Internal Nitrogen Cycling in Tropical Forest Soils

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Summary

Growing human population and activities such as the continued increase in fossil fuel consumption, fertilizer use, and biomass burning have considerably increased the amount of atmospheric nitrogen (N) deposition. Until recently, elevated N deposition has been mainly a concern in temperate, highly industrialized regions but deposition of N increasingly occurs also in fast-developing and industrializing tropical regions like Latin America and South-East Asia. The most harmful impacts of elevated N deposition are increases in soil N_2O (a long-lived greenhouse gas also contributing to the depletion of stratospheric ozone) and NO emissions (important for the formation of tropospheric ozone and acid rain) as well as N-leaching to ground and surface waters. The few studies that have been conducted in tropical forests showed that the N status (i.e. high or low N availability) of ecosystems is the key to estimate reactions to elevated N input.

For this reason, the present thesis assessed the internal N cycling of tropical soils under old-growth forests in Ecuador, Costa Rica and Panama. Beforehand, two ways of measuring gross N cycling rates in soils were contrasted, one based on in-situ processing and incubation of soil samples and the other one based on laboratory processing and incubation. Both treatments were followed by the ^{15}N pool dilution technique. This first investigation revealed that reliable data of gross N cycling rates in tropical forest soils can only be obtained from samples that were processed (i.e. injected, incubated and extracted) in-situ. In view of decreasing gross N mineralization rates and increasing gross nitrification rates occurring from soil storage and laboratory incubation before analysis, it followed that the processing of soils has to be undertaken in-situ.

Subsequently, we followed this methodology to examine the gross rates of soil N cycling in forest sites across two Andosol toposequences in Ecuador and Costa Rica and across one Ferralsol toposequence in Costa Rica. The two Andosol sequences showed opposite trends in soil N cycling changes with elevation. While gross N mineralization rates decreased across the altitudinal gradient in Ecuador, an increasing pattern was observed in Costa Rica. For the Costa Rican Ferralsol toposequence, we obtained a decrease in gross mineral N production with elevation. Comparing young Andosols with old, strongly weathered Ferralsols, our data revealed similar gross N production rates. Nevertheless, net N mineralization rates (used as an indicator for plant available N) in Andosols exceeded those of Ferralsols, a finding that was attributed to a lower

microbial NH_4^+ immobilization in these Andosols. This was in contrast to the general assumption that old, highly weathered soils do not limit plant growth by the insufficient supply of N but instead by the limitation of rock derived nutrients. On the other hand, young soils were reported to have relatively low available N.

Furthermore, we assessed the factors that determined the changes in gross N mineralization across these three altitudinal gradients. In Ecuador, changes were paralleled by a declining degree of soil development, while this was not observed along the Costa Rican toposequences. In all toposequences, mean annual air temperature controlled gross N mineralization rates in soils either directly or indirectly by influencing the organic layers. In Costa Rica the mean annual precipitation appeared to be an additional factor behind the mineral N production rates, again, by controlling the organic layers across both altitudinal gradients. In general, organic layers of sites had a great influence on mineral N production in soils. This was manifested in the Ferralsol toposequence, where both quantity and quality of the organic material in topsoil controlled the gross N mineralization rates by regulating the microbial biomass. In the Costa Rican Andosols, it was only the quality of organic substrate that was responsible for changes in mineral N production by controlling the microbial biomass. Across all gradients, we also consider the abundance of N fixing legumes to be responsible for the changes in N cycling rates we observed. No doubt that these factors controlling the soil N cycling are interdependent to some extent. But the fact that these factors varied among the three toposequences suggests that the effect of one factor may counteract and possibly outweigh another, dependent on the soil characteristics and locations.

Finally, we measured changes in soil N cycling rates after experimental chronic N-addition in a tropical lowland forest in Panama. Elevated N input resulted in increasing rates of gross N mineralization, induced by the improving quality of incoming organic substrate. Chronic N-addition decreased pH and tended to reduce the microbial biomass in the top soil. This was reflected in lower microbial NH_4^+ immobilization rates in the N-addition plots. Due to these changes, more NH_4^+ was available for nitrification which was manifested in higher soil extractable NO_3^- concentrations.

Based on these results, we expect gross N mineralization rates to increase with elevated atmospheric N deposition in the tested sites in Ecuador and Costa Rica. The extent may vary according to the factors that controlled the gross N mineralization rates at each of the three gradients. We expect this increase in gross N mineralization rates to

be paralleled by increasing N losses through soil gaseous N-oxide emissions and/or leaching. In general, we assume that possible losses may follow the patterns of mineral N availability (gross N mineralization rates) across the tested toposequences since patterns of nitrification rates resembled the trends of N availability and N losses are strongly connected with nitrification activities.

