 1.1 and 1.5 ± 1.1 kg in the N and N+P treatments, respectively, compared to the control treatments, which is equivalent to 2.5 ± 1.5 and 2.0 ± 1.5 %, respectively, of the applied N. A higher percentage of the applied N (11 ± SD 13% and 15 ± 10%) was returned to the soil with litterfall in the N and N+P treatments, respectively (Fig. B-5). The jumps in the lines of figure B-5 are a consequence of the fact that immediately after the application of more fertilizer, we switched the basis to which the percentage refer to the double and triple of the first fertilizer application rate and are thus a mathematical artifact.

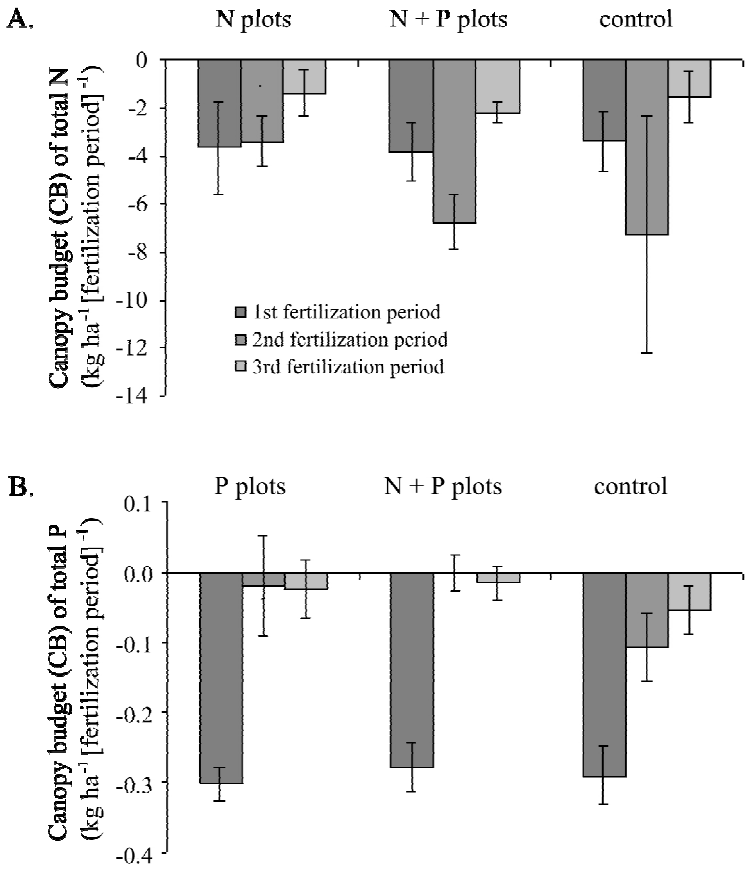

Fig. B- 4: Canopy budget (CB) of (A) total N and (B) total P for the three fertilization periods. Negative values of CB indicate net retention in the canopy. Error bars represent standard deviation.

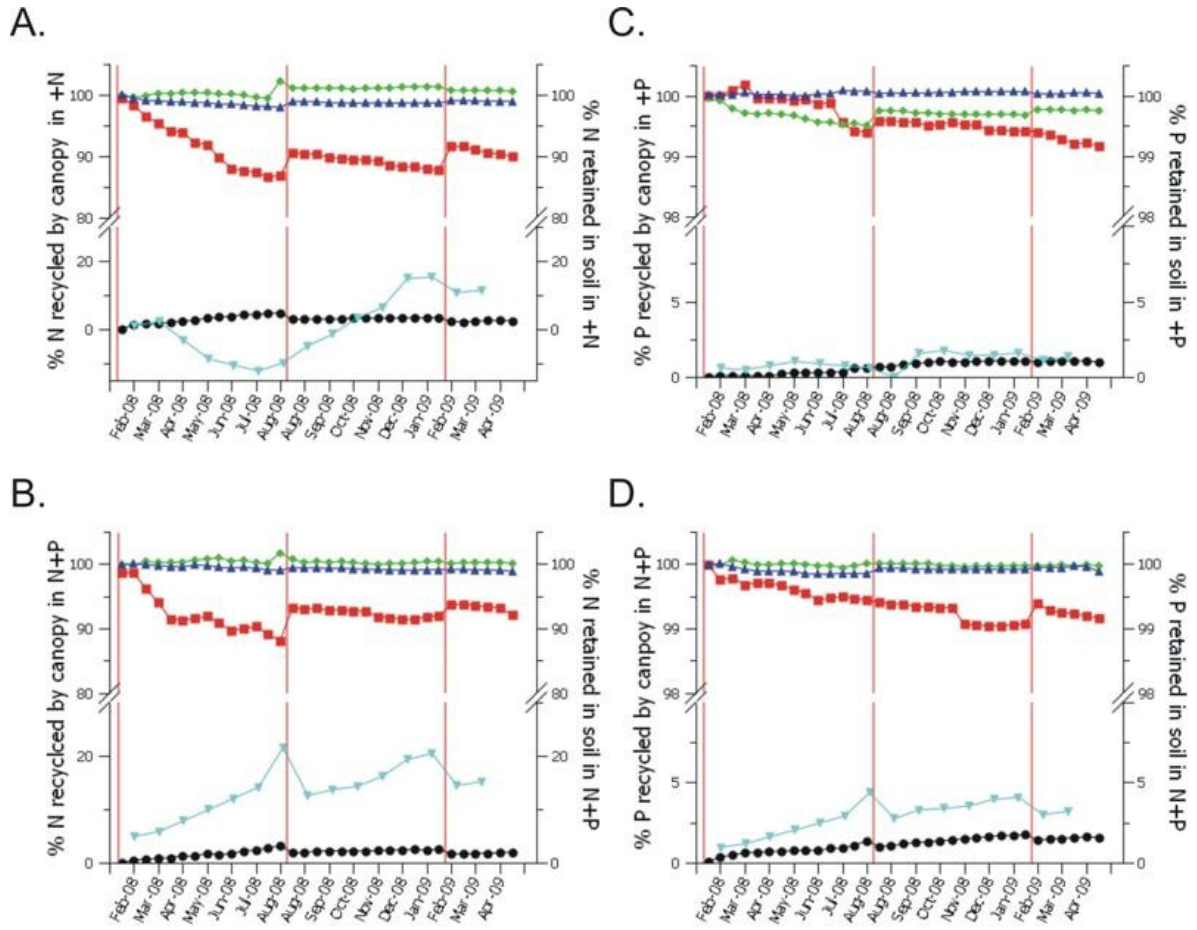

Fig. B- 5: Cumulative losses of N or P from the canopy with throughfall (●) and litterfall (▼) and cumulative retention of applied N and P in soil: litter leachate (■), soil solution at 0.15 m (♦), and soil solution at 0.30 m (▲). (A) N in N plots, (B) N in N+P plots, (C) P in P plots, (D) P in N+P plots. The shown values were calculated as differences between fertilized and unfertilized control treatments. Fertilizer application dates are indicated by vertical red lines.

Total fine litterfall production in the N (4.8 ± SD 1.3 t ha^{-1} yr^{-1}) and N+P (4.9 ± 0.4 t ha^{-1} yr^{-1}) treatments (for the whole observation period) was not significantly different from that of the control treatment (4.2 ± 1.1 t ha^{-1} yr^{-1}). This was also true when the period before the first fertilization and the three periods after the fertilizer applications were evaluated separately (results not shown). Concentrations and fluxes of N in litterfall and N use efficiency did not change significantly after N addition in the N treatments. However, the mean N concentrations in litterfall of the tree fertilization periods were 4 and 6% higher in the N and N+P treatments, respectively, than in the control treatment. In the N+P treatments, mean total N flux in litterfall of

the three fertilization periods was significantly higher (P = 0.010, than in the unfertilized control treatments; Fig. B-5). Tree diameter growth increased after addition of N+P but the increase was not significant in the first year after fertilization started. The N treatment showed a small decrease in plot basal area growth compared to the control (own unpublished data).

4.4. Effect of P addition on P concentrations and fluxes

Before nutrient application, P concentrations and fluxes in throughfall, litter leachate and litterfall did not differ significantly among the fertilized and unfertilized control treatments. The total P concentrations in mineral soil solution were below the detection limit of 0.012 mg l^{-1}, probably because of the precipitation of aluminium phosphates in the studied acid soils. After three fertilizer applications, total P concentrations (P = 0.013 and 0.012) and fluxes (P = 0.023 and 0.049) in litter leachate were significantly higher in the P and N+P treatments, respectively, than in the unfertilized control treatments (Fig. B-5 & B-6). Nevertheless, after three fertilizer application periods, only 0.8 ± SD 0.6% and 0.8 ± 0.5% of the applied P was leached from the organic layer in the P and N+P treatments, respectively and hence, left the rooting zone.

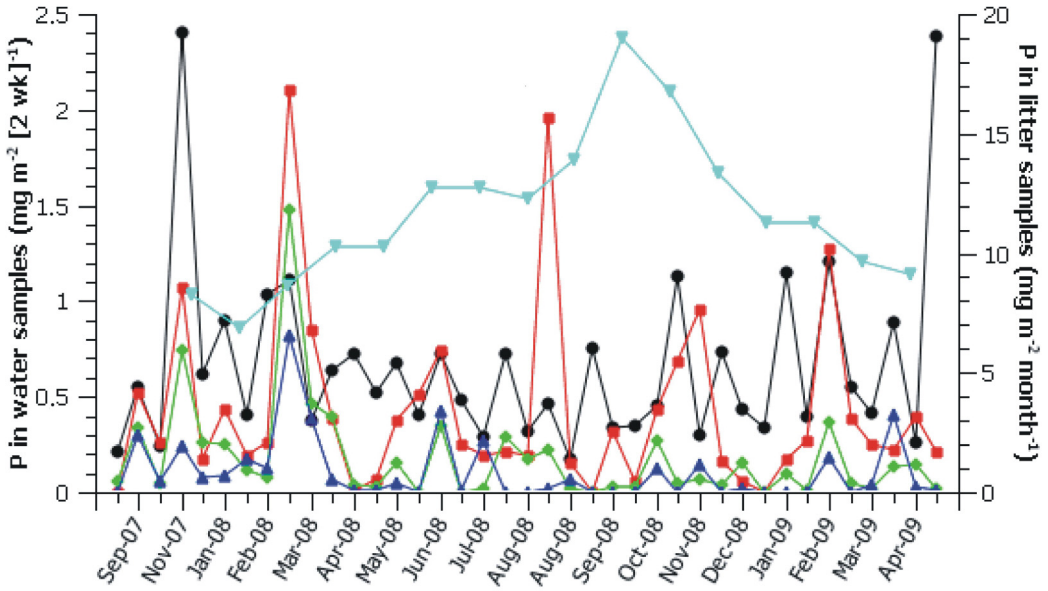

Fig. B- 6: Total P fluxes with throughfall (●), litter leachate (■), soil solution at 0.15 m (♦), soil solution at 0.30 m (▲) and litterfall (▼) in the control treatment.

In all treatments, CB_P was negative (i.e. P was retained in the canopy). In the unfertilized control treatment, 77, 59 and 49% of TD_P were retained by the canopy in the first, second and third fertilization periods, respectively. In the P treatment, 70, 11 and 21% of TD_P were retained by the canopy in the first, second and third fertilization periods, respectively, while this was 62, 1 and 11% respectively in the N+P treatment. This indicates that, similar to N, the canopy uptake of P reduced in P-applied plots (Fig. B-4). Although the differences in mean CB_P of the P and the unfertilized control treatment were not significant for any fertilization period, the reduced canopy uptake of P resulted in significantly increased throughfall P concentrations (P = 0.041 and < 0.001) and fluxes (P = 0.025 and < 0.001; Fig. B-5) of the P and N+P treatments, respectively. After three fertilization periods, the reduced canopy uptake of P resulted in a cumulative extra P flux to the soil with throughfall of 0.15 ± SD 0.07 kg and 0.24 ± 0.06 kg in the P and N+P treatments, respectively, compared to the control treatment, which is equivalent to 1.0 ± 0.4% and 1.6 ± 0.4%, respectively, of the applied P. A higher percentage of the applied P (1.3 ± SD 2.4% and 3.2 ± 2.2%, respectively) was returned to the soil with litterfall in the P and N+P treatments.

Similar to the N and N+P treatments, the total fine litterfall production (3.9 ± 0.9 t ha^{-1} yr^{-1}) did not differ significantly between the P and the control treatments. Mean P concentrations in litterfall of the three fertilization periods were 18 and 11% higher in the P and N+P treatments, respectively, than in the control treatments. The P concentrations in litterfall were only significantly higher in the P than the control treatments (P = 0.044), while the P flux with litterfall was only significantly higher in the N+P than the control treatments (P = 0.037). Furthermore, the mean P use efficiency of the three fertilization periods was lower in the P and N+P than the control treatments (P = 0.050 and 0.055, respectively). The P treatment showed a small but not significant increase in plot basal area growth compared to the control (own unpublished results).

5. Discussion

5.1. Impact of enhanced N depositions

Studies in which low rates of N (around 50 kg ha^{-1} yr^{-1}) are added to tropical forest ecosystems to mimic future ambient N depositions as predicted by Galloway et al. (2004) and Phoenix et al. (2006) are scarce. We are only aware of one report of the effects of slightly elevated N depositions from south China where under low N additions an old-growth forest exhibited larger N losses than younger forests (Fang et al. 2008, 2009). Differences in N losses among the forests in different succession stages became smaller under higher N input. A perfect imitation of future N depositions in our study was not possible. (i) Because of logistic reasons, we were only able to split the N application in two dates. (ii) We applied N directly to the soil while the future increased N deposition will have to first pass the canopy where it might be retained or converted to other N forms (Parker 1983). (iii) While the deposition is usually dominated by NH_4^+, NO_3^- and HNO_3 (Galloway et al. 2004), we used urea as N source because of its commercial availability and because of security restrictions to the import of NO_3^- salts. Currently, atmospheric NH_4-N deposition at our study site is 3-5 times higher than NO_3-N input (Fabian et al. 2005, Boy et al. 2008b), which is similar to findings of Clark et al. (1998) in Costa Rica where annual fluxes of NH_4-N were about twice as high as NO_3-N fluxes. However, to test a possible N limitation, the fertilized N form does not matter because of a similar response of plant growth to all types of N fertilizer (LeBauer and Tresender 2008). After fertilization with urea NH_3 losses of <1% to >50% were reported depending on soil type and environmental conditions at the time of application (Sharpe and Harper 1995, Krupa 2003, Kissel et al. 2004, Cabrera et al. 2010). The largest part of NH_3 volatilization in forested ecosystems occurs during the first days following fertilizer application (Mugasha and Pluth 1995, Sharpe and Harper 1995, Palma et al. 1998). High NH_3 volatilization rates after urea addition are specific for soils with high pH. At at our study site, the pH of the organic layer is acidic (Table B-2) and therefore does not favor NH_3 volatilization although in the immediate vicinity of fertilizer grains the local pH might be shortly higher than measured in our solutions. Two months after the second fertilizer addition, the equilibrium soil pH in the fertilized plots was not significantly different from the control treatment (Table B-2). Comparatively low temperature and wind speed in the stand also do not favour NH_3 volatilization. Furthermore, in well-drained soils, like the organic layer at our study site, NH_3 volatilization is less than in poorly drained soils (Mugasha and Pluth 1995, Kissel et al. 2004). The only physico-chemical condition favoring NH_3 volatilization at our study site is

the high relative air humidity (Kissel et al. 2009, Cabrera et al. 2010). In summary, we conclude that the likelihood of significant losses of NH_3 after urea application is small. Furthermore, if there was volatilization of NH_3, the released N would be either lost from our budget, taken up by the canopy (and then contribute to the underestimation of N uptake by the canopy) or dissolved in throughfall. This would not change our interpretation that a certain percentage of the applied N is recycled with throughfall although the underlying mechanism would be different.