Zusammenfassung

Wachsende Bevölkerungszahlen und Maßnahmen wie der kontinuierlich steigende Verbrauch fossiler Brennstoffe, der Einsatz von Mineraldünger sowie die Verbrennung von Biomasse haben zu einem erheblichen Anstieg der Deposition atmosphärischen Stickstoffs (N) geführt. Bis vor kurzem gab der Anstieg der N-Deposition nur in hoch industrialisierten Gebieten der gemäßigten Zone Anlass zur Sorge. Mittlerweile ist dieses Problem jedoch auch in tropischen Regionen Lateinamerikas und Südostasiens, in denen eine schnelle Entwicklung und Industrialisierung stattfindet, ernst zu nehmen. Die schädlichsten Auswirkungen einer erhöhten N-Deposition sind die Zunahme der Emissionen von N_2O (ein langlebiges Treibhausgas, welches zum Abbau der stratosphärischen Ozonschicht beiträgt) und NO (welches an der Bildung von troposphärischem Ozon und saurem Regen beteiligt ist) sowie Grund- und Oberflächenwasserbelastungen durch N-Auswaschung. Die wenigen Studien, die sich mit den Auswirkungen erhöhter N-Deposition in tropischen Regenwäldern beschäftigt haben, konnten zeigen, dass der N-Status (das heißt hohe oder geringe Verfügbarkeit von N) der Schlüssel zur Abschätzung zukünftiger Reaktionen auf den erhöhten N-Eintrag ist. Aus diesem Grund wurden in der vorliegenden Arbeit mit Hilfe der ^{15}N Pool Dilution Methode die bodeninternen Brutto-N-Umsatzraten in tropischen Naturwäldern in Ecuador, Costa Rica und Panama untersucht. Die Brutto-N-Mineralisierungsraten zeigen die Verfügbarkeit von mineralischem N an.

Im Vorfeld wurde getestet, ob sich Unterschiede in der Vorbehandlung der Bodenproben auf die Analyse der Brutto-N-Transformationsraten auswirken. Hierfür wurde ein Teil der Bodenproben sofort nach der Probenahme im Wald verarbeitet (^{15}N-Injektion, auf die eine Extraktion mit K_2SO_4 folgte) und *in-situ* inkubiert, wohingegen der andere Teil der Bodenproben vor der Verarbeitung zwei oder dreißig Tage gekühlt im Labor gelagert wurde. Die ^{15}N-Injektion, die K_2SO_4-Extraktion sowie die Inkubation bei standorttypischer Temperatur haben für diesen Teil der Proben im Labor stattgefunden. Diese erste Studie der vorliegenden Dissertation machte deutlich, dass die erhobenen Daten der Brutto-N-Transformationsraten nur verlässlich sind, wenn die Verarbeitung der Proben *in-situ* stattgefunden hat. Die Lagerung und Inkubation im Labor führten zu einer Verminderung der Brutto-N-Mineralisierungsraten in den Proben sowie zu einem deutlichen Anstieg der Brutto-Nitrifikationsraten. Aus diesem Grund

wurden die ^{15}N Injektion, die K_2SO_4-Extraktion und die Inkubation der Proben in den darauf folgenden Teilen dieser Arbeit direkt am Probennahmeort durchgeführt.

Im zweiten Teil der Dissertation wurden die Brutto-N-Umsatzraten in Regenwaldböden entlang zweier Andosol-Höhensequenzen in Ecuador und Costa Rica und einer Ferralsol-Höhensequenz in Costa Rica bestimmt. Die Brutto-N-Mineralisierungssraten veränderten sich in unterschiedlicher Form entlang der beiden Höhensequenzen von Andosolen. Während die Brutto-N-Mineralisierungsraten des Höhengradienten in Ecuador mit zunehmender Höhe abnahmen, stiegen sie entlang der Höhensequenz von Andosolen in Costa Rica an. Entlang der Ferralsol-Höhensequenz wurde eine Abnahme der Brutto-N-Mineralisierung mit zunehmender Höhe festgestellt. Bei dem Vergleich der zwei verschiedenen Bodentypen (relativ junge Andosole und alte, stark verwitterte Ferralsole) wurde kein Unterschied in den Brutto-N-Mineralisierungsraten festgestellt. Allerdings wiesen die Andosole deutlich höhere Raten der Netto-N-Mineralisierung (die als Indikator für den pflanzenverfügbaren N dient) auf als die Ferralsole. Der Grund hierfür liegt in einer geringeren NH_4^+-Immobilisierung durch die mikrobielle Biomasse in den Andosolen. Dieses Ergebnis entspricht nicht der allgemeinen Theorie, dass alte, stark verwitterte Böden das Pflanzenwachstum weniger durch eine unzureichende N-Verfügbarkeit limitieren, als durch die mangelnde Verfügbarkeit von Nährstoffen aus dem Ausgangsgestein, wohingegen junge Böden als eher N-limitiert gelten.

Des Weiteren wurde in dieser Arbeit untersucht, welche Faktoren die Veränderungen der Brutto-N-Mineralisierungsraten entlang der Höhensequenzen verursacht haben. In Ecuador wurde die Abnahme der Brutto-N-Mineralisierungsraten von einer abnehmenden Bodenentwicklung begleitet. Dies konnte in Costa Rica nicht festgestellt werden. Entlang aller Höhensequenzen wurde die Brutto-N-Mineralisierung entweder direkt oder indirekt von der durchschnittlichen Jahrestemperatur mitbestimmt. Indirekt war es die Einflussnahme auf die organische Auflage, die wiederum die mikrobielle Biomasse in den Böden steuerte. Neben der Temperatur hatte in Costa Rica auch der durchschnittliche Jahresniederschlag einen signifikanten Einfluss auf die Brutto-N-Mineralisierungsraten. Der Grund hierfür ist wiederum in einer Einflussnahme auf die organische Auflage zu sehen, welche für den Umfang der mikrobiellen Biomasse und somit für die N-Mineralisierungsraten entlang beider Höhensequenzen verantwortlich war. Die Bedeutung der organischen Auflage der Standorte war zweigeteilt. Entlang der Ferralsol-Höhensequenz wurde die mikrobielle Biomasse

sowohl von der Quantität als auch von der Qualität des organischen Materials im Boden gesteuert. Entlang der Costa Ricanischen Andosol-Höhensequenz war es allein die Qualität, die die mikrobiellen Biomasse regulierte und somit für die Veränderungen der Brutto-N-Mineralisierung verantwortlich war. Es wird angenommen, dass darüber hinaus auch das Vorkommen von N-fixierenden Leguminosen einen Einfluss auf die sich mit der Höhe verändernden N-Produktionsraten hatte. Ohne Zweifel sind die vorgestellten Faktoren, die die Brutto-N-Mineralisierungsraten kontrolliert haben, in einem gewissen Ausmaß voneinander abhängig. Allerdings zeigt die Tatsache, dass diese Faktoren zwischen den beprobten Höhensequenzen variierten, dass der Einfluss eines Faktors durchaus von einem anderen vermindert oder ausgeglichen werden kann. Dies geschieht in Abhängigkeit von der Boden- und der Standortbeschaffenheit.

Im letzten Teil der Arbeit wurden die Effekte einer experimentell erhöhten N-Zufuhr auf die N-Umsatzraten in einem tropischen Regenwald im Tiefland Panamas untersucht. Der erhöhte N-Eintrag hatte eine Steigerung der Brutto-N-Mineralisierungsraten zur Folge. Die Ursache dafür war die verbesserte Qualität (engeres C:N Verhältnis) der Blattstreu, die für die Mineralisierung zur Verfügung stand. Durch die chronischen N-Gaben wurde der pH-Wert des Oberbodens gesenkt. Die dadurch verursachte leichte Abnahme der mikrobiellen Biomasse wirkte sich in signifikant geringeren NH_4^+-Immobilisierungsraten durch die mikrobielle Biomasse aus. Diese Veränderung führte dazu, dass mehr NH_4^+ für die Nitrifikation zur Verfügung stand, was sich in erhöhten Konzentrationen an extrahierbarem NO_3^- im Boden widerspiegelte.

Basierend auf diesen Erkenntnissen erwarten wir, dass die Brutto-N-Mineralisierungsraten in den untersuchten Standorten in Ecuador und Costa Rica mit zunehmender N-Deposition ansteigen werden. Das Ausmaß wird voraussichtlich in Abhängigkeit der Faktoren, die die N-Mineralisierung entlang der Höhensequenzen steuern, variieren. Es wird damit gerechnet, dass die Erhöhung der Brutto-N-Mineralisierungsraten mit gesteigerten gasförmigen N-Verlusten und vermehrter N-Auswaschung aus dem Boden einhergeht. Da die Aktivität der Nitrifikanten, welche eng mit möglichen N-Verlusten in Verbindung steht, einem ähnlichen Trend folgte wie die mineralische N-Verfügbarkeit (Brutto-N-Mineralisierungsraten) im Boden, ist es wahrscheinlich, dass das Ausmaß der N-Verluste denselben Verlauf entlang der Höhensequenzen einnimmt.