Almost all tree roots are located in the organic layer and it is therefore reasonable to assume that elements leaching from the organic layer are no longer plant-available. Less than 10 ± SD 2.9% of the in total applied 75 kg N ha^{-1} leached from the organic layer in the N treatments and 8.0 ± 2.5% from that of the N+P treatments until the end of our observation period and hence, ≥90% was retained in the organic layer and aboveground biomass by adsorption, microbial immobilization and uptake by plants followed by storage in below or aboveground biomass if NH_3 volatilization was negligible (Fig. B-5). In several tropical forests, it was observed that fast microbial immobilization of NH_4^+ and conversion to organic N forms occurred in the topsoil (Corre et al. 2006, Sotta et al. 2008, Arnold et al. 2009). The lower C:N ratio in the organic layer of the N and N+P treatments nine months after the start of fertilization supports this assumption (Table B-2) and is in line with results from European forests where under higher N deposition changes in the N status of the vegetation and organic layer but not of the mineral soil were observed (Gundersen et al. 1998, Tietema et al. 1998). The assumption that part of the added N is taken up by the plants is supported by the increased N fluxes in throughfall and litterfall of the N and N+P treatments (Fig. B-5). Part of the added N might also be denitrified. However, preliminary results of an accompanying working group indicated that the denitrification loss is only around 0.2 kg ha^{-1} yr^{-1} ($NO + N_2O$; personal communication by G.O. Martinson and collaborators), which is 0.4% of the applied N. The even smaller N losses in the N+P than in the N treatments suggest that the simultaneous addition of P stimulates N uptake because after N addition P quickly falls short of supply. This is in line with previous reports that modest additions of N induce a P limitation in tropical forests (Davidson and Howarth 2007, Elser et al. 2007). Concentrations and fluxes of N in the mineral soil were small and most of them did not differ among the treatments (Fig. B-5). We attribute the lower total N concentrations in the N and the higher total N fluxes at 0.30 m soil depth in the N+P treatments to spatial

variations. Consequently, losses of N to the streams draining the montane forest were small during the observation period.

The canopy of our studied forest retained N (Fig. B-4). Nitrogen uptake by the canopy was also reported earlier at our study site (Boy et al. 2008a) and might partly be attributed to the abundant epiphytes (Fleischbein et al. 2005) that are known to be able to absorb N from deposition (Chuyong et al. 2004). In the canopy of a tropical lowland forest in Costa Rica, dissolved organic N was net retained mainly by epiphytic mosses (Umana and Wanek 2010). Retention of N deposited from the atmosphere is frequently observed in N-limited forests (Clark et al. 1998, Laclau et al. 2003, Oyarzun et al. 2004). For example, NH_4^+ retention from rainfall within the canopy or a conversion from NH_4^+ to NO_3^- or organic N by epiphytic bacteria or lichens was observed in an evergreen rainforest in Chile (Oyarzun et al. 2004). A tropical montane forest in Costa Rica retained NH_4-N and NO_3-N (Clark et al. 1998). However, similar N fluxes in throughfall than in incident rainfall were reported from forests in Colombia (Veneklaas 1990) and Panama (Cavelier et al. 1997). In a forest in China, even slightly higher N fluxes in throughfall compared to incident rainfall were observed (Liu et al. 2003). At some sites in south China, N was on balance retained in the canopy while at other sites N was released (Chen and Mulder 2007). However, in the studies of Veneklaas (1990), Cavelier et al. (1997), Liu et al. (2003) and Chen and Mulder (2007) dry deposition was not quantified. The elevated N fluxes in throughfall of the N and N+P compared to the unfertilized control treatments were not attributable to N leaching from the canopy but reflect the reduced canopy retention of deposited N and thus further support that the study forest is N-limited.

The fact that we did not detect differences in litterfall production might be related with the short observation period of 1.5 yr while the mean leaf lifespan in the research area is around two years (Moser et al. 2007). In the N and N+P treatments, 11 ± SD 13% and 15 ± 10% of the applied 75 kg N ha^{-1}, respectively, were recycled to the soil as litterfall until the end of our observation period (Fig. B-5). Increased N fluxes with litterfall were also reported by Campo et al. (2007) after N application. Vitousek (1982) suggested that tropical trees retranslocate only a small proportion of N from their leaves prior to abscission in contrast to trees in the temperate zone which might explain that the nutrient use efficiency did not change after N application.

The concentrations of N in litterfall of around 10 g kg^{-1} in all treatments were at the lower end of the range in N-rich tropical forests which may contain up to 17 g kg^{-1} (Vitousek 1984). However, N concentrations in plant tissue strongly depend on genetic N demand (Harrington et

al. 2001, Hedin et al. 2009). The N use efficiency did not change during our observation period in the fertilized treatments. An evaluation of canopy properties of forests in Hawaii which were fertilized for 6-11 yr, showed that the N use efficiency did not change at N-limited sites because the increase in tissue N stock was of the same size as the increase in biomass production (Harrington et al. 2001).

5.2. Impact of enhanced P depositions

Similar to our study on N, a perfect imitation of future P depositions in our study was not possible because (i) we added P as a salt whereby P becomes readily biological available as $H_2PO_4^-$ after dissolution. This P form differs from the mineral aerosol dust that dominates global P deposition (Mahowald et al. 2008). Mahowald et al. (2008) estimated that around one sixth of atmospheric P is in the form of phosphate. (ii) Phosphorus was directly applied to the soil and hence, did not pass the canopy before reaching the soil as would P deposited from the atmosphere.

After three P applications, the Oe horizons of the P and N+P treatments had increased P concentrations compared to the unfertilized control treatment illustrating that the added P was retained in the organic layer. The fact that we only observed a P accumulation in the Oe but not in Oi and Oa horizons is likely related to the time after the first fertilization in relation to the turnover times of the respective horizons. While the Oi is completely turned over within ca. 1 yr and P turnover times in the Oi horizon are even shorter (0.73 ± SD 0.07 yr, Wilcke et al. 2002) than our observation period, the turnover time of the whole organic layer is far longer (12 ± 1.4 yr for the mass and 11 ± 1.5 yr for P) than our observation period.

From the 15 kg P ha^{-1} we added, only 0.84 ± SD 0.62% and 0.84 ± 0.53% leached to below the organic layer in the P and N+P treatments, respectively, until the end of our observation period and thus was considered to leave the rooting zone (Fig. B-5). The most likely explanation for the retention of fertilized P in the organic layer is microbial immobilization, because the C:P ratio was 1117 ± SD 147 far above the value of 100 which is considered as initiating strong P immobilization (White 2000). This suggests P limitation of the microorganisms which will be affected by nutrient limitation prior to trees (Kaspari and Yanoviak 2008). A general inhibition of microbial turnover is also indicated by the thick organic layers (Table B-2). Alternatively, P might be precipitated as Al phosphate because the organic

layer contained a roughly estimated 10% of mineral soil material and stored 768 ± 199 kg Al ha^{-1} (own unpublished result) although most of the Al will be organically complexed.

Deposited P from the atmosphere was retained by the canopy in all treatments. Elevated P fluxes in throughfall of the P and N+P compared to the unfertilized control treatments were not attributable to P leaching from the canopy but reflected reduced canopy uptake in response to the fertilizer application. This indicates that the trees took up part of the applied P which is further confirmed by higher P concentrations in litterfall of the P and larger P fluxes with litterfall in the N+P treatments. A similar observation was reported from a tropical rain forest in Borneo (Mirmanto et al., 1999) and from a P-limited forest in Hawaii (Harrington et al., 2001) where the addition of P and N+P also resulted in increased P fluxes. Up to 5% of the applied P was recycled with throughfall in the P and N+P plots.

6. Conclusions

The studied forest seems to be co-limited by N and P as illustrated by the consistent retention of both nutrients from deposition in the canopy and by the negligibly small losses of <10% and around 1% of the applied N and P, respectively, to below the organic layer which contains almost all roots. Up to 20 and 5% of the applied N and P, respectively, was cycled through the vegetation and returned to soil via throughfall and litterfall until the end of our observation period suggesting that the total uptake of fertilizer N and P was even higher because of N and P accretion in wood and roots which was not measured. The most likely reason for the retention of N and P in the ecosystem was microbial immobilization in the organic layer and plant uptake, while precipitation of P as aluminium phosphate played a minor role. These short-term ecosystem responses suggest that the expected increasing atmospheric N and P depositions will remain in the forest ecosystem likely resulting in higher biomass production. Leaching losses to the ground and surface waters are at least in the short term not to be expected. However, short-term effects are not necessarily similar to long-term effects calling for extended observation periods of fertilizer experiments.

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C Response of the Ca cycle of an old-growth montane forest in Ecuador to experimental Ca amendments

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1. Abstract

The Eastern slope of the Ecuadorian Andes receives calcium (Ca) from long-range transport originating in the Sahara during La Niña events. The shortening of the El Niño Southern Oscillation might result in higher Ca inputs in the future. We investigated the short-term response of the plants and the Ca concentrations and fluxes within a north Andean tropical montane forest at 2100 m above sea level to elevated Ca input. We added 10 kg ha^{-1} yr^{-1} Ca as $CaCl_2$, i.e. without pH effect, split into two applications to fourfold replicated experimental plots. The results were compared with an also fourfold replicated untreated control. We determined the response of fine root biomass, total fine litterfall, leaf area index, leaf area and specific leaf area, tree diameter growth and basal area increment. We furthermore collected litter leachate, mineral soil solution (0.15 and 0.30 m depths), and throughfall before the treatment began and 16 months after the first nutrient application and determined the Ca concentrations and fluxes. One year after Ca addition, fine root biomass had decreased significantly. The leaf size of the four most common tree species also tended to decrease and the specific leaf area increased significantly in *Graffenrieda emarginata* Triana, the most common tree species. The plant response suggests decreased nutrient stress. As aluminium (Al) toxicity was unlikely, because of almost complete organo-complexation of Al and high molar Ca:Al concentration ratios in solution, this response cannot be attributed to alleviated Al stress. Less than 5% of the applied Ca leached below the organic layer which contained almost all roots in spite of the low pH (3.5-4.8) and no significant leaching losses of Ca to below 0.15 m mineral soil depth occurred. Calcium retention from atmospheric deposition by the canopy was reduced in the Ca treatment compared to the control treatment, resulting in higher Ca concentrations and fluxes in throughfall and litterfall in the Ca treatment. Up to 21% of the applied Ca was recycled to the soil with litterfall. Our results strongly suggest that increased Ca input will reduce nutrient stress in the study forest.

2. Introduction

Besides numerous reports about the limitation of tropical forests by nitrogen (N) and phosphorus (P) (Tanner et al. 1998), there are indications that calcium (Ca) can also be a limiting element for forest growth (McLaughlin and Wimmer 1999, Paoli and Curran 2007, Boy and Wilcke 2008). Calcium plays an essential role in regulating numerous physiological processes in plants like cell division, synthesis and function of membranes and cell walls, stomatal regulation, activation of the enzyme system involved in the plant response to environmental stimuli like low temperature, carbohydrate metabolism, disease resistance and wound repair (McLaughlin and Wimmer 1999).

A significant source of Ca for transatlantic ecosystems is Saharan mineral dust (Kaufman et al. 2005, Boy and Wilcke 2008, Pett-Ridge et al. 2009b). Pett-Ridge et al. (2009a) reported that 83% of the atmospheric Ca input in a watershed in Puerto Rico originated from Saharan dust and only 17% from sea salt. In south Ecuador, Boy and Wilcke (2008) demonstrated that during a strong La Niña event in 1999/2000, Ca and magnesium (Mg) which originated from the Sahara, were deposited from the atmosphere to the east exposed slope of the eastern Andean cordillera. The Saharan dust was transported across the Amazon basin during dry weather conditions along the wind trajectories. Boy and Wilcke (2008) therefore hypothesized that the Ca supply by Saharan dust deposition is linked with the El Niño Southern Oscillation (ENSO). The amplitude or frequency of ENSO might change as an effect of global climate change, possibly resulting in a shortening of the currently approximately 7-yr cycle (Timmermann et al. 1999, Richardson et al. 2009). A consequence could be increased Ca deposition via Saharan dust to the east-exposed slopes of the northern Andes.

In acid mineral soils, Ca deficiency and aluminium (Al) toxicity can appear simultaneously (Marschner 1993) which makes it difficult to distinguish between both growth limiting factors. The total Al concentration in soil solution often failed to describe Al toxicity most probably because of the differential toxicity of the various Al species. While the free Al^{3+} ion is phytotoxic and the $Al(OH)^{2+}$ and $Al(OH)_2^{+}$ ions are potentially toxic, organically complexed Al is not toxic (Alva et al. 1986, Savory and Wills 1991, Cronan and Grigal 1995). In the thick organic layers of tropical montane forest soils, where most roots are located (Soethe et al. 2006), Al is most likely almost entirely organically complexed.

The response of tropical montane forest to nutrient inputs can either be determined by observing plant growth and change of plant morphological properties or by assessing changes in

the nutrient cycling. Nutrient retention from rainfall in the canopy can be interpreted as an indication of a scarcity of the considered nutrient (Ulrich 1983). To determine nutrient retention in the canopy, a full budget of wet and dry deposition and leaching is necessary. In a montane forest of southern Ecuador, Boy and Wilcke (2008) observed Ca retention in the forest canopy during elevated Ca deposition from the atmosphere suggesting that there was a periodical Ca deficiency.

In response of transatlantic forest ecosystems to Sahara dust-derived Ca depositions, the potential Ca effect cannot be separated from the simultaneously occurring pH effect because Ca is deposited as carbonate salt as indicated by elevated pH values of rainfall (Boy and Wilcke 2008). An increased pH in the soil solution likely stimulates organic matter turnover and thus the release of organically bound nutrients such as N. Soethe et al. (2006) observed a non-significant increase in fine root growth in an Ecuadorian tropical montane forest after the addition of lime to an ingrowth core. Again, it was not possible to separate the effects of Ca and pH. To study the Ca effect alone, a pH-neutral salt such as $CaCl_2$ needs to be added experimentally.

We aimed to determine the short-term response of the Ca cycle and plant performance of a montane old-growth forest in southern Ecuador to changed Ca depositions by simulating possibly increased future Ca depositions. We hypothesized that the addition of Ca (i) reduces plant nutrient stress because Ca is at least co-limiting and (ii) alleviates Al toxicity and (iii) Ca remains in the ecosystem as indicated by Ca retention in the forest canopy and little Ca leaching to greater soil depths.

3. Materials and methods

3.1. Study area

The studied forest is located in southern Ecuador on the eastern slope of the Cordillera Real of the Andes (i.e. the eastern cordillera) at an altitude between 2020 and 2120 m a.s.l. (3°59´S, 79°05´W), in the Reserva Biológica San Francisco (RBSF) in the deeply incised valley of the Rio San Francisco draining to the Amazon. The vegetation at the study site can be classified as “evergreen lower montane forest” according to Homeier et al. (2008). Mean canopy height at the study site is about 12-14 m. The estimated crown radius for canopy trees is between 2-4 m, some of the biggest trees may reach 5-6 m. In the study area, more than 280 tree species have been identified so far with Lauraceae, Melastomataceae and Rubiaceae as the most

abundant plant families (Homeier and Werner 2007). *Graffenrieda emarginata* Triana (Melastomaceae) is the most abundant tree species with a diameter at breast height ≥10 cm.

The 4-yr mean annual rainfall (2004-2008) ranged between 2527 ± standard deviation (SD) 400 and 2611 ± 397 mm at two rainfall gauging stations (Wullaert et al. 2009). Rainfall has a unimodal distribution with a maximum between April and September and without a pronounced dry season (Fleischbein et al. 2005, 2006). Annual bulk Ca depositions with rainfall ranged between 4.4 and 29 kg ha^{-1} yr^{-1} for the period 1998-2003 (Boy and Wilcke 2008). Mean annual temperature at 1950 m a.s.l. is 15.2 °C. The coldest months are June and July with a mean temperature of 14.4 °C; the warmest month is November with a mean temperature of 16.1 °C (Bendix et al. 2008). The soil is a Stagnic Cambisol (Hyperdystric, Chromic) (IUSS Working Group WRB 2007) developed from Palaeozoic phyllites, quartzites and metasandstones.

3.2. Experimental design

Our study was conducted in the framework of the interdisciplinary NUtrient Manipulation EXperiment (NUMEX). The study site was located on the upper slope near a major ridge and has an average slope of 51% (range: 25% to 84%). The experiment consists of Ca addition and unfertilized control plots. Each treatment was fourfold replicated in a randomized block design with the restriction that the unfertilized control plots were located upslope to avoid nutrient leaching from fertilized to control plots. Three blocks had a north aspect and the fourth block a south-southwest aspect. The location of each plot was selected in a way that the vegetation was representative of the area and similar on all plots. Each plot was 400 m² (20 m x 20 m) large and the distance between the plots was at least 10 m (Fig. C-1). There is no data available about the root radius of canopy trees. Therefore, we cannot fully rule out that roots of trees growing close to the edge of a plot reach another plot.

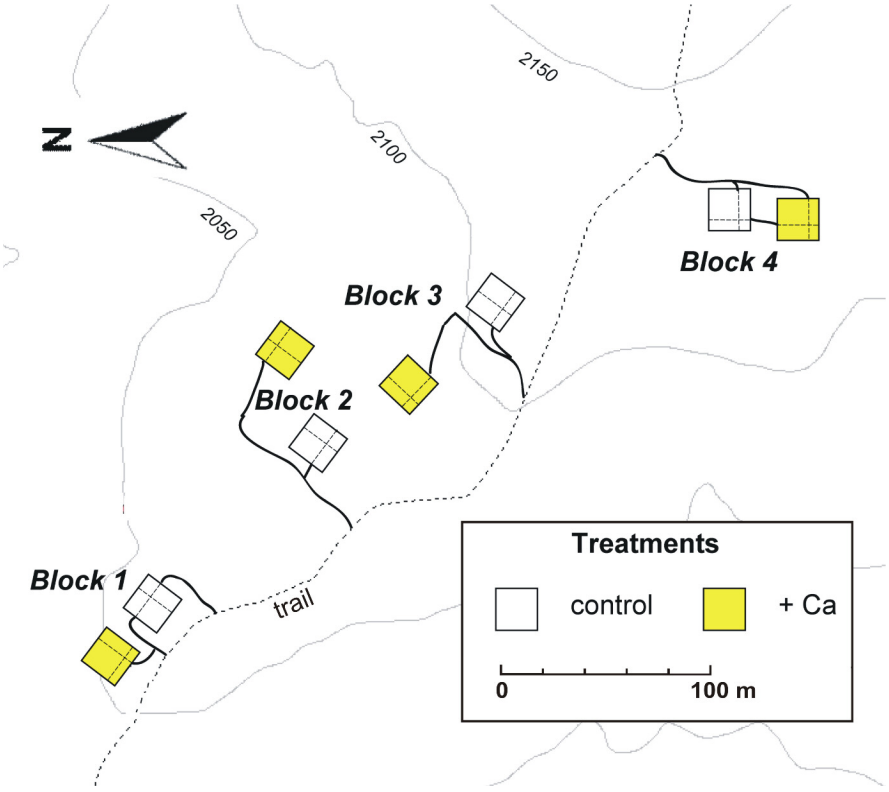

Fig. C- 1: Location of the study sites. Dotted lines within plots represent transects and continuous lines between the plots represent the access paths.

The Ca-addition plots received 10 kg Ca ha^{-1} yr^{-1}, split in two applications per year. Calcium was applied manually as $CaCl_2 \cdot 2H_2O$ in *pro analysi* quality. Calcium additions started in January 2008, followed by two further applications in August 2008 and February 2009. Calcium was applied as a salt. The salt was applied manually by four persons walking parallel in strips of 5 m. Throughfall collectors and collecting bottles of litter percolate were covered with plastic bags during nutrient application. The research equipment (except throughfall collectors) was installed in subplots of 2 m x 2 m. Six subplots were located along two perpendicular random-transects within the 20 m x 20 m plots and the transects were at least 4 m from the plot border so that the subplots were located at minimum 2 m from the plot border to avoid edge effects (Fig. C-1).

3.3. Sampling

3.3.1. Soil and fine root sampling

Before the first nutrient application in August 2007, we sampled the Oa, A and B horizon of each plot from hand-dug profiles (where we later installed the zero-tension lysimeters) within 2 weeks. After two nutrient applications (October 2008), the Oi, Oe, Oa, A and B horizons were re-sampled with an Edelman auger. At both sampling dates we sampled to a depth of about 0.5 m in the mineral soil. Furthermore, the horizons of the organic layer were sampled in a representative way per plot after the third fertilizer application between February and May 2009 for Ca analyses. The soil sampled after the second or third nutrient application was collected with a soil auger from a location within the subplots which we considered as not disturbed.

In order to assess fine root biomass, soil samples were taken from the organic layer and the upper 20 cm of the mineral soil with a soil corer (3.5 cm in diameter) in each subplot per experimental plot. To assess fine root biomass, the soil samples were transferred to plastic bags and transported to the laboratory, where processing of the stored samples (4 °C) took place within six weeks.

3.3.2. Litterfall and leaf sampling

Fine litterfall was collected once per month from six litter traps (0.60 m x 0.60 m) per plot from October 2007 until March 2008. From April 2008 on, only two out of the six litterfall traps were sampled where the litterfall remained only seven days in the field. The two litterfall traps were randomly chosen. Samples of the pre-treatment months were bulked to one sample per plot. After the first nutrient application, samples from subplots were bulked per plot and per month for further analyses.

Leaf samples from sun-exposed branches of the four most common tree species (*Alchornea grandiflora* Müll. Arg., *G. emarginata*, *Hieronyma moritziana* Pax & K.Hoffm. and *Myrcia* sp.) from 4-5 individual trees per treatment and species were collected in January 2009 to quantify changes in leaf morphology and foliar nutrient concentrations one year after the first fertilization. We sampled 10-20 fully developed leaves without visible damages from two different branches of each tree.

3.3.3. Water sampling

Throughfall was collected with 20 fixed-positioned funnel gauges in each plot. Each of the perpendicular transects contained 10 throughfall collectors randomly distributed along the transect in a way that the collectors were at least 2 m away from the plot border to avoid edge effects. The funnel gauges consisted of a 2-l polyethylene sampling bottle and a polyethylene funnel with 115 mm diameter. The rims of the funnels were 0.3 m above the soil surface to avoid splash-in effects from the forest floor. The funnels were vertical to avoid splash-out (around 0.1 m vertical edge). To reduce evaporation, a table-tennis ball was used in the funnel and the collecting bottle was wrapped with aluminium foil. Throughfall volumes of the 20 collectors per plot were measured with a graduated cylinder in the field and were volume-weighted bulked to result in one sample per plot per collecting date.

Zero-tension lysimeters were used to collect leachate from the organic layer. They were made of plastic boxes with a 0.15 m x 0.15 m collection area and covered with a polyethylene net with a mesh of 0.5 mm. The organic layer remained intact during and after installation. A tube connected the lysimeter to a collection bottle which was wrapped in aluminium foil to reduce the impact of radiation. Three replicates were installed per plot but at each sampling the leachates were bulked to one sample per plot.

Soil solution at 0.15 and 0.30 m mineral depth was collected with suction lysimeters which had a ceramic cup with 1 µm pore size. To produce spatially representative samples, three suction lysimeters were installed per plot at a distance of 0.40 m from each other. The set of three suction lysimeters per plot were connected with tubes to the same collecting bottle so that the collected soil solution was bulked *in situ*. Based on our visual inspection, we estimate that the total fine root fraction in the organic layer is >95% so that the collected soil solution can be considered as from below the rooting zone. The collecting bottles for the soil solution were brown-coloured and were placed in a closed bucket to reduce the radiation impact.

Throughfall, litter leachate and mineral soil solution were sampled fortnightly. After each sampling, we applied a vacuum of -0.6 bar to the suction lysimeters in order to collect sufficient sample for the next sampling period. Sampling of throughfall, litter percolate and soil solution took place from August 2007 until April 2009 of which the first five months were before the first nutrient addition.

3.4. Physical and chemical analyses

Bulk density was measured by the soil core method (Blake and Hartge 1986) at four soil profiles.

Per leaf sample, 10 - 20 fresh leaves were scanned using a flat bed scanner (CanonScan LIDE 30, Canon). Subsequently, images were analysed with the software WinFolia 2001a (Regent Instruments Inc., Canada) for calculation of the average single leaf area (LA). Leaves were then dried at 60 °C to constant mass and specific leaf area (SLA) was calculated as the ratio of LA to leaf dry weight.

The soil samples for fine root analysis were soaked in water and cleaned from soil residues using a sieve with a mesh size of 0.25 mm. Only fine roots (roots <2mm in diameter) of trees were considered for analysis. Live fine roots (biomass) were separated from dead rootlets (necromass) under the stereomicroscope based on colour, root elasticity, and the degree of cohesion of cortex, periderm and stele. A dark cortex and stele, or a white, but non-turgid cortex, or the complete loss of the stele and cortex with only the periderm being present, were used as indicators of root death (Persson 1978, Leuschner et al. 2001). The fine root biomass of each sample was dried at 70 °C for 48 h and weighed. The data were expressed as fine root abundance ($g\ m^{-2}$).

Soil and fine litterfall samples were dried at 40 °C to constant mass after field collection. Dried litterfall samples and samples of the organic layer were ground with a ballmill at 650 rounds per minute for 10 min. Dried mineral soil samples were sieved (<2 mm) before further analysis. Soil pH was measured in a soil:deionized water mixture (ratio of 1:10 and 1:2.5 for the organic horizons and mineral soil, respectively). To measure the effective cation-exchange capacity (ECEC) of mineral soil samples, 5 g of sample were shaken in 100 ml unbuffered 1 mol l^{-1} NH_4NO_3 for one h, then filtered and analysed for Al, Ca, K, Mg and Na concentrations. Base saturation (BS) was calculated as the percentage of the sum of charge equivalents of the base metal cations of the ECEC.

Litterfall and leaf samples were digested with 65% HNO_3 under pressure in a microwave system (Mars Xpress, CEM GmbH, Kamp-Lintfort, Germany).

After collection in the field, throughfall, litter leachate and soil solution samples were transported to our field laboratory where pH (Sentix HWS, WTW GmbH, Weilheim, Germany) was immediately measured in an aliquot of each sample within <24 h. Another aliquot was filtered (ashless filters with pore size 4-7µm, folded filter type 389; Munktell & Filtrak GmbH,

Bärenstein, Germany) and frozen until transport to Germany for further analysis. During transport to Germany, samples were stored cool at <4 °C and transport did not take longer than one week.

Concentrations of Ca in water samples and litterfall digests and concentrations of Al, Ca, K, Mg and Na in soil extracts were determined with flame atomic absorption spectrometry (AAS, Varian AA240FS, Thermo Fisher, Darmstadt, Germany). Elemental concentrations of Ca and Al in digests of fresh leaves were determined with an Inductively Coupled Plasma Analyzer (Optima 5300DV ICP-OES, Perkin Elmer, Rodgau, Germany). Water samples were furthermore analysed for concentrations of total dissolved Cl^- using continuous flow analysis (CFA, Bran+Luebbe GmbH, Norderstedt, Germany).

Total organic carbon in solution (TOC) in litter leachate was measured with a high temperature TOC analyzer (high TOC *II*, Elementar Analysensysteme, Hanau, Germany).

To assess the precision of the Ca and TOC analysis, we analysed the concentration of an internal reference standard (a throughfall water sample from our field site in Ecuador) in every laboratory run of about 100 samples. The relative standard deviations of Ca and TOC concentrations in the reference standard were 11 and 8.6%, respectively (n = 15 for Ca and 25 for TOC). The relative standard deviations of Na were relatively high because the Na concentration in the reference solution was close to the detection limit of 0.009 mg l^{-1}.

3.5. Stem diameter growth and plot basal area increment

Stem diameter growth of all 384 trees with a diameter at breast height (dbh) ≥10 cm on our study plots was monitored 6-weekly with permanent girth-increment tapes (D1 dendrometer, UMS, Munich, Germany) at breast height (1.3 m). The increase of plot basal area was calculated as the sum of all individual tree increments from 15.2.2008 (after the first fertilization) until 25.4.2009.

3.6. Leaf area index

The leaf area index (LAI) was quantified with two LAI-2000 plant canopy analyzers (LI-COR Inc., Lincoln, NE, USA). The LAI measurements were conducted in the remote mode, i.e. by synchronous readings below the canopy at 2 m height above the forest floor and in a nearby open area (“above-canopy” reading) using two devices. One measurement was done above each litter trap and a second at the same time outside the forest. The LAI measurements were realized

in January 2008 (before the first fertilization) and in January 2009 (one year after the first fertilization). All measurements were conducted during periods of overcast sky. To avoid reductions in the sky sector seen by the LAI-2000 fish-eye lens (by high mountains or trees at the horizon), only data of the three inner rings (0-43° from zenith) were analysed.

3.7. Meteorological data

For the calculation of reference evapotranspiration, we used meteorological data obtained from the automatic meteorological station on a clear-cut area in ridge-top position at 1950 m a.s.l. Air temperature was measured at a height of 2 m above soil surface with an electronic sensor (Pt100). Relative air humidity was measured with a hygrometer at 2 m above soil surface. Wind velocity was measured with a four-cup anemometer at 2.5 m above soil surface. Total radiation was measured with a pyranometer at 2 m above soil surface with a sensor after WMO and ISO 9060 standards. Rainfall was measured with a tipping-bucket funnel gauge. All meteorological data were recorded with measuring intervals of 1 h and stored by a data logger (Logger DL 15).

3.8. Speciation of aluminium

The speciation of Al in litter leachate was calculated with Visual MINTEQ (Version 2.61). We used pH values and TOC concentrations from our own measurements in the NUMEX site to run the programme. Since no Al values are available from this study site, we used Al data from three adjacent sites in the same study forest to estimate Al speciation. These three sites are located in an adjacent forested catchment at similar altitude (between 1900 and 2010 m a.s.l.) but at the lower slope instead of the upper slope to ridge position like in our study plots. All sites: our NUMEX study site and the adjacent sites, as described in Boy et al. (2008a), have similar total Al in the organic layer and similar exchangeable Al in the mineral soil. We therefore regard it as correct to use Al data from adjacent sites to estimate Al speciation in our studied NUMEX site. We also inferred that the concentrations of Ca, Mg, Na, K, SO_4^{2-}, Cl^- and NO_3^- at our study site were similar as at the adjacent forest sites for which a 5-yr data set was available (Wilcke et al. 2001, Boy et al. 2008b) in order to calculate the ionic strength of the litter leachate.

3.9. Hydrological calculations

Leaching losses from the organic layer (LO) were calculated using a one-dimensional soil water balance model from throughfall (Th), transpiration (Tr) and change in water storage (ΔS; t_2-t_1) of the organic layer (Eq. C-1).

$$LO = Th - Tr - \Delta S \quad (1)$$

More details are described in DVWK (1996) and Oelmann et al. (2007). Reference evapotranspiration was calculated with REF-ET (University of Idaho and Dr. R.G. Allen; version 2.0) using the ASCE Penman-Monteith method based on daily mean wind speed, air humidity and irradiation, daily precipitation sum and daily minimum and maximum temperature. Transpiration losses were determined as the difference between reference evapotranspiration and interception losses. For the calculation of transpiration, we assumed direct evaporation from the soil as negligible. Interception losses were directly measured as the difference between incident rainfall and throughfall. We used frequency domain reflectometry (FDR) probes to calculate change in water storage in the organic layer and the mineral soil. FDR probes were installed at 0.10, 0.20, 0.30 and 0.40 m mineral soil depth and one in the organic layer. No upward flux from the mineral soil to the organic layer was allowed (Eq. C-2) and hence, when

$$Tr > Th - \Delta S \text{ ; then } LO = 0 \quad (2)$$

leaching losses from the organic layer were used as an input for the calculation of the leaching losses of the mineral soil (LM, Eq. C-3),

$$LM = LO - \Delta S_{M0.15 \text{ or } 0.30} \quad (3)$$

where $\Delta S_{M0.15 \text{ or } 0.30}$ is the change in water storage in the mineral soil (0-0.15 or 0.15-0.30 m depth). To determine the soil water content per plot we used data of tensiometers which were installed in each plot close to the suction lysimeters and data of electronic FDR and electronic tensiometer sensors which were installed in the middle of the whole research site.

We assumed that water uptake from the mineral soil was negligible because of the almost complete lack of roots in the mineral soil. From earlier results in our research area, Boy et al. (2008b) calculated that <10% of the total water flux contributed to lateral flow. We therefore assumed it reasonable to use a one-dimensional soil water balance model to calculate water fluxes in the mineral soil. Nutrient fluxes were calculated as the product of fortnightly fluxes and nutrient concentrations of the respective flux type.