1 Introduction

1.1 Anthropogenic alterations of the global nitrogen cycle

Nitrogen (N) is a key element controlling species composition, diversity, dynamics, and functioning of ecosystems (Vitousek et al., 1997). Since world population has increased by 78% from 1970, the global N-cycle is subject to huge alterations due to human activities, such as the combustion of fossil fuels, production of N-fertilizers, cultivation of N-fixing crops, and other activities (Galloway et al., 1995, 2008). Nitrogen is mainly atmosphere-based and only a small part of the global N is available to organisms. The most fundamental anthropogenic change to the global N-cycle is the doubling of transfer from the huge unreactive atmospheric N pool to biologically available forms (Vitousek et al., 1997). This N fixation caused by human activities has doubled the quantity of N entering terrestrial ecosystems in the past decades (Smil, 1990; Galloway et al., 1995; Vitousek et al., 1997). Moreover, the mobility of fixed N within and between ecosystems increased as a consequence of land use changes, biomass burning, wet land drainage and other anthropogenic modifications (Vitousek and Matson, 1993; Vitousek et al., 1997). Fixed N can occur in various forms and its spreading can range from a regional (e.g. as mineral N deposited on land) to global distribution (e.g. as potent greenhouse gas).

These changes to the global N cycle have strongly increased the deposition of atmospheric N compounds to terrestrial ecosystems. N deposition through rainfall and gas may reach ecosystems far away from conurbations where it originated. Until recently, the increase in N fixation and the resulting increase in N deposition were concentrated on highly industrialized temperate regions including intensive agriculture but studies predict that the rates of N deposition in the tropics will also increase by several hundred percent by the year 2025 (Matson et al., 1999). Above all, economically emerging tropical regions such as South-East Asia and Latin America will be affected by increasing N deposition as a result of large demands for food by a growing population with increasing per capita use of N and increasing energy consumption by their growing industries (Galloway et al., 2004).

1.2 Impacts of elevated N deposition on terrestrial ecosystems

The impact of increased N deposition has been well-studied in temperate ecosystems of Europe and North America where this consequence of industrialization and intensive agriculture emerged decades ago. Investigations showed that elevated N input to terrestrial ecosystems can change the composition of plant species and consequently affect the occurrence of higher organisms (Vitousek et al., 1997). High N deposition has been reported to decrease plant diversity (Phoenix, 2006; Bobbink et al., 1998), either by promoting the disappearance of species adapted to the efficient use of N, or by making some plants more susceptible to stresses such as drought and diseases. Elevated N input can also lead to soil acidification (van Breemen et al., 1982) and declining soil fertility as it may cause the loss of soil nutrients that are important for the long-term fertility of soils. Other consequences may be the pollution of ground and surface water by NO_3^- leaching (Aber et al., 1998; Schulze, 1989) and increasing emissions of the potent greenhouse gas N_2O and NO that drives the formation of photochemical smog and contributes to acid rain (Vitousek et al., 1997).

A status where the availability of N exceeds the capacity of an ecosystem to accumulate N through uptake by plants and soil biotic and abiotic processes is described as N saturation. For temperate forests, Aber et al. (1998) summarized the results of European and North American studies into a conceptual model of N saturation showing that the rate at which a forest ecosystem moves towards N saturation is regulated by two main factors: the inherent N status (i.e. low or high N availability) of the ecosystem and the rate of N input. The N status of an ecosystem is mainly determined by the type of soil and vegetation and by land use history.

In contrast to most temperate forest ecosystems, where plant growth is limited by N and elevated N input results in an increase in aboveground biomass, many tropical forest ecosystems are expected to have N in relative excess. This might lead to large gaseous and leaching losses of N in case of augmented N deposition. There is only sparse information on the N status of tropical forest ecosystems; hence reactions to augmented N deposition are difficult to predict until now. Most of the studies conducted so far support the hypothesis that old-growth tropical lowland forests provide a relative excess in N as it is accumulating from the atmosphere during pedogenesis, while nutrients derived from rock weathering (e.g. phosphorus (P)) become progressively unavailable during soil development (Vitousek and Farrington, 1997; Walker and Syers,

1976). In contrast, tropical montane forests which are located on relatively young soils due to erosion and slope processes (Tanner et al., 1998) are supposed to have N as the most limiting nutrient, while rock derived nutrients should be in sufficient supply. In a study conducted in montane forests in Hawaii, Hall and Matson (2003) revealed that in contrast to an N-limited forest on relatively young volcanic ash soil, a P-limited forest on old weathered soil reacted to N-additions with large and immediate soil gaseous losses. Studies conducted in tropical lowland forests in Australia and Brazil showed that N was in relative excess in these forests and they measured high net mineralization rates coupled with high emissions of N oxides (Breuer et al., 2000; Kiese and Butterbach-Bahl, 2002; Verchot, 1999). These studies propose that the soil nutrient status of an ecosystem is the key to analyze its reaction on elevated N input. Nevertheless, there is evidence that also other factors like legume abundance contribute to the N status of ecosystems.

1.3 The N cycle

An atom of N can emerge in many different forms while passing the N cycle, each with its own properties, behaviors, and consequences for the ecosystem (Brady and Weil, 2002). It may appear in inorganic or organic forms as well as in various oxidation states. The N cycle (Figure 1-1) describes the movement of this element between the atmosphere, biosphere and geosphere. Major processes constituting it are N fixation, N assimilation, N mineralization, nitrification, and denitrification. Microorganisms, particularly bacteria, play a major role in all of the principal nitrogen transformations.

a) Fixation of N means the conversion of atmospheric N (mainly dinitrogen (N_2)) to plant available forms and hence successional available to animals and human. Most atmospheric N is fixed in biological processes by either symbiotically-living or free-living bacteria. Fixed N is usually converted to ammonia (NH_3) followed by the transformation to ammonium (NH_4^+). High energy natural events such as lightning can also lead to N fixation.

b) Assimilation of N by plants usually occurs in the form of NH_4^+ and nitrate (NO_3^-) but plants may also assimilate soluble organic N. Absorbed NO_3^- is first reduced to nitrite (NO_2^-) followed by the reduction to NH_4^+. Heterotrophic organisms consume N by the uptake of plants.

c) N mineralization describes the transformation of organic N (e.g. from dead plant material) into NH_4^+. Decomposing organisms such as bacteria, fungi and protozoa attack amino groups of dead biomass and simple amino compounds are formed. These amino compounds are hydrolyzed and released as NH_4^+.

d) Nitrification is the conversion of NH_4^+ to NO_3^- consisting of two steps: The oxidation of NH_4^+ to NO_2^- followed by the oxidation of NO_2^- to NO_3^-. The first step is performed by oxidizing bacteria and archaea (Treusch et al., 2005). The second oxidation is mainly conducted by nitrobacter bacteria. Nitrification requires the presence of oxygen and holds some important consequences for ecosystems. While NH_4^+-ions are positively charged and stick to negatively charged clay particles and soil organic matter, the negatively charged NO_3^--ions are not held by soil particles and can be washed out easily, leading to decreased soil fertility and NO_3^- enrichment of downstream, surface and groundwater. Furthermore, gaseous N-oxide losses from soils occur throughout the process of nitrification.

e) Denitrification is known as the conversion of NO_3^- and NO_2^- to N_2. This process is anaerobic and is carried out by denitrifying bacteria (e.g. Pseudomonas). During denitrification, gaseous N losses occur in form of N-oxides contributing to environmental pollution and global warming.

One major part of the entire N cycle is the soil internal N cycle. Beside N mineralization and nitrification, there are three other transformation processes that are important for the N cycling within the soil. First, the immobilization of mineral and organic N through the incorporation into microbial biomass that is released again after the organisms die. Second, the abiotic NH_4^+ and NO_3^- retention by NH_4^+ fixation to clay minerals (Davidson et al., 1991) or physical condensation reactions with phenolic compounds (Nömmik, 1970; Nömmik and Vahtras, 1982; Johnson et al., 2000), and NO_3^- reduction to NO_2^- which readily reacts with soil organic matter (Smith and Chalk, 1980; Azhar et al., 1986, Thorn and Mikita, 2000). Third, the conversion of NO_3^- to NO_2^-, and than to NH_4^+ which is known as the dissimilatory nitrate reduction to ammonium (Silver et al., 2001). These mechanisms display the production and retention processes of N within the soil and since soils reflect the N status of ecosystem, information on the soil internal N cycle helps to project the ecosystem reactions to elevated N deposition.