3.10. Canopy budget

To set up the canopy budget (CB) of Ca, we used the model of Ulrich (1983), where CB is calculated as the difference between throughfall deposition (TFD) and total deposition (TD, Eq. C-4).

$$CB_{Ca} = TFD_{Ca} - TD_{Ca} \quad (4)$$

We did not include stemflow in our experimental setup since in most tropical forests including the north Andean montane forest, stemflow does not exceed 2% of rainfall (Lloyd and Marques 1988, Wilcke et al. 2001, Lilienfein and Wilcke 2004, Fleischbein et al. 2006). Positive values of CB indicate leaching, negative ones uptake of Ca by the canopy. Total deposition (TD) of Ca, was calculated with Eq. C-5:

$$TD_{Ca} = BD_{Ca} + DD_{Ca} \quad (5)$$

where bulk deposition (BD) refers to bulk rainfall deposition. Dry deposition (DD) was estimated with Eq. C-6.

$$DD_{Ca} = [(TFD_{Cl} / BD_{Cl}) \times BD_{Ca}] - BD_{Ca} \quad (6)$$

In Eq. C-6, TFD_{Cl} represents the throughfall deposition and BD_{Cl} the bulk deposition of Cl^-. It is assumed that Cl^- is a non-reactive tracer which was confirmed in earlier work at the same study site (Boy et al. 2008a, Boy and Wilcke 2008, Wilcke et al. 2009). Chloride concentrations were volume-weighted averaged for the period before the first fertilization and the periods between the first and the second and the second and the third fertilizer fertilization to reduce the influence of measurement uncertainty of Cl^- on the canopy budget calculations.

3.11. Statistical analyses

To test for differences in fine root biomass between the Ca and control treatment, subplots were considered as independent replicates because the distance between the sample locations was large enough. We used a one-factorial non-parametric Kruskal-Wallis test followed by a post-hoc paired comparison (Wilcoxon-U-test).

To test if there were *a priori* differences in Ca concentrations and fluxes in throughfall, litter, litter leachate, soil solution between the Ca and control treatment, the time period before the first nutrient application was assessed. The whole period after the first nutrient application was analysed separately from the period before the first nutrient application. Changes in Ca concentrations in throughfall, litterfall, litter leachate and soil solution were analysed by linear mixed effects models. We used this type of model since it allowed us to analyse the data set even

when several data were missing because of e.g., a lack of sufficient amount of sample to analyse. The treatment was defined as fixed effects and the four plot replicates as random effects.

For soil parameters, LAI, tree diameter growth, leaf morphology and foliar Ca and Al concentrations, the effects of Ca application were assessed using a *t*-test for independent data sets. Significance was set at $P \leq 0.05$. All analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA), except statistics of root biomass data was performed with SAS software (version 8.2, SAS Institute, Cary, NC, USA).

4. Results

4.1. Plants

After two Ca fertilization periods, mean living fine root biomass was with ca. 370 g m^{-2} significantly lower (16.5%; $P < 0.05$) in the Ca than the control treatment (Fig. C-2; data provided by D. Hertel). At the same time, mean dead fine root biomass tended to be lower in the Ca treatment but was not statistically significant from the control (ca. 400 vs. 425 g m^{-2}). Accordingly, the mean live:dead ratio in fine root mass was lower in the Ca than the control treatment.

Graffenrieda emarginata was the most common tree species contributing 28.3% of all trees having a dbh ≥10 cm. After three Ca applications, 15 months after the first Ca application, the mean cumulative tree diameter increment tended to increase slightly in all species, including three of the four most common tree species (*A. grandiflora, G. emarginata, H. moritziana*) but did not differ significantly between Ca and control treatment. In contrast, the mean cumulative tree diameter increment of *Myrcia* sp. was less in the Ca than the control treatment ($P = 0.049$) (Table C-1). The mean total basal area increment of the plots did not differ between the Ca and control treatment (1719 ± SD 484 and 1327 ± 433 cm^2 ha^{-1}, respectively).

Fig. C- 2: Living and dead fine root biomass. Data in the Ca treatment is presented as percent deviation from the control treatment. A star (*) represents that the mean in the Ca treatment is significantly different ($P < 0.05$) from the control treatment. Data kindly provided by Dietrich Hertel.

Total fine litterfall production in the Ca (4.2 ± SD 1.0 t ha^{-1} yr^{-1}) treatment was not significantly different from that of the control treatment (4.2 ± 1.1 t ha^{-1} yr^{-1}). The mean LAI increased between January 2008 and January 2009 from 4.72 ± SD 0.77 to 4.80 ± 0.92 in the Ca treatment and from 4.60 ± 0.93 up to 4.69 ± 1.01 in the control treatment, but the mean LAI increment was not significantly different between the Ca and control treatment. The average LA of the four most common tree species (*A. grandiflora, G. emarginata, H. moritziana* and *Myrcia* sp.) was smaller one year after the first Ca application in the Ca than the control treatment but changes were not significant. Leaves of the most common tree specie, *G. emarginata*, became significantly thinner since the SLA in the Ca treatment was significantly higher than in the control treatment ($P = 0.016$, Table C-1).

Mean concentrations of Ca and Al, in fresh leaves did not differ significantly between the Ca and control treatment (Table C-1).

Table C - 1: Calcium and Al concentrations and Ca:Al ratios in fresh leaves and (specific) leaf area and cumulative tree diameter increment for the four most common tree species. Data represent mean values with standard deviation.

	Alchornea grandiflora Müll. Arg.		*Graffenrieda emarginata* Triana		*Hieronyma moritziana* Pax & K.Hoffm.		*Myrcia* sp.	
	Control	Ca treatm.	Control	Ca treatm.	Control	Ca treatm.	Control	Ca treatm.
Ca (mg g^{-1})	2.37 ± 0.70	3.23 ± 0.92	1.39 ± 0.31	1.55 ± 0.64	2.20 ± 0.49	2.06 ± 0.89	0.93 ± 0.17	0.94 ± 0.21
Al (mg g^{-1})	0.02 ± 0.02	0.04 ± 0.01	3.38 ± 1.14	3.46 ± 0.92	0.04 ± 0.04	0.02 ± 0.02	0.08 ± 0.02	0.08 ± 0.01
Ca:Al ratio [a]	76 ± 27	67 ± 29	0.30 ± 0.10	0.30 ± 0.06	96 ± 109	82 ± 62	8.1 ± 3.2	8.5 ± 3.4
LA[b] (cm^2)	30.5 ± 10.9	26.2 ± 6.1	178 ± 38	155 ± 78	26.8 ± 7.5	24.3 ± 4.9	17.9 ± 3.0	16.1 ± 4.3
SLA[c] (cm^2 g^{-1})	40.9 ± 5.5	49.8 ± 8.5	38 ± 3	45 ± 4 *	69.3 ± 8.8	62.7 ± 6.8	40.2 ± 4.3	38.4 ± 4.2
Cumulative tree diameter increment (cm)	0.016 ± 0.018	0.029 ± 0.047	0.104 ± 0.095	0.115 ± 0.090	0.013 ± 0.015	0.039 ± 0.067	0.091 ± 0.069	0.049 ± 0.052 *

* Mean significantly different from control treatment ($P < 0.05$).

[a] Ratio is expressed as molar ratio as suggested by Cronan and Grigal (1995).

[b] LA is the leaf area.

[c] SLA is the specific leaf area.

Table C - 2: Selected soil properties of the Ca and control treatments (means ± SD; n = 4) in the study area determined in October 2008, two months after the second nutrient application.

Property	Control treatment	Ca treatment
	Organic layer	
	Oi horizon	
pH range (1:10 H_2O)	4.52 – 5.07	4.58 – 5.24
Ca (mg g^{-1}) [a]	1.9 ± 0.3	3.6 ± 0.9 *
Ca : Al ratio [b]	0.58 ± 0.09	1.5 ± 0.5 *
	Oe horizon	
pH range (1:10 H_2O)	3.92 – 4.48	4.08 – 4.75
Ca (mg g^{-1}) [a]	0.81 ± 0.44	2.2 ± 1.3
Ca : Al ratio [b]	0.13 ± 0.08	0.42 ± 0.35
	Oa horizon	
pH range (1:10 H_2O)	3.35 – 3.91	3.63 – 3.88
Ca (mg g^{-1}) [a]	0.16 ± 0.17	0.16 ± 0.13
Ca : Al ratio [b]	0.033 ± 0.027	0.033 ± 0.031
	Mineral soil	
	A horizon	
pH range (1:2.5 H_2O)	3.67 – 3.81	3.69 – 3.80
ECEC ($mmol_c$ kg^{-1}) [c]	26 ± 10	41 ± 13
BS (%) [d]	3.6 ± 1.5	2.3 ± 1.1
	B horizon	
pH range (1:2.5 H_2O)	4.01 – 4.20	4.04 – 4.14
ECEC ($mmol_c$ kg^{-1}) [c]	37 ± 2	37 ± 5
BS (%) [d]	0.9 ± 0.3	0.9 ± 0.1

[a] Determined after three nutrient applications

[b] Ratio is expressed as molar ratio as suggested by Cronan and Grigal (1995).

[c] Effective cation exchange capacity

[d] Base saturation

* Data significantly different from control treatment ($P < 0.05$).

4.2. Soil solid phase

The Ca concentration in the organic layer increased in the Ca treatment after three Ca applications in the Oi and Oe horizons but not (yet) in the Oa horizon. However, the differences in mean Ca concentrations between the Ca and control treatment were only significant for the Oi horizon (P = 0.013). Consequently, the molar ratio of total Ca:Al concentrations increased significantly after three Ca applications in the Oi horizon (P = 0.037). The exchangeable Ca concentration in the mineral soil did not differ between the Ca and control treatment after two Ca applications (Table C-2). After two Ca applications, the pH in the forest floor and mineral soil as well as the ECEC and BS in the mineral soil did not show significant differences between the Ca and control treatment (Table C-2).

4.3. Water fluxes

There were no significant differences in water fluxes between the Ca and control treatment for the whole observation period. We also did not observe any differences in water fluxes when the 5-month period before the first fertilization and each period after the three fertilizer applications were evaluated separately (results not shown). Figure C-3 summarizes the mean water fluxes of all study plots.

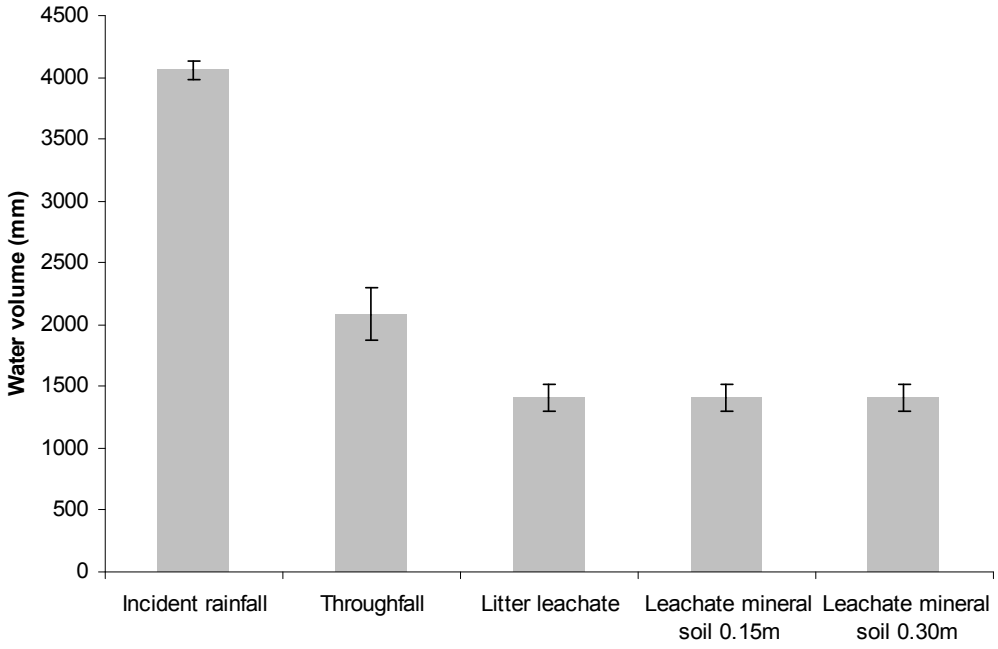

Fig. C- 3: Water budget during the study period (19/08/2007 – 25/04/2009). Error bars represent standard deviation.

4.4. Calcium concentrations and fluxes

Calcium flux in litter leachate and soil solution at 0.15 and 0.30 m depth in the control plots were very similar. For most weeks, the Ca flux in throughfall from the control plots was slightly greater than the Ca flux in litter leachate and soil solution at the 0.15 and 0.30 m depths from the same control plots (Fig. C-4). Before nutrient application, the Ca concentrations and fluxes in litter leachate did not differ between the Ca and control treatment (not shown). After three Ca applications, only non-significant 0.6 kg ha^{-1} Ca had cumulatively leached more from the organic layer in the Ca treatment than in the control treatment. The 0.6 kg ha^{-1} Ca corresponds to 4.0 ± 5.1% of the 15 kg ha^{-1} Ca applied during this period and is small compared to the Ca stock in the mineral soil. Therefore, applied Ca did not leach significantly with the soil solution to the 0.15 m and 0.30 m soil depths (Fig. C-5). The jumps in the lines of Fig. C-5 are a consequence of the fact that immediately after the application of more fertilizer, we switched the basis to which the percentage refer to the double and triple of the first fertilizer application rate and are thus a mathematical artifact.

In all plots, Ca was retained by the canopy from TD. A total of 3.5 kg ha^{-1} yr^{-1} entered the ecosystem by incident rainfall and 1.0 ± SD 2.3 kg ha^{-1} yr^{-1} as DD in the control treatment in the first year after the onset of the Ca addition. In the same period, 2.2 ± SD 0.8 kg ha^{-1} yr^{-1} Ca was deposited to the forest soil via throughfall in the control treatment resulting in a negative canopy budget, indicating that Ca was retained (Fig. C-6). In the unfertilized control treatment, 42, 61 and 28% of TD was retained by the canopy in the first, second and third fertilization periods, respectively. Overall, there was less Ca uptake (i.e. higher Ca flux in throughfall) in the Ca treatment compared to the control treatment, although the differences in CB of Ca between the treatments were only marginally significant (P = 0.09) over the three fertilization periods. This illustrates that Ca application tended to increase the Ca concentration in througfall (i.e. decreased the Ca uptake in the canopy) in the Ca treatment (Fig. C-5). After three fertilization periods, the reduced canopy uptake of Ca resulted in a cumulative extra Ca flux to the soil with throughfall of 0.26 ± SD 0.53 kg ha^{-1} in the Ca compared to the control treatment, which is equivalent to 1.7 ± 3.5% of the applied Ca.

A higher percentage of the applied Ca (20 ± SD 27%) was returned to the soil with litterfall in the Ca treatment (Fig. C-5). When Ca is transported in the stem, it can be bound to negatively charged sorption sites of the xylem and hence, total uptake of the fertilized Ca by the plants likely exceeded 20%.

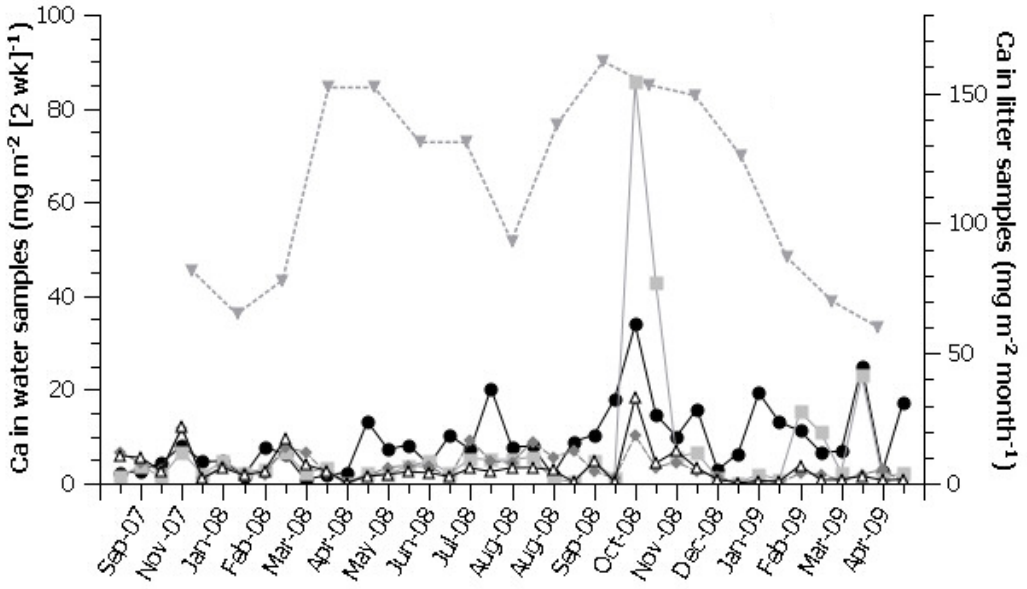

Fig. C- 4: Mean Ca fluxes in throughfall (—●—), litter leachate ( ), soil solution at 0.15 m (—◆—), soil solution at 0.30 m (—△—) and litterfall ( ) in the control treatment. Calcium application dates are indicated by vertical lines.

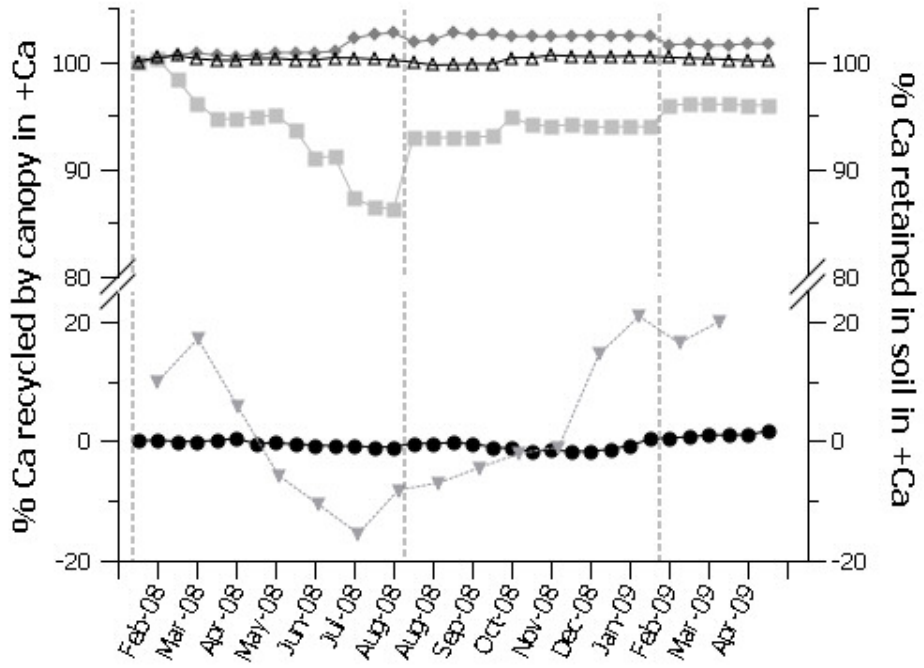

Fig. C- 5: Cumulative leaching by throughfall (—●—) and litterfall ( ) from the canopy and % retention in the soil of applied Ca: litter leachate ( ), soil solution at 0.15m (—◆—), soil solution at 0.30m (—△—). All values were calculated as differences between the Ca and control treatment. The vertical dashed grey lines indicate the times of Ca application.

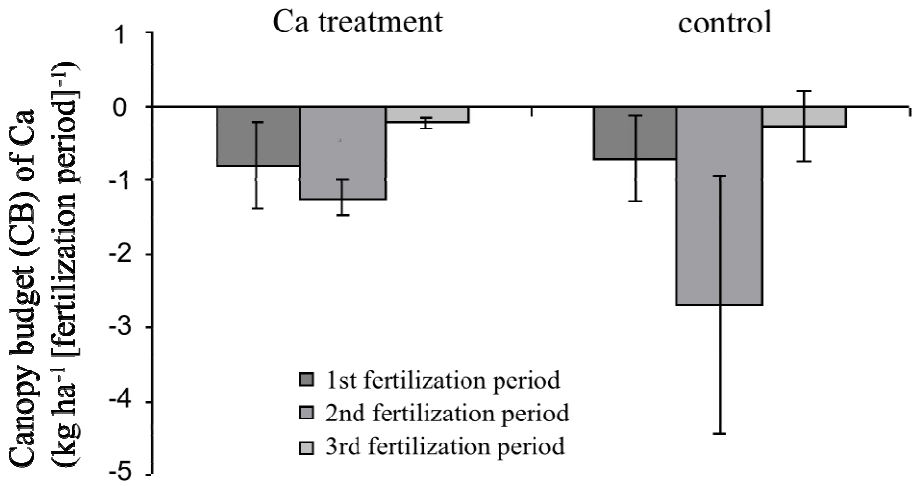

Fig. C- 6: Canopy budget (CB) of Ca for the three fertilization periods. Negative values of CB indicate net retention in the canopy. Error bars represent standard deviation.

4.5. Speciation of aluminium

To assess the possible Al toxicity, the properties of litter leachate reflect the root environment. Our chemical speciation modelling revealed that in the organic layer almost no free Al^{3+} occurred in solution. The minimum 5-yr average Al concentration in litter leachate was 0.05 mg l^{-1} and the maximum 5-yr average 0.7 mg l^{-1}. For the minimum Al^{3+} concentration and assuming different ionic strengths of the litter leachate, no free Al^{3+} in solution was modelled. All Al was complexed by organic material and thus non-toxic. When the Al speciation was calculated for 0.7 mg l^{-1} total Al, the free Al^{3+} concentrations was calculated to be between 0.014% and 0.04% of the total Al under different assumed ionic strengths of the litter leachate. This represents 0.1 and 0.3 µg l^{-1} Al^{3+}, respectively. According to our speciation calculation neither $Al(OH)^{2+}$ nor $Al(OH)_2^{+}$, which may potentially also be phytotoxic (Marschner 1993), occurred in litter leachate under any of the assumed conditions.

5. Discussion

5.1. Stimulation of plant performance

Nutrient limitation results in morphological and physiological adaptations such as e.g, root/shoot ratio, slow growth rate, increased leaf longevity, dense and shallow root mats, and small trees with thickened leaves (low SLA).

Living fine root biomass was reduced in the Ca treatment compared with the control. This suggests that the trees invested less C (and energy) in fine root mass because the additional Ca improved the growth conditions. The reason for improved growth conditions may be related with the alleviation of Ca deficiency or of Al toxicity. Decreased fine root necromass is attributable to the relative high fine root turnover (Graefe et al. 2008a, b). Mean root longevity in the research site at 1890 m was 467 ± 67 days (Graefe et al. 2008a).

Graffenrieda emarginata, the most common tree species in the study area, was the only tree species that reacted rapidly with an increased SLA which indicates thinner leaves. Thinner leaves are a morphological adaptation to less stress or higher nutrient availabilities (McLaughlin and Wimmer 1999). We interprete this as an indication of improved nutrient availability. As at the same time the living fine root biomass was reduced by Ca addition, it is unlikely that the higher nutrient availability to the plants is associated with a more extensive exploration of the soil. Therefore, it is reasonable to assume that the added Ca improved the nutrient availability which is an indication of Ca deficiency.

We hypothesize that on the long run, we will observe significantly increased tree growth which was the case in a Canadian forest three years after Ca addition as $CaCl_2$. In the same experiment, significantly increased foliar Ca concentrations were only reported six years after Ca addition (Huggett et al. 2007).

5.2. Alleviation of aluminium toxicity

In acid soils, Al becomes a strong competitor of Ca and interferes with Ca uptake in roots and root growth (McLaughlin and Wimmer 1999). Therefore many of the symptoms of Ca deficiency are associated with Al toxicity (Edwards et al. 1976). One factor limiting forest growth might therefore be Al toxicity. Cronan and Grigal (1995) reported in their review several threshold conditions to detect Al stress in forests including (i) soil BS <15% of ECEC, (ii) molar Ca:Al concentration ratio <1 in soil solution and (iii) a molar foliar tissue Ca:Al concentration ratio ≤12.5. After two Ca applications at our study plots, the BS in the A horizon of the Ca treatment was 2.3 ± 1.1%, i.e. clearly <15% (Table C-2). The fact that the majority of the tree roots are located in the thick organic layer might therefore be an adaptation in order to avoid Al toxicity in the mineral soil. If this was true, the soil solution of the organic layer should not contain free Al^{3+} at toxic concentration. Consequently, we modelled Al speciation in soil solution with Visual MINTEQ using solution properties of the litter leachate. The modelling results

indicate that $Al(OH)^{2+}$ and $Al(OH)_2^{+}$ were not present in the soil solution of the organic layer as approximated by the litter leachate. However, we might expect a maximum concentration of 0.3 µg l^{-1} Al^{3+} in the litter leachate, equivalent to 0.01 µmol l^{-1} Al^{3+}. The remaining part of the total Al concentration in litter leachate (699.7 µg l^{-1}, 99.96% of the total Al concentration) was organically complexed. Wheeler et al. (1992) reviewed the Al tolerance of 34 plant species (including 87 cultivars) growing in diluted nutrient concentrations and concluded that in order to reduce the shoot dry weight by 50%, for the most sensitive genotypes Al^{3+} concentration <1 µmol l^{-1} and for more tolerant genotypes more than 30 µmol l^{-1} were necessary. Our modelled free Al^{3+} concentration is far below this toxic concentration range suggesting that Al toxicity in the organic layer, where the majority of the tree roots is located, is unlikely.

At the adjacent study sites between 2000-2003, the mean molar Ca:Al ratio was 50 ± 73, 10 ± 17, 12 ± 17 in litter leachate, soil solution at 0.15 m and soil solution at 0.30 m mineral soil depth, respectively (Boy et al. 2008b), i.e. well above the toxicity threshold value. However, our study site is more acid and more base metal-depleted than the adjacent site, which might result in higher Al and lower Ca concentrations in the soil solution. The Ca concentrations in our control treatment in the Oi, Oe and Oa horizons were about 5, 8 and 30 times and the storage (kg ha^{-1}) about 18, 13 and 15 times smaller, respectively, compared to the adjacent study sites. Nevertheless, it can be assumed that the molar Ca:Al molar ratio does not indicate potential toxicity, particularly if it is furthermore considered that a large part of Al will be organically complexed.

Of the studied most common trees, only *G. emarginata* had a mean foliar molar Ca:Al concentration ratio of 0.3 which is clearly below the threshold value of ≤12.5. However, *G. emarginata* had up to 300 times higher foliar Al concentrations compared to the other analysed species (Table C-1). *Graffenrieda emarginata* belongs to the plant family of Melastomataceae which are are known to contain several species that accumulate Al (Cuenca et al. 1990). *Myrcia* sp. had a foliar molar Ca:Al concentration ratio around eight and both *A. grandiflora* and *H. moritziana* around six times the threshold ratio.

These results demonstrate that there is little indication of Al toxicity. Consequently it is highly unlikely that the observed Ca effect is attributable to an alleviation of Al toxicity.

5.3. Calcium cycling

The addition of Ca to the soil changed the canopy budget of Ca. Calcium was generally retained in the canopy suggesting a Ca demand of the canopy plants, their epiphytes or epiphylls. However, the addition of Ca to the soil decreased the uptake of Ca by the canopy from total deposition (i.e., wet and dry deposition) although the differences in the canopy budgets of Ca between Ca and control treatment were only marginally significant (Fig. C-6). Obviously, our Ca application reduced the Ca deficiency because the plants took up more Ca from the soil.

The fact that only 4% of the applied Ca was leached from the organic layer into the mineral soil indicates that Ca is taken up by the plants and soil organisms and – given the very acid soil reaction – to a small extent adsorbed in the organic layer. Based on the Ca fluxes with litterfall, 20% of the added Ca was taken up by the plants and recycled to the soil. To this percentage the unknown amount of Ca accreted in wood must be added. In red spruce, Ca can occupy around 60-80% of the cation exchange sites in the xylem (Momoshima and Bondietti 1990). In a Puerto Rican rain forest 2.4-5.1 kg yr^{-1} Ca were accreted (McDowell and Asbury 1994) which would correspond to ca. 25-50% of the amount of added Ca.

The Ca losses to the deeper subsoil were negligibly small. This is reflected by the small calculated fluxes and the fact that the BS of the A horizon did not differ between the soil samples collected prior to the first Ca application and those collected after two Ca applications. The leached Ca from the organic layer only accounted for 0.8% of the exchangeable Ca pool in the A horizon. Interestingly, Ca leaching to the deeper subsoil seemed to be reduced in the Ca compared with the control treatment indicating that the Ca addition even increased Ca retention in the ecosystem similar to a priming effect. This finding is in line with a previous report of Boy and Wilcke (2008) that – paradoxically – in phases of high Ca inputs e.g., because Sahara dust-derived Ca reached the study ecosystem, more Ca was retained in the canopy and on ecosystem level, compared to periods of low Ca input.

If microorganisms and plants lacked Ca, the higher Ca uptake in the second and third application periods compared to the first period could also be explained as consequence of a priming effect in the first application period in line with the observation of reduced Ca leaching to the deeper subsoil during the second and third fertilization periods.

6. Conclusions

The reduced fine root biomass in the Ca treatment compared to the control suggests that Ca was a (co-)limiting element resulting – on the long run – in increased tree diameter growth which is already indicated 1.5 yr after the first Ca addition (although not yet statistically significant).

The low Al concentrations inferred from an adjacent site, the high molar Ca:Al concentration ratios in soil solution and plant tissue (with the exception of the Al-accumulating *G. emarginata*), and the almost complete organic complexation of dissolved Al in the soil solution of the organic layer prior to the first Ca application and in the control treatment all suggest that the positive response of the vegetation to Ca application is not attributable to an alleviation of Al toxicity.

Retention of Ca in the canopy from total atmospheric deposition and small leaching losses of applied Ca (<4%) from the organic layer to the mineral soil indicate that the added Ca is almost entirely retained in the aboveground part of the ecosystem including the soil organic layer. We interpret this as a consequence of reduced nutrient stress by Ca application.

From these results, we conclude that a future elevated Ca deposition would stimulate tree growth in the study forest.

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Appendix A:

Spatial throughfall heterogeneity in a montane rain forest in Ecuador: Extent, temporal stability and drivers *

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1. Abstract

The drivers of spatial throughfall heterogeneity are still not fully understood. At an undisturbed forest site in the Ecuadorian Andes with ca. 2600 mm of annual rainfall we determined the accuracy of throughfall measurements by comparing Hellmann-type funnel gauges with troughs. At the same undisturbed and a managed, selectively-logged forest site we determined spatial variability of throughfall, temporal stability of spatial variability and the controls of spatial throughfall variability using a 4-yr dataset in weekly resolution. There were no systematic differences between the collected volumes of funnel gauges and troughs. Based on the statistical distribution of annual throughfall volumes, a high number of 27 funnel-type rainfall collectors were required in the undisturbed forest and 20 in the managed forest to estimate throughfall with an error of 10% and a confidence interval of 95%. Spatial throughfall variability in the studied forests was high, markedly stable during four years and similar in six selected rain events suggesting that a stable canopy structure controlled throughfall variability. After mathematically eliminating the canopy influence, no meteorological variable had a significant effect on throughfall variability. We conclude that the high spatial variability of throughfall in the study forest, mainly controlled by a long-term stable canopy structure, contributes to the creation of different ecological niches which are a prerequisite for the enormous biological diversity of the north Andean forests.

2. Introduction

Incident rainfall in forests is partitioned into throughfall, stemflow, and interception loss. Throughfall is the portion of rainfall reaching the forest floor as direct throughfall (without interception by the canopy), cascading through the canopy, and dripping from the crowns after temporal storage in the canopy. Throughfall in tropical montane rain forests ranges from 62% (Cavelier et al. 1997) to 97% (Chuyong et al. 2004) of incident rainfall. In general, it represents approximately four-fifths of incident rainfall (Levia and Frost 2006). Water running down the trunks is referred to as stemflow but comprises a minor contribution to the water flux in a forest ranging from 0.1% (Veneklaas 1990) to an exceptional 12.2% (Mamanteo and Veracion 1985). In most tropical forests including the north Andean montane forest, stemflow does not exceed 2% of rainfall (e.g., Lloyd and Marques 1988, Cavelier et al. 1997, Wilcke et al. 2001, Chuyong et al. 2004, Lilienfein and Wilcke 2004). The difference between incident rainfall and the sum of throughfall and stemflow is interception loss.

Throughfall is known to show considerable spatial variability posing severe problems for representative sampling (Levia and Frost 2006). Throughfall can be measured with troughs or with funnel gauges. Troughs have higher surface areas than funnel gauges allowing to be exposed to more drip points (Kostelnik et al. 1989) but funnel gauges can more easily be manifold replicated. Furthermore, the representativity of sampling can be improved by roving the samplers after each sample collection (Kimmins 1973, Lloyd and Marques 1988). There are a number of studies in which the sample size for representative measurement of throughfall was determined for temperate forests and a tropical lowland forest (Levia and Frost 2006) but not yet for a tropical montane forest. To estimate throughfall volume with an error of not more than 10% with a 95% confidence interval, one needs 9-90 funnel gauges in a 30-40 year old coniferous stand in a temperate region (Kimmins 1973) or 7-44 funnel gauges in a temperate hardwood forest (Houle et al. 1999).