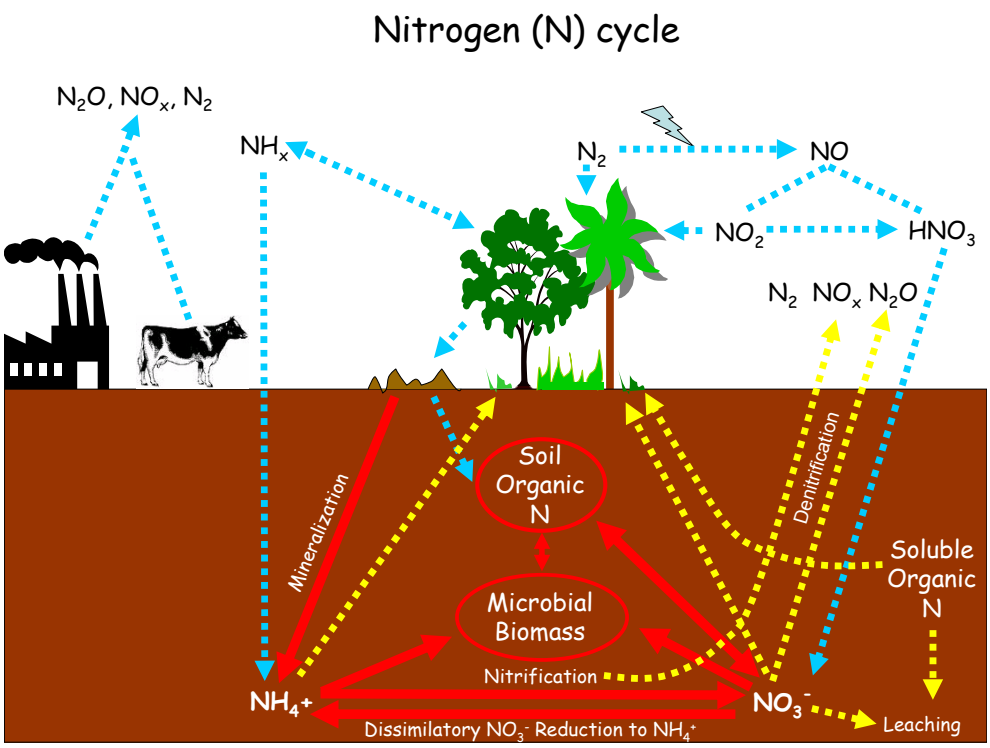

Figure 1-1: A simplified model of the nitrogen cycle, emphasizing the internal nitrogen cycle in soil (red arrows), nitrogen assimilation or losses from the soil nitrogen cycle (yellow arrows), atmospheric nitrogen transformations and nitrogen entering the soil nitrogen cycle (blue arrows).

1.4 Methods to study the soil internal N cycle

Most previous studies on the soil N status of ecosystems are restricted by measuring only net N mineralization and net nitrification rates that do not allow conclusion on the entire internal N cycle in soils. The main shortcoming of measuring only net rates is the failure to consider the retention processes of the produced NH_4^+ and NO_3^- such as biotic and abiotic N immobilization. Hence, net rate analyses state the mineral N availability in soils but not the amount of N that is mineralized or nitrified, since the transformed N might be immobilized during the incubation period. The ^{15}N pool dilution technique (Kirkham and Bartholomew, 1954; Davidson et al., 1991) used for the present thesis makes it possible to quantify the gross rates of N mineralization and nitrification, such that the incidence of produced mineral N can be considered. By injecting solutions of either $^{15}NH_4^+$ or $^{15}NO_3^-$ into intact soil cores, gross rates are calculated from the dilution of ^{15}N by incoming ^{14}N during 24 h of incubation. The

measurement of biotic and abiotic immobilization of NH_4^+ and NO_3^- may provide important information for estimating the projected N-losses from the soil.

Presently available data on gross N transformation rates in tropical forests using this technique derives from soil samples that were stored before processing. Comparing these data with ours (obtained from in-situ processing) led to the suspicion that nitrification rates and nitrate concentrations might have been overestimated as a consequence of sample storage, since most of the data differed in the same manner from our results. Using this methodology for in-situ measurements (extraction and incubation conducted in the field) from undisturbed soil cores is novel and generates new insights into the N cycle of tropical forest soils.

1.5 Objectives and outline of the thesis

The objectives of the study presented here are threefold and all measurements were conducted in old growth tropical forests to minimize the effect of land use history.

i) The first objective was to clarify how storage of soil samples affects the measurement of soil N cycling rates. Hence, we assessed N production and retention rates from Ecuadorian and Panamanian soil samples under in-situ processing and processing in the laboratory after sample storage.

ii) The second goal was to examine the N status of soils under old growth forest across three altitudinal gradients. We chose toposequences with either relatively young Andosols (FAO classification)/Andisols (USDA classification) or old and highly weathered Ferralsols (FAO classification)/Oxisols (USDA classification) to investigate how the N status changes with elevation and if the soil N status is highly dependent on the development stage of soils. Furthermore, we aimed to explore the factors that control the soil N status of these contrasting soil types and to investigate whether these factors vary in soils of different age. We measured the internal soil N cycling along a toposequence of Andosols in Ecuador and along two altitudinal sequences in Costa Rica, one consisting of Andosols and the other one consisting of Ferralsols. The Costa Rican Andosol toposequence included similar elevations as the Andosol toposequence in Ecuador.

iii) The last objective of this study was to asses how soil N cycling will change under elevated N input. Therefore, we simulated the scenario of increased N deposition in a Panamanian lowland forest under deeply-weathered soil. We tested the impact of chronic experimental N-addition on mineral N production and retention processes.

The present work provides important information for cross-site synthesis of studies on N cycling in the tropics and gives insights into the inherent N status of different old growth tropical forest ecosystems helping to predict their probable reactions to increasing N deposition.