Throughfall heterogeneity is influenced by canopy architecture. A higher epiphytic load of the canopy and certain leaf morphologies can provide more drip points, leading to more throughfall variability (Fleischbein et al. 2005, Staelens et al. 2006). Several studies tried to relate throughfall variability with canopy cover and gauge position, such as distance from gauge to stem. While Robson et al. (1994) found that throughfall decreased with increasing distance from the tree stem in a beech forest in southern England, Beier et al. (1993) reported the reverse in a spruce forest in Denmark. Loustau et al. (1992) did not observe any correlation between

throughfall volume and distance from tree stem in a maritime pine stand in France. Loescher et al. (2002) observed a weak relationship (r = 0.33) between percent plant cover and throughfall volume. In the study of Fleischbein et al. (2005), direct throughfall was negatively correlated with leaf area index. In a tropical rainforest in Kalimantan, Indonesia, interception losses decreased from 11% to 6% because of logging and throughfall volume increased correspondingly (Asdak et al. 1998). Thus, manipulations of the forest cover e.g., by improvement felling (i.e. logging of selected trees to improve growth of potential crop trees) are expected to have an impact on throughfall heterogeneity.

As throughfall influences local soil moisture (Schaap and Bouten 1997) and carries directly plant-available nutrients to the soil, throughfall heterogeneity possibly creates a small-scale heterogeneity of ecological niches (Parker 1983). Wilcke et al. (2002) demonstrated that soil nutrients are heterogeneously distributed in the organic layer of a tropical montane rain forest but were not able to offer a mechanistic explanation for this heterogeneity. Throughfall could contribute to explain the spatial variability of soil fertility if the spatial throughfall pattern was stable in the long run. Thus, temporal stability of spatial patterns is an ecologically important issue. A visual way to represent the stability of spatial variability in throughfall volume is by using so-called time-stability plots (Raat et al. 2002, Staelens et al. 2006). This technique was first applied by Vachaud et al. (1985) to describe soil water storage variability and transferred by Raat et al. (2002) to spatial throughfall variability. In a 3-month study in a tropical montane rainforest in Ecuador, Zimmermann et al. (2007) analysed five throughfall events and found that throughfall is spatially heterogeneous (mean coefficient of variation of 53%) and that throughfall patterns were stable during the monitored period.

In several studies it was shown that meteorological conditions influence spatial throughfall heterogeneity (Levia and Frost 2006). Usually, increasing rainfall volume decreases the variability of throughfall within an event often until a threshold water input is reached after which the variability stabilizes (Llorens et al. 1997, Levia and Frost 2006). Furthermore, rainfall event duration and intensity influence throughfall variability although observations are not consistent with respect to the direction of these effects. However, a separation of the effects of canopy properties and meteorological conditions was not attempted in any of the published studies.

Our objectives were to: (i) determine if throughfall volumes collected by troughs and funnel gauges differ from each other, (ii) quantify the spatial variability of annual throughfall in

an undisturbed and a moderately thinned Ecuadorian tropical montane forest and derive the necessary number of funnel gauges for representative measurement, (iii) assess the temporal stability of the throughfall patterns for a period of four years and (iv) investigate into the meteorological controls of spatial throughfall heterogeneity after accounting for the influence of canopy properties. We hypothesized that: (i) troughs and funnel gauges collect the same throughfall volume, (ii) the variability of throughfall in an undisturbed tropical montane forest is high and is increased by thinning (iii) that throughfall patterns in the study forest are temporally highly stable even at the scale of several years, and (iv) that meteorological variables are important drivers of throughfall heterogeneity even after mathematical elimination of the canopy influence.

3. Materials and methods

3.1. Study area

The studied forest is located in south Ecuador on the eastern slope of the Cordillera Real of the Andes (i.e. the eastern cordillera) at an altitude between 1900 and 2000 m a.s.l. (3°58´S, 79°05´W), near the research station Estación Cientifica San Francisco in the deeply incised valley of the Rio San Francisco that drains into the Amazon. Our studied forest is located on the north-facing flank of the San Francisco valley. The vegetation of the study site is a Lower Montane Forest according to Bruijnzeel and Hamilton (2000). In the study area, more than 250 tree species have been identified so far with an above average abundance of the plant families Lauraceae, Melastomataceae and Rubiaceae. Most of the tree species are evergreen (Bendix et al. 2006). *Graffenrieda emarginata* (Melastomaceae) is one of the most common tree species. The high plant species diversity in the whole research area is also characterized by about 140 climber species, more than 400 angiosperm epiphytes, up to 98 vascular epiphytes species on single trees, 248 ferns and ferns allies and more than 525 bryophytes (Homeier et al. 2008). The forest has a mean canopy height of 18.9 m, and 80 trees – which were reaching the lower canopy – had a mean diameter at breast height (dbh) of 122 mm. Leaf area index ranges between 5.2-9.3 m^2 m^{-2} (Fleischbein et al. 2005).

Mean annual temperature in the research area is 15.2 °C. The coldest months are June and July, respectively, with a mean temperature of 14.4 °C; the warmest month is November with a mean temperature of 16.1 °C. The average temperature gradient between a meteorological station at 1952 m and another station at 2927 m a.s.l. is 0.61 °C per 100 m increase in elevation (Bendix

et al. 2008). The distribution of the annual rainfall is unimodal with a maximum between April and September and without a dry season (Fleischbein et al. 2005), which is typical for eastern Andean slopes at altitudes between 1000 and 3600 m a.s.l. Mean annual rainfall is 2569 mm yr^{-1}. During a 1468 day-period of meteorological observations at the meteorological station, 2971 rainfall events were registered. The events had a mean intensity of 0.41 mm h^{-1} and a mean duration of 9 h and 18 min. Events were separated by a dry period of at least 2 h, which was the time considered to dry the canopy completely. Seventy-three percent of the events had 0-2 mm of rain, 20% were larger than 2 mm and smaller than 10 mm, and only 7% were larger than 10 mm (Fleischbein et al. 2006). Horizontal rain in the study forest was small, accounting for <6% of the incident rainfall (Bendix et al. 2008). Mean humidity was 86% (with 90% continuously from April to June 2001) and 79% in November 2000. The mean speed of the mainly easterly winds for the period between April 1998 and April 2001 was 1.5 m s^{-1} with a maximum of 7.9 m s^{-1}.

3.2. Experimental design

Between May 2004 and May 2008, throughfall samples were collected in weekly intervals from three 20 m-long transects at lower slope position covering 10 altitudinal meter from 1900-1910, 1950-1960, and 2000-2010 m a.s.l. in a 9.1 ha large undisturbed microcatchment. We decided to collect rainfall samples in weekly intervals instead of sampling individual events because of the frequent slight rain events and because the rainfall samples are the basis for long-term nutrient budgets. Additionally, in the managed forest catchment which was ca. 12.1 ha large, throughfall samples were collected from three ca. 50 x 50 m large plots at 1875-2055 m a.s.l. where improvement fellings took place in June 2004. On the experimentally thinned site, all trees with dbh >0.20 m were registered out of a list of defined timber species, including *Tabebuia chrysantha*, *Cedrela* sp., and *Podocarpus oleifolius*, being the most valuable ones, followed by *Nectandra membranacea*, *Hyeronima asperifolia* and *H. moritziana*, and finally *Inga acreana*, *Clusia cf ducuoides*, and *Ficus subandina* with reduced economic value. Before the improvement felling, basal area of the site was 24.3 ± s.e. 1.8 m^2 ha^{-1} which represent all trees and palms with dbh ≥0.20 m. On four ha, some 215 selected individuals on a list of defined timber species were considered as potential crop trees (PCTs). The most effective crown competitors of these trees (accounting for 13.5% of the original tree basal area) were felled in June 2004 in order to stimulate growth and natural regeneration of the PCTs. Silvicultural measures usually always go along with damages to the remnant stand. In the thinned stand, 8.6% (3.8-16.8%) of the forest

surface suffered collision damage from the felled crowns (Günter et al. 2008, Wilcke et al. 2009). Along each transect of 20 m in the undisturbed forest, 20 fixed-positioned funnel gauges were randomly distributed. On each plot of the managed forest, 20 fixed-positioned funnel gauges were regularly distributed along four diagonals in a star shape. Funnel gauges consisted of a 2-l polyethylene sampling bottle and a polyethylene funnel with 115 mm diameter. The rims of the funnels were 0.3 m above the soil surface to avoid splash-in effects from the forest floor. The funnels were vertical to avoid splash-out. To reduce evaporation, a table-tennis ball was used in the funnel and the collecting bottle was wrapped with aluminium foil.

Furthermore, from May 2002 to May 2003, six troughs were installed at the lowermost transect in the undisturbed forest in addition to the funnel gauges for comparison. Each trough had a length of 3 m and a width of 0.1 m. It consisted of a plastic pipe cut in half. Throughfall volume of these two halves was collected in the same collecting tank. Each trough was installed according to Van Ek and Draaijers (1994) with a constant angle of 25°. This was accomplished through a flexible construction that enabled adjusting the angle in case of disturbances. The total horizontally-projected collecting area of the troughs was 1.63 m^2. Throughfall volume of troughs between May 2002 and May 2003 was compared with throughfall volume collected by 52 funnel gauges in the undisturbed patch of forest in the same period (in total representing a collecting area of 0.54 m^2). Throughfall volumes were measured with a graduated cylinder in the field.

3.3. Meteorological data

Meteorological data was obtained from the automatic meteorological station on a clear-cut area in ridge-top position at 1952 m a.s.l. Air temperature was measured at a height of 2 m above soil surface with an electronic sensor (Pt100). Relative air humidity was measured with a hygrometer at 2 m above soil surface. Wind velocity was measured with a four-cup anemometer at 2.5 m above soil surface. Total radiation was measured with a pyranometer at 2 m above soil surface with a sensor after WMO and ISO 9060 standards. Rainfall was measured with a tipping-bucket funnel gauge. All meteorological data were recorded with measuring intervals of 1 h and stored with a data logger (Logger DL 15). Since our dataset of throughfall volume is on weekly basis, we used weekly mean meteorological parameters for our analyses. Data of the meteorological station was only available for an almost 3-yr period from 19 May 2004 to 4 April 2007. We calculated weekly means of temperature (T_M, °C), relative humidity (h, %), wind velocity (u, m/s) and we calculated weekly sums of both total radiation (G, W/m^2), and incident

rainfall (RF, mm). During the 3-yr period, there was only 1 week with only a single rain event. Therefore, we allowed a maximum of 4-h dry period between two rain events in order to regard it as one single event, which was arbitrarily done as well by Rodrigo and Àvila (2001). This resulted in six weeks with only one rain event (on 19/01/2005, 13/07/2005, 20/07/2005, 21/09/2005, 24/05/2006, and 21/02/2007).

3.4. Calculations and statistical analyses

3.4.1. Annual measures

Prior to data analyses we excluded all dry weeks (collected volume in the field ≤1 mm) to yield unimodal distributions. For a probability distribution function of yearly rainfall, weekly totals were summed up to yearly totals per rain gauge. We used oneway ANOVA to test the homogeneity of the three mean annual throughfall values of each transect (number of replicate measurement stations = 3, number of gauges per station = 20) of both, the undisturbed and managed forest site, respectively, prior to combining the results of all 60 samplers to one large data set per forest treatment (undisturbed/managed). The Kolmogorov-Smirnov test was used to test normality of the dataset. To estimate a required number of collectors with a certain acceptable error and confidence interval for the undisturbed and managed forests, mean annual throughfall was calculated from our 4-yr dataset of 60 rainfall collectors and taken as the true throughfall amount. Furthermore, the coefficient of variation (*CV*) of annual throughfall heterogeneity among *n* collectors was calculated for annual throughfall volumes. This information was used as input for Eq. 1 (Kimmins 1973).

$$m = \frac{t^2_{(\alpha,n-1)} CV^2}{E^2} \qquad (1)$$

where *m* is the estimated amount of required collectors, *t* is the Student´s *t*-value with an error probability of α and *n* - 1 degrees of freedom, *n* is the number of used samplers (i.e. 60) and *E* is the acceptable error expressed as a percentage of the mean.

3.4.2. Weekly measures

Every meteorological variable was normally distributed except weekly throughfall of the 20 funnel gauges in the undisturbed forest (although the annual throughfall totals were normally distributed). Therefore, we calculated interquartile ranges (IQR), i.e. the 50% range of data between the upper and the lower quartile, to express spatial throughfall variation. The IQR is a

robust measure of variability and does not require normality of the data as it is the case for the standard deviation. In the case of a normally distributed variable, IQR includes 50%, whereas the standard deviation only accounts for 34.1% of the data.

As weekly throughfall volumes were frequently not normally distributed, we used medians instead of arithmetic means for the calculation of time-stability plots. The deviation of the throughfall volume of an individual sampler from the overall median of all samplers for a given week for each of the undisturbed and managed forests was calculated with Eq. 2:

$$\delta_{t,j} = \left(TF_{t,j} - \tilde{TF}_t \right) \left(\tilde{TF}_t \right)^{-1} \quad (2)$$

where $\delta_{t,j}$ is the deviation of the throughfall volume at time t and location j from the median throughfall volume of all 60 samplers in undisturbed and managed forest, respectively, normalized to the median throughfall of all locations j at time t ($\tilde{TF}_t$) in the undisturbed and managed forests, respectively, and $TF_{t,j}$ is the throughfall volume at time t and location j. Thereafter, we calculated the temporal median of $\delta_{t,j}$ (i.e. $\tilde{\delta}_j$) for each individual sampler and arranged the $\tilde{\delta}_j$ values from the smallest to the largest. This resulted in a graphical representation of the deviation of the throughfall at a given location from the overall median of all 60 samplers at all times ($\tilde{TF}_t$) in each of the undisturbed and managed forests. Locations with $\delta_{t,j}$ <0 or $\delta_{t,j}$ >0, collect less or more throughfall, respectively, than the overall median at time t. To be able to assess the influence of meteorological conditions on spatial throughfall variability, the part of the spatial variability which is attributable to canopy structure must be eliminated prior to analyzing the relationship between meteorological conditions and throughfall variability. To eliminate the effect of the canopy structure on throughfall variability we used our time-stability plots (Fig. 4). We attributed the differences between the throughfall median at one sample location over the whole research period (further called time-median of a collector at location j; $\tilde{TF}_j$) and the overall throughfall median at all locations for the whole research period ($\tilde{TF}$) to canopy structure alone and assumed that the scatter around the time-median from each collector was produced by other factors including meteorological conditions and temporal changes of the canopy (which we did not determine). Therefore, we rescaled the time-medians of all collectors according to Eq. 3 to calculate modified throughfall volumes ($MTV_{t,j}$) for each monitored week at each sampler.

$$MTV_{t,j} = TF_{t,j} \frac{\tilde{TF}}{\tilde{TF}_j} \quad (3)$$

This scaling removed the systematic differences of the time-medians of the individual collectors which we attributed to canopy structure but still allowed for a scatter around the adjusted time-median that was attributable to other controls.

Significance was set at $P \leq 0.05$. Statistical analyses were run using R version 2.4.1 (2006) and SPSS 14.0.

4. Results and discussion

4.1. Accuracy of rainfall and throughfall measurement

The accuracy of our rainfall measurement was tested by comparing eight different recording and collecting techniques (Rollenbeck et al. 2007). It was shown that sampling with our funnel gauges deviated by <10% from a tipping bucket-based measurement with a Hellmann-type collector at the same site and was similarly close to all other rainfall measurements.

Before starting the 4-yr throughfall monitoring, we assessed the accuracy of our funnel-based throughfall measurement by comparison with independent measurements using troughs in the undisturbed forest. Mean weekly throughfall of the 52 funnel gauges correlated closely with mean weekly throughfall of the six troughs (r = 0.86, Fig. 1). The regression line had a slope close to one (b = 1.01) and illustrates that there are no systematic deviations (P = 0.71) in collected throughfall volumes between both collector types. These results demonstrate that the accuracy of the throughfall measurement with the later installed 60 funnel gauges (total horizontally-projected surface area: 0.62 m^2) is similar to that of the measurement with troughs (surface area: 1.63 m^2). Our results are similar to those of Llorens et al. (1997) who reported a slope of regression not different from unity for the regression line of throughfall collected by funnel gauges on throughfall collected by troughs in a Mediterranean pine forest. Reynolds and Neal (1991) observed the same close correlation between funnel gauges and troughs under 24-yr old conifer stands and concluded that annual total throughfall amounts collected by troughs or funnel gauges are not significantly different and that there was no evidence to reject a specific type of collector based on the accuracy.