1.6 Working hypothesis

i) For the first objective ('How do N production and retention rates vary under in-situ and laboratory processing?') we hypothesized that laboratory processing (including the storage of samples) does not give an accurate picture of the in-situ soil N transformation rates. As NO_3^- production and concentrations in previous studies, including the storage of samples, were relatively high compared to our results obtained from in-situ measurements, we speculated that an overestimation of nitrification rates and NO_3^- concentrations occurred in these studies. Thus, we expect higher nitrification rates in the stored-sample treatment than in the in-situ processed treatment.

ii) For the second objective ('How does the soil N status vary with elevation, is it highly dependent on the development stage of soils and which factors control the N status?') our hypothesis follows the theory of Walker and Syers (1976) that young soils presumably have low N availability while old and heavily weathered soils have a relative excess of N. Since montane soils are more likely to be young due to more recent erosion and slope processes (Tanner et al., 1977), we project N availability to decrease with elevation. Comparing the two different soil types, we expect that the younger Andosol sites have relatively low available N while the Ferralsols are relatively rich in N. For the factors that control the soil N status across the altitudinal gradients, we hypothesized that aside from the development stage of soil, abundance of legumes, the presence of an organic layer and climatic factors are responsible for N dynamics within the soil. Erickson et al. (2001) conducted a study in Puerto Rico where the abundance of legumes correlated with increased N cycling and N losses. This may also lead to

decreasing rates of N transformations across the elevation sequences as the presence of legumes is reported to decline with elevation (Hartshorn and Peralta, 1987). Additionally, the accumulation of an organic layer is influenced by altitude. In a montane forest in Jamaica, forests on ridge tops had thicker organic layers than forests on slopes or in gaps (Tanner, 1977; Hafkenscheid, 2000). Hence we assume that increasing thickness of the organic layer across the toposequences implies higher N cycling rates, although other factors associated with altitude (e.g. climatic conditions) may mitigate or outweigh this effect across the toposequences. A study of Schuur and Matson (2001) showed that foliar N and soil N availability decreased with augmenting precipitation. Thus, we expect correlations of rainfall and temperature with N dynamics across the tested toposequences.

iii) For the third goal ('How does soil N cycling change under elevated N input?') we hypothesized alterations in mineral N production and retention after chronic N addition. Serial elevated N input may have changed the amount and composition of microbial biomass and improved the quality of plant-derived inputs.

2 Comparison of nitrogen cycling rates from in-situ processed soil samples and after cold storage and laboratory incubation*

2.1 Abstract

Measurements of N transformation rates in tropical forest soils are commonly conducted in the laboratory from disturbed or intact soil cores. On four sites with Andisol soils under old-growth forests of Panama and Ecuador, we compared N transformation rates measured from laboratory incubation (at soil temperatures of the sites) of intact soil cores after a period of cold storage (at 5 °C) with measurements conducted in-situ. Laboratory measurements from stored soil cores showed lower gross N mineralization and NH_4^+ consumption rates and higher gross nitrification and NO_3^- immobilization rates than the in-situ measurements. We conclude that cold storage and laboratory incubation change the soils to such an extent that N cycling rates do not reflect field conditions. The only reliable way to measure N transformation rates of tropical forest soils is in-situ incubation and mineral N extraction in the field.

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

2.2 Introduction

Nitrogen (N) status of tropical forest soils has been shown to be the key to analyze how forest ecosystems will react to predicted changes in N deposition in the tropics (Vitousek and Farrington, 1997; Tanner et al., 1998). Soil N status has been commonly assessed by measurements of mineral N concentrations and N transformation rates. These are preferably done on fresh soil samples. However, often this is done in the laboratory which involves cold storage and pre-incubation. Cold storage of soil samples at 2-5 °C is recommended for temperate soils (Wollum, 1982; Hart et al., 1994a) and is widely used when soils cannot be processed directly after sampling. Refrigeration is supposed to decelerate microbial growth and to decrease disturbance effects associated with sampling. While microbial populations in temperate soils are adapted to a large range of temperature, including values below the freezing point, microorganisms in tropical soils are accustomed to relatively high temperatures with small fluctuations. Thus, cooling to temperatures of 2-5 °C is abnormal for microbial biomass in these soils and repression or stimulation of microbial processes, such as mineralization and nitrification, may occur during cold storage or rewarming of samples after storage. Hence, cold storage and subsequent laboratory incubation may lead to significant alterations in microbial activities, resulting in modified nutrient availability. Moreover, the delay between sampling and measurement of N transformation rates in tropical soils, which commonly have high N cycling rates, may falsify results more seriously than in temperate soils which usually have lower N cycling rates.