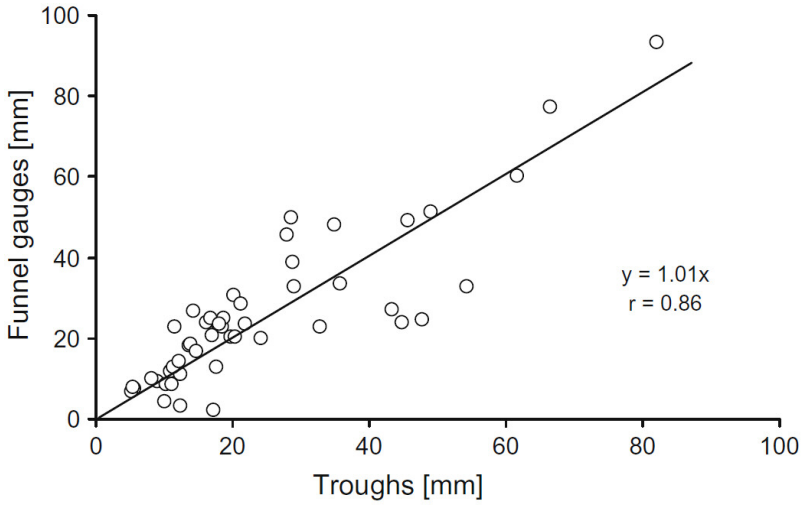

Fig. 1: Relationship between weekly throughfall volume measured with troughs and funnel gauges (May 2002-2003).

4.2. Annual rainfall and throughfall

Annual rainfall and throughfall volumes were calculated as sums of weekly volumes for each individual collector. Incident rainfall at the managed forest plots (RF2) did not differ significantly ($P = 0.94$) from that at the undisturbed forest (RF1, Table 1). Mean annual incident rainfall was similar to the mean annual incident rainfall of the period 1998–2003 (Wilcke et al. 2008). Thus, the monitored period represented average rainfall conditions.

Mean annual throughfall volume of the three measurement transects in the undisturbed forest (TF-U1, TF-U2, TF-U3) were not significantly different from each other ($P = 0.47$) according to the one-way ANOVA. The same was true for the three plots in the managed forest (TF-M1, TF-M2, TF-M3, $P = 0.07$). Consequently, each of the samples of the three transects in the undisturbed forest and the three plots in the managed forest belong to the same population. Therefore, we grouped the samples to one large population of $n = 60$ samplers for the undisturbed and managed forests, respectively. Annual throughfall volume in both the undisturbed and the managed forests was normally distributed and thus the prerequisite for the use of parametric statistics was met.

Mean annual throughfall in the undisturbed forest was significantly lower ($P < 0.001$) than in the managed forest. This is attributable to the a priori higher canopy openness in the managed forest already before the improvement fellings (Günter et al. 2008). The further significant shift in canopy openness after the improvement fellings that removed 13.5% of the

initial basal area from 24.3 ± 1.8 m^2 ha^{-1} to 21.8 ± 1.9 m^2 ha^{-1} of trees with a dbh of >0.2 m did not change the throughfall significantly (Wilcke et al. 2009).

Table 1: Means and ranges of annual incident rainfall (RF) at two rainfall gauging stations (1 and 2) and throughfall (TF) at three throughfall gauging stations in each of undisturbed (U1-U3) and managed forests (M1-M3, n = 5 for the incident rainfall gauging stations and n = 20 for the throughfall gauging stations).

	RF1	RF2	TF-U1	TF-U2	TF-U3	TF-M1	TF-M2	TF-M3
Mean annual water volume (mm)	2527	2611	1419	1470	1498	1682	1912	1636
Standard deviation water volume (mm)	400	397	244	171	238	257	228	229
Coefficient of variation (%)	16	15	17	12	16	15	12	14

Mean annual throughfall were 58% and 67% of incident rainfall in the undisturbed and managed forests, respectively (Table 1). Throughfall values are at the lower end of throughfall percentage of rainfall in other tropical montane forests accounting for 62.4% in Panama (Cavelier et al. 1997), 70% in Costa Rica (Hölscher et al. 2004), 73-77% in Puerto Rico (Holwerda et al. 2006) and 92-97% in Cameroon (Chuyong et al. 2004). Similarly low values were previously reported by Fleischbein et al. (2005) and Wilcke et al. (2008) for the same study site. While particularly the study of Fleischbein et al. (2005) might have suffered from a too low number of throughfall samplers, we are now confident that our measurement of throughfall is correct based on the comparison measurements outlined in the previous section. Thus, the extremely high interception losses must be considered as realistic. We attribute the high interception losses to the dominating slight drizzle and advected energy from the lowland by mountainvalley wind systems as well as the high epiphytic and bryophytic load in the canopy. There were 3 weeks in the undisturbed and 4 weeks in the managed forest for which volume of throughfall was higher than that of incident rainfall (Fig. 2). This is attributed to extreme fog events where fog entrapment by the canopy leads to enhanced throughfall volume (Wilcke et al. 2001, Fleischbein et al. 2006).

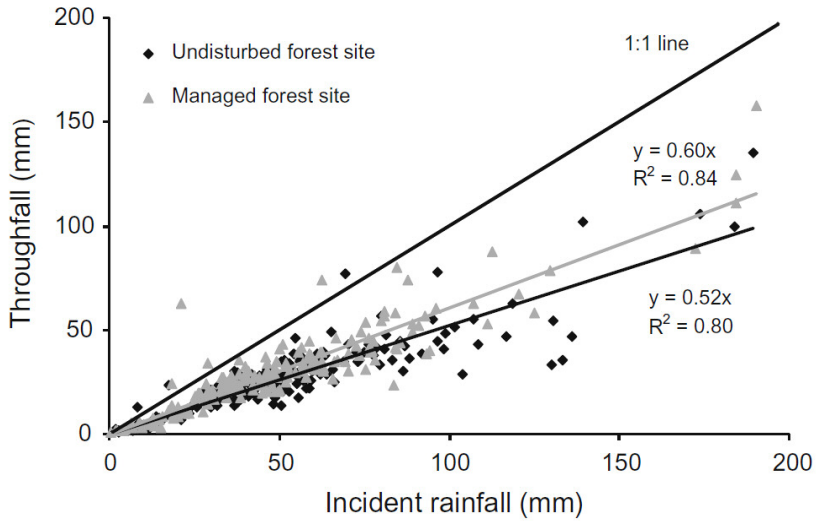

Fig. 2: Relationship between weekly mean incident rainfall and median throughfall in the undisturbed and managed forests.

4.3. Spatial throughfall variability

In a first step, we calculated the required number of throughfall samplers in the undisturbed and the managed forests, respectively, to determine annual means with an acceptable error by assuming that the mean annual throughfall of all 60 samplers represents the true value. For the undisturbed forest, a minimum sampler number of $n = 27$ is required for an estimation of mean annual throughfall within an acceptable error of $E = 10\%$ ($\alpha = 0.05$, Fig. 3a). However, the required sample size decreases to $n = 15$, if an error of $E = 15\%$ ($\alpha = 0.05$) is accepted.

In the managed forest, fewer gauges ($n = 20$) are needed compared to the undisturbed forest to represent throughfall with the same error of 10% (Fig. 3b). The required sample size was $n = 9$ gauges if the error of $E = 15\%$ is accepted. The higher canopy openness in the managed forest might reduce potential drip points (Loescher et al. 2002) and hence produce less spatial variability in throughfall as suggested by Carlyle-Moses et al. (2004). One might expect that a forest with a multi-layered, particularly dense canopy is homogeneous and therefore would cause a relative low spatial variability. Opening the canopy by removing some trees in the stand should therefore increase the spatial throughfall variability. Our calculations show the opposite result. Selective logging of trees decreased spatial variability. The high spatial throughfall variability can only be attributed to the high heterogeneity of the canopy structure in the undisturbed forest caused by the 250 tree species present in the forested region and the presence of numerous species of climbers, angiosperm and vascular epiphytes, ferns and bryophytes.

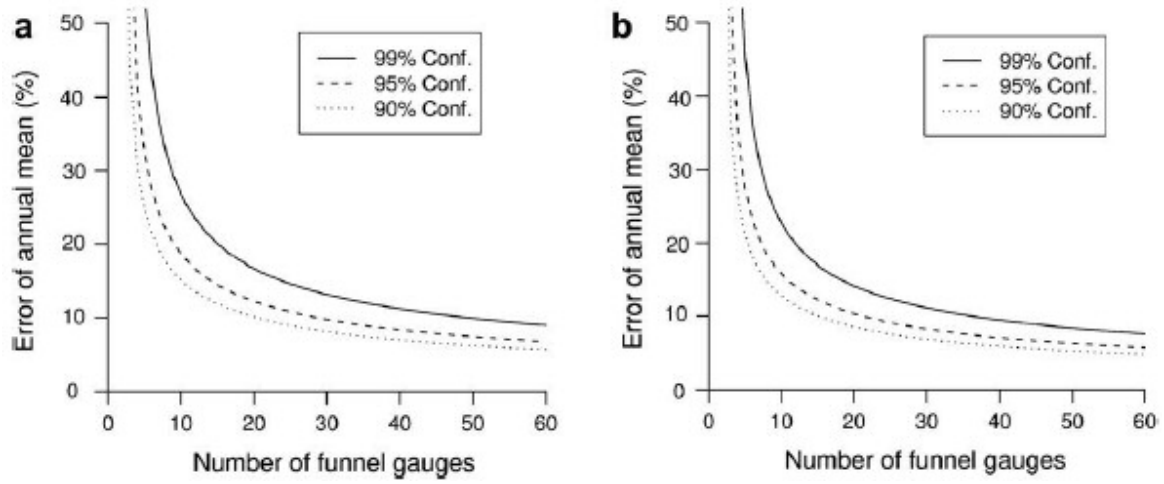

Fig. 3: Error of annual throughfall volume as related to the number of throughfall samplers for different confidence intervals in: (a) undisturbed forest and (b) managed forest.

Table 2: Number of collectors needed to calculate throughfall with a given maximum error in different forest ecosystems. Only publications with more than 15 collectors were taken into account.

Calculated number of gauges[a]	Number of used funnel gauges	Forest type and location	Size of gauges (cm^2)	Duration of study	Reference
27	60	Undisturbed forest, Ecuador; 4°S, 79°W	104	208 Weekly collections	This study
20	60	Managed forest, Ecuador; 4°S, 79°W	104	208 Weekly collections	This study
28[b]	36	Hardwood forest; 46°N, 71°W	577	163 Rain events	Houle et al. (1999)
9–90[b,c]	43	30–40 year olds coniferous stand,	121	8 Weekly collections	Kimmins (1973)
8–21[d]	40	Even-aged mixed hardwood forest, temperate; 40°N, 78°W	1134	6 Rain events	Kostelnik et al. (1989)
8–52, 24[e]	40	Coniferous forest, temperate zone; 45°N, 68 W	342	23 Monthly collections	Lawrence and Fernandez (1993)
8–14[d]	50	Hardwood forest, temperate; 39°N, 77°W	182	5 Rain events	Pucket (1991)
3, 9 [f]	25	Subtropical forest; 24°N, 99°W	n.a.	18 Rain events	Carlyle-Moses et al. (2004)
9, 11[g]	32	Evergreen Mediterranean forest; 41°N, 2°E	79	144 and 178 Rain events[h]	Rodrigo and Àvila (2001)
2–15, 6 (mean)[d,h]	96	Oak-hickory forest	189	5 Rain events	Peterson and Rolfe (1979)
11[b,h]	100	Bialowieza National Park	n.a.	26 Rain events	Czarnowski and Olszewski (1970)

n.a.: Data not available.

[a] Number of gauges necessary to measure throughfall volume with an error of 10% and a confidence interval of 95%.

[b] Data were read from a graph.

[c] Data represent a range of four different forest ecosystem types.

[d] This range is the minimum and maximum of calculated required sample sizes.

[e] Data represent number of necessary collectors needed if calculating with minimum, maximum and median coefficient of variation, respectively.

[f] The first number indicates the required number of gauges for a cumulative study period of 18 rain events and the latter number indicates the mean of required number of gauges analysing single rain events.

[g] Numbers indicate the required number of gauges for a whole study period at two different study sites.

[h] These data were calculated from available data in the publication.

The required sample number at our study site is high compared to most other studies (Table 2) indicating that throughfall at our study site was particularly heterogeneous. The fact that tropical montane forests are characterized by abundant epiphytes in the canopy providing many potential drip points might be an explanation (Fleischbein et al. 2005). Particularly, the high number of epiphytic bromeliads might contribute to the extraordinary variability of throughfall because the bromeliads can store high water volumes, which can suddenly be released resulting in a strong drip point (Hölscher et al. 2004).

4.4. Time stability of spatial throughfall variability

In the undisturbed forest, the sampler with the lowest median of the weekly throughfall collected 65% of the weekly median of all 60 samplers during the 4-yr monitoring period while the sampler with the highest median of the weekly throughfall collected 181% (Fig. 4a). The spatial throughfall pattern in the managed forest (Fig. 4b) was comparable to the undisturbed forest with relative differences among the individual samplers ranging from 70% to 156%. In a 2-yr study at a mixed deciduous forest in Belgium, Staelens et al. (2006) observed a time-averaged relative difference of less than 20% between the sampler collecting the least and that collecting the most throughfall. Thus, the differences in relative throughfall among the various sampling sites of our study forest are comparatively high.

The average temporal IQR of throughfall collectors with the lowest weekly median throughfall (Fig. 4) from the undisturbed and managed forests (IQR = 0.50 and 0.47, respectively) were about two times smaller compared to the temporal IQR of throughfall collectors with the highest weekly median throughfall. Hence, throughfall at dryer spots in the forest shows a considerably smaller temporal variability than at wetter spots in the forest. As described by Zimmermann et al. (2007), the dryer spots likely belong to locations covered by a shelter such as thick moss mats that can store much water and act as a buffer for throughfall. In contrast, throughfall collectors at wetter locations might be placed below drip points like from bromeliads that can store water in a tank structure but once saturated, provide a huge overrun of water (Zotz and Thomas 1999).

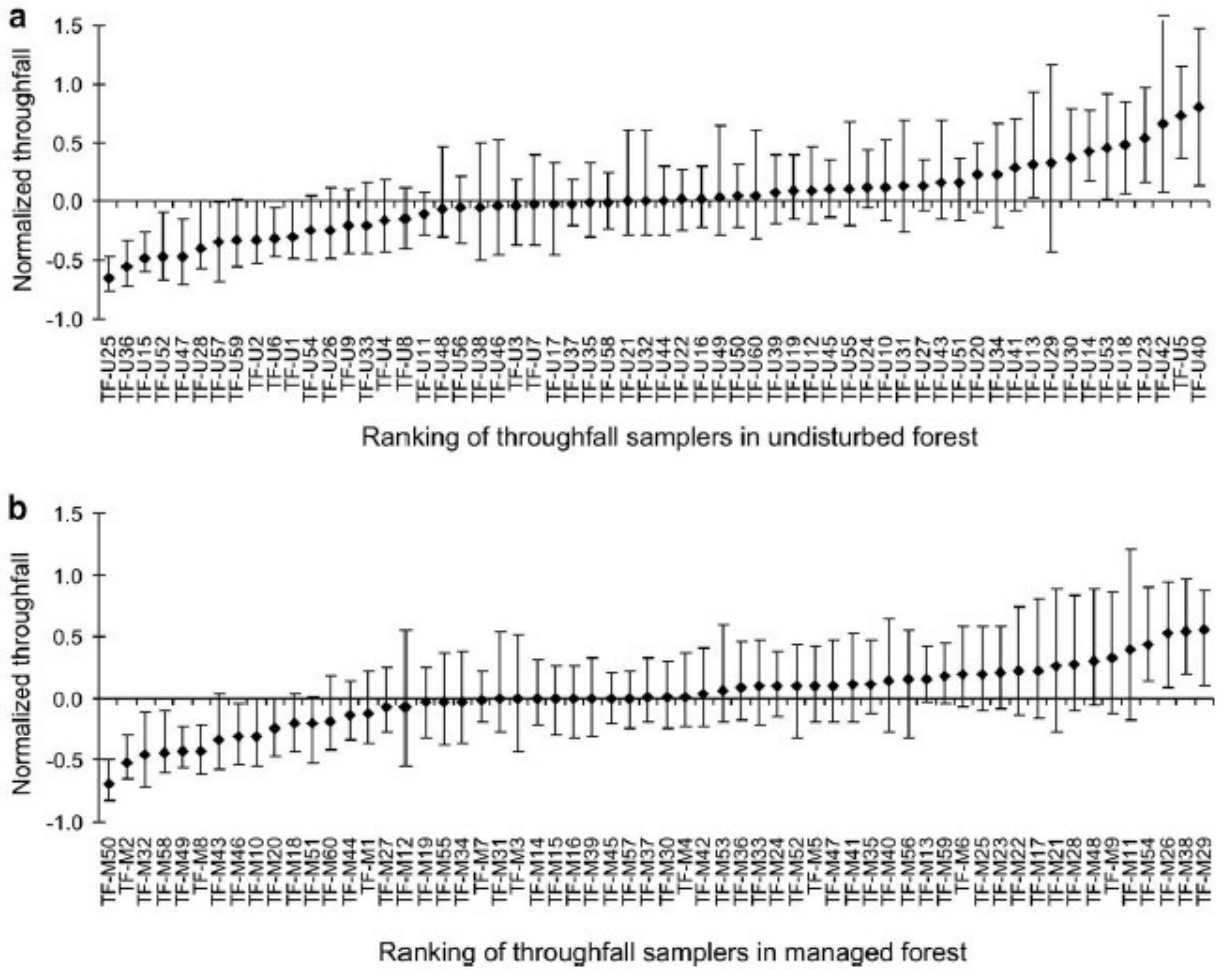

Fig. 4: Time stability plots of weekly throughfall for: (a) undisturbed forest and (b) managed forest. Normalized throughfall calculated according to Eq. 2. Lower error whisker shows 1st quartile, upper error whisker shows 3rd quartile.

As the time stability might be influenced by the duration of the observation period for which throughfall data are averaged, we additionally drew time-stability plots for the selected six weeks with one rain event and for monthly throughfall means. Figure 5 illustrates that, independent of the time scale, all time-stability plots indicate extreme stability. The time stability is even more pronounced for the individual rain events than for the longer-term intervals while the plots of weekly and monthly intervals are not different from each other. Given the low number of considered individual rain events and the overlap of the temporal IQRs of throughfall (shown as error bars in Fig. 5) collected by all individual samplers at all three time scales we conclude that there is no indication that the duration of the time interval used for constructing

time-stability plots had an influence on the finding of high time stability of throughfall at our study site.

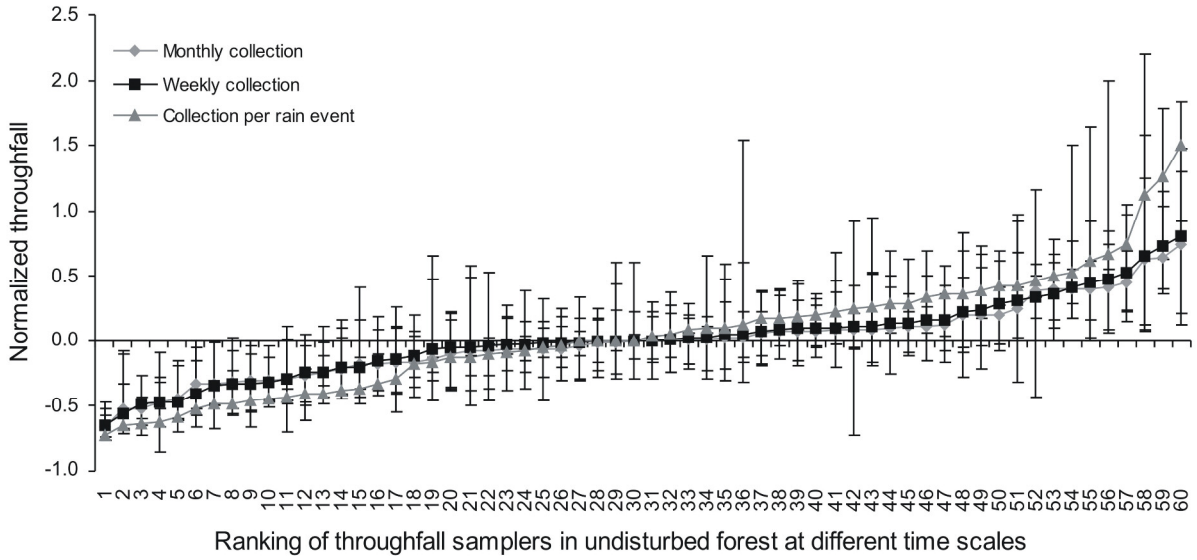

Fig. 5: Time stability plots in the undisturbed forest at different time intervals: one rain event per week, weekly and monthly data collection. Normalized throughfall calculated according to Eq. (2). Lower error whisker shows 1st quartile, upper error whisker shows 3rd quartile. Note that the ranking of the individual samplers was slightly different among the different time scales and therefore samplers are only continuously numbered in the order of increasing time-medians but not labelled with the sampler designation.

Our finding of a high time stability of spatial throughfall patterns during the whole monitored period of four years, which is to our knowledge the longest period ever used for drawing time-stability plots, suggests that throughfall variability indeed results in the creation of substantially different living conditions for soil organisms and plant growth because of the persistently different water and nutrient availability. Thus, throughfall variability may contribute to explain the extremely high heterogeneity of soil properties at our study site (Wilcke et al. 2002).

4.5. Controls of spatial throughfall variability

In the review of Levia and Frost (2006) it is stated that there is still a knowledge gap about the effect of meteorological conditions on the variability of throughfall volume. However, to be able to assess the influence of meteorological conditions, the part of the spatial variability

which is attributable to canopy structure must be eliminated prior to analyzing the relationship between meteorological conditions and throughfall variability.

The high time stability of the spatial throughfall pattern at our study site suggests that canopy structure was the dominating control rather than meteorological conditions because the canopy structure varies less in time compared to meteorological conditions. It therefore seems reasonable to assume that the differences among the normalized median throughfall of the different samplers (Fig. 4) can be attributed to the canopy structure alone. The influence of the canopy structure on the time stability of spatial throughfall can be mathematically eliminated. To achieve this, we rescaled all measured throughfall volumes of all individual collectors (Eq. 3). In the rescaled data set, the remaining spatial variation, expressed as spatial IQR of the rescaled throughfall data can be attributed to other reasons such as variable meteorological conditions or changes in canopy properties (which we did not measure).

To detect the possible influence of meteorological conditions on the remaining spatial IQR of throughfall after mathematical elimination of the canopy influence, we regressed selected meteorological variables (weekly means of relative humidity, temperature, and wind speed, and weekly sums of global radiation and precipitation) on the spatial IQRs of the rescaled throughfall data. We tested linear and exponential fits but did not detect any significant correlation between meteorological conditions and the canopy structure-independent spatial variability. This indicates that meteorological conditions did not have an influence on spatial throughfall variability at our study site.

Furthermore, the ranking of throughfall samplers in the time-stability plot of the selected six individual rain events was similar to the ranking of the samplers in the time-stability plot for a 4-yr period at weekly resolution both in the undisturbed and managed forests (not shown). This further supports our view that throughfall is mainly controlled by canopy structure but not by meteorological conditions.

A further indication of the dominating role of canopy structure for spatial throughfall variability is the differences between the undisturbed and the managed forest sites. These sites are located close to each other and consequently have similar meteorological conditions but differ in canopy properties. Thus, the facts that fewer gauges are needed in the managed than in the undisturbed forest to precisely determine annual throughfall and that the relative differences between the sampler collecting the least and the most throughfall were smaller can only be attributed to the different canopy properties (Fig. 3). Therefore, we reject hypothesis (iv) and

conclude that the canopy structure is the main driver of throughfall heterogeneity. This might be characteristic of evergreen tropical forest. However, Staelens et al. (2006) also pointed at the importance of the canopy cover (tree branches and canopy leaves) on the spatial throughfall variability in the leafy period of a temperate forest.

5. Conclusions

Troughs and (a sufficiently high number of) funnel samplers (n = 52) did not collect different annual throughfall amounts in spite of the three times higher collecting surface of the troughs.

The spatial variability of throughfall in the studied humid Andean tropical montane forest is high requiring 27 throughfall samplers for measurement of annual throughfall volumes in an undisturbed forest with an accepted error of <10% of the annual mean. A higher canopy openness as e.g., induced by thinning of the forest decreased the variability of throughfall at our study site.

Spatial throughfall patterns showed a high temporal stability during the monitored period of four years, which might further enhance the differentiation of ecological niches, contributing to explain the extraordinary plant species richness of the north Andean tropical montane forests. If the high species richness is also a reason for high throughfall heterogeneity, this would be a positive feedback mechanism.

After mathematically eliminating the influence of the canopy structure on spatial throughfall variability, we did not observe an effect of meteorological conditions on spatial throughfall variability and therefore conclude that meteorological conditions are not important drivers of spatial throughfall variability at our study site with its evergreen tropical forest.

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Appendix B

The Appendix is attached as a CD containing the following files (letters at the beginning of the file name indicate the respective sections):

- Contents of Appendix **(Contents.doc)**
- Bulk density of organic layers and mineral soil **(BC_Bulk density.xls)**
- C, N and S concentrations and $\delta^{13}C$ and $\delta^{15}N$ values of soil horizons **(BC_CNS_soil.xls)**
- Electrical conductivity of throughfall, litter leachate and soil solution samples **(BC_Conductivity.xls)**
- Logged tensiometer and FDR data **(BC_Datalogger_NUMEX.xls)**
- Concentrations of base metals and P in the organic layer before fertilization **(BC_destruction organic layer.xls)**
- Effective cation exchange capacity of A and B horizons **(BC_ECEC and BS.xls)**
- Mass, C, Ca, K, Mg, N, Na and P concentrations, $\delta^{13}C$ and $\delta^{15}N$ values and C/N ratios of monthly litterfall samples. **(BC_Litterfall)**
- Concentrations of Cl^-, PO_4^{3-}, total P, NO_3^-, NH_4^+, total N, total dissolved N, K, Na, Mg, Ca, total dissolved organic C and H^+ in litter leachate **(BC_Lysimeter)**
- Equilibrium pH values of soil horizons **(BC_pH soil samples)**
- Concentrations of Cl^-, PO_4^{3-},total P, NO_3^-, NH_4^+, total N, total dissolved N, K, Na, Mg, Ca, total organic C in solution and H^+ in soil solution at 0.15 m mineral soil depth **(BC_SC15.xls)**
- Concentrations of Cl^-, PO_4^{3-},total P, NO_3^-, NH_4^+, total N, total dissolved N, K, Na, Mg, Ca, total organic C in solution and H^+ in soil solution at 0.30 m mineral soil depth **(BC_SC30.xls)**
- Slope of NUMEX plots **(BC_Slope in NUMEX plots.xls)**
- Soil texture of mineral soil **(BC_Soil texture.xls)**
- Manually read tensiometers values **(BC_Tensiometers.xls)**
- Thickness of the soil horizons **(BC_Thickness soil layers.xls)**
- Concentrations of Cl^-, PO_4^{3-},total P, NO_3^-, NH_4^+, total N, total dissolved N, K, Na, Mg, Ca, total organic C in solution and H^+ in throughfall **(BC_Throughfall.xls)**

- Climate data of the meteorological station and incident rainfall and throughfall volumes in MC2 and MC5 (2004-2008) **(D_4-year_climate data.xls)**
- Comparison of throughfall data collected with Hellmann- versus trough-type collectors **(D_trough vs. Hellmann.xls)**

CURRICULUM VITAE

PROJECTS AND LEADERSHIP

Sustainable management of a mega-diverse forest ecosystem, Ecuador (Earth System Science Research Centre, Geographic Institute; Mainz, Germany) **since 2007**

Evaluation of elevated atmospheric nitrogen, phosphorus and calcium input on forest biogeochemical cycles. I planned and organized equipment installation and field work. I have guided the field and laboratory work of about 15 students. In cooperation with the National University of Loja, Ecuador and the NGO Nature and Culture International.

NITROF-project, Panama (Buesgen Institute – Soil Science of Tropical and Subtropical Ecosystems; Göttingen, Germany) **2006**

Measuring the direct impact of elevated atmospheric nitrogen depositions on greenhouse gas emissions of an old-growth forest. In cooperation with Smithsonian Tropical Research Institute, Panama.

Cost – Benefit Analysis of land-use types in a watershed, Lao PDR (Faculty of Forest Sciences and Forest Ecology; Göttingen, Germany) **2006**

Groupleader during fieldwork. In cooperation with the German Technical Cooperation (GTZ); the Mekong River Commission and the National University of Lao PDR.

Green North Sea Docks Project (Centre for Surface Chemistry and Catalysis; Leuven, Belgium) **2003–2004**

Project in waste water treatment: Development of the best environmental practice for decontaminating tributyltin, zinc and copper from dockyard waste waters. I worked on a decontamination method based on sorbents.

BELTO-NVA (BELgique-TOgo – Nouvelle Vision Agricole), Togo **2003 – 2008**

After an agricultural training, I raised funds to buy vans and to ship them to Togo. The vans were filled with goods (e.g., chairs and tables for a school) to help the local communities and improve their living conditions. With these buses, the local community got access to markets, allowing them to get higher revenue for their agricultural products. Owing to this, the local community could finance the construction of a agricultural school by themselves.

EXTRA WORKING EXPERIENCES

- Scientific advisor to the Belgian government delegation in the field of forestry during UN Climate Change Conference in Copenhagen (COP 15) and Bonn **2009**
- Scientific assistant (Faculty of Resources management, University of Applied Sciences and Arts, Göttingen, Germany) **2005**
  Responsible for the transformation and maintenance of a testing plant for biogas production. The goal was to produce energy from biological waste and manure.

ACADEMICAL EDUCATION

M.Sc. in Tropical and International Forestry **2006**
Georg-August University Göttingen, Germany

M.Sc. in Bio-engineering in Environmental Technology; secondary specialisation: Forestry **2004**
Catholic University Leuven, Belgium

B.Sc. in Bio-engineering **2001**
University of Antwerp, Belgium

EXTRA CURRICULAR COURSES

- "Irrigation field day"-basics of practical irrigation technology (Oct. 2007)
- Conference attendance: Tropentag 2006 – Conference on International Agricultural Research for Development, University of Bonn, Germany (Oct. 2006)
- Conference attendance: 2nd Göttingen GIS & Remote Sensing Days – Global Change Issues in Developing and Emerging Countries, University of Göttingen, Germany (Oct. 2006)
- Course in Excellency in Global Forestry, University of Freiburg, Germany (June 2005)
- Workshop: 19th Forest Versatility, University of Brno, Czech Republic (April 2005)
- Workshop: Non Timber Forest Products: a Way to Preserve Forests, University of Göttingen, Germany (Dec. 2004)

LANGUAGE SKILLS

Language	Verbal proficiency	Written proficiency
Dutch	Mother tongue	Mother tongue
English	Very good	Very good
German	Very good	Very good
French	Good	Good
Spanish	Good	Intermediate

PRICES AND AWARDS

My written research proposal got financed by the DAAD-RISE programme (Research Internships in Science and Engineering) with a 12-week stay of a Canadian student as intern, who I guided during her laboratory work. (2009)

Winner of the Bio-medical science award by the University of Antwerp obtained for an excellent presentation about genetic modification of trees. (1999)

MEMBERSHIPS

- IUFRO: International Union of Forest Research Organisations (since 2009)
- DBG: German Soil Science Society (since 2009)
- K VIV: Royal Flemish Society of Engineers (since 2004)
- Natuurpunt: a society in Belgium with a focus on nature and landscape (since 2003)

PEER-REVIEWED PUBLICATIONS

Wullaert H., Homeier J., Valarezo C., Wilcke W. Response of the N and P cycles of an old-growth montane forest in Ecuador to experimental low-level N and P amendments. Submitted on 04/03/2010; manuscript no. FORECO7533; *Forest Ecology and Management*.

Wullaert H., Pohlert T. , Boy J. , Valarezo C. , Wilcke W. (2009). Spatial throughfall heterogeneity in a montane rain forest in Ecuador: Extent, temporal stability and drivers. *Journal of Hydrology.* 377:71-79.

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

Vreysen S., Maes A., **Wullaert H.** (2008). Removal of organotin compounds, Cu and Zn from shipyard wastewaters by adsorption - flocculation: A technical and economical analysis. *Marine Pollution Bulletin*. 56:106-115.

A complete list of abstracts/conference contributions and other publications are available on request or on my website: http://www.staff.uni-mainz.de/wullaert/pub.html.