Nevertheless, most previous studies investigating N dynamics in tropical forest soils involve cold storage of samples between collection and laboratory measurement (e.g. Neill et al., 1999; Hall and Matson, 2003; Corre et al., 2006; Sotta et al., 2008). The extractable NH_4^+:NO_3^- ratios have been used to indicate N status in ecosystems, with a declining ratio when N availability increases (Vitousek et al., 1982; Davidson et al., 2000). This statement may not be applicable for measurements conducted from stored laboratory-incubated soils, if storage and laboratory incubation lead to a shift in NH_4^+:NO_3^- ratios due to changes in mineral N production rates and/or microbial biomass. Our objective was to investigate how cold storage and laboratory incubation of tropical forest soils change N transformation rates compared to in-situ measurement.

2.3 Materials and methods

The study was conducted in Andisols under old-growth forests in Panama and north western Ecuador. In Panama (Fortuna site, 1200 masl), the soil is classified as a Hapludand. We sampled at 8 sampling points (considered as replicates) with a minimum distance of 80 m. At each point, 12 intact soil cores were taken within a 0.6-m^2 area using stainless steel cores of 5-cm height and 8-cm diameter. Soil cores were taken after removing fresh and partially decomposed litter, and hence the soil samples encompassed the horizon below this loose litter down to 5-cm depth. Six of the cores were incubated in-situ and extracted for mineral N right in the field by bringing prepared bottles of 150 ml 0.5M K_2SO_4 solution to which soil samples (approximate solution to dry mass soil ratio of 5) were added (hereafter referred to as in-situ measurement). The soil-K_2SO_4 bottles were brought in a cooler from the field to the laboratory, shaken for 1 hour, filtered, and the extracts were frozen immediately. The other 6 cores were put in a cooler in the field and brought to the laboratory where they were refrigerated at 5 °C for 2 days followed by 3-d acclimatization, incubation and extraction in the laboratory at 20 °C (soil temperature of the site) (referred to as stored-laboratory measurement). The 3-d acclimatization was undertaken to recondition the microbial activity and to avoid artificially low N cycling rates possibly due to the cooling. In Ecuador, the soils are classified as Fulvudands for Pitzara (300 masl) and La Bilsa (630 masl), and Hapludand for Mindo (1500 masl). At each site, 5 sampling points (or replicates) spaced between 25 m - 50 m were randomly selected. We sampled 6 cores from each point as described above. Soils were sampled in 2005 and 2006 during the rainy season. The 2005 samples were cooled immediately after sampling and were stored at 5 °C for 30 days followed by 3-d acclimatization, incubation and extraction in the laboratory at temperatures similar to the soil conditions of the sites (23 °C for Pitzara, 22 °C for La Bilsa, and 18 °C for Mindo). The 2006 samples were incubated and extracted in-situ, as described for in-situ measurement of the Panama site. Time between field extraction and filtration ranged from 3 hours (Pitzara) to 7 hours (Fortuna and Mindo), depending on the distance of the sites to the laboratory.

For each method (in-situ and stored-laboratory measurements), 4 of the 6 soil cores were used for the determination of gross rates of N cycling using the ^{15}N pool dilution techniques (Davidson et al., 1991; Hart et al., 1994a). Two cores were each injected with 130 µg N-$(NH_4)_2SO_4$ (96 % ^{15}N) contained in 5 ml solution (for gross N

mineralization and NH_4^+ consumption), and each of the other two cores with 130 μg N-KNO_3 (99 % ^{15}N) in 5 ml (for gross nitrification and NO_3^- immobilization). These are equivalent to a rate of 1 μg N g^{-1}. One core of each labelled pair was extracted with 0.5M K_2SO_4 after 10 minutes and the other core was incubated for 1 day and then extracted. Microbial immobilization of NO_3^- was determined by 5-d $CHCl_3$-fumigation of the 1-d incubated, $^{15}NO_3^-$-injected cores. This was only measured from the Ecuador sites because immediate fumigation was not possible for the Panama site. The two remaining cores were used for measurements of initial mineral N concentrations and net N transformation rates with 7-d incubation period. Soil extracts remained frozen during transport by air to the University of Goettingen (Germany), where ^{15}N diffusion and mineral N analyses were conducted. For ^{15}N diffusion, 50 ml of extract was placed in a 150 ml glass bottle. NH_4^+ was diffused from the $^{15}NH_4^+$-labeled cores by adding MgO to the extracts, placing immediately the acid trap (2 discs of 7-mm diameter glass fiber filter paper acidified with 20 μl of 2.5 M $KHSO_4$ and encased in 5-cm wide Teflon tape) on the mouth of the bottle, and fastening the lid tightly. Diffusion proceeded for 6 days. NO_3^- was diffused from the $^{15}NO_3^-$-labeled cores by first adding MgO to the extracts and leaving the bottles open for 6 days to get rid of NH_4^+, followed by 6 days of diffusion after adding Devarda's alloy to convert NO_3^- to NH_4^+ and eventually to NH_3 (Corre et al., 2006; Sotta et al., 2008). For the 1-d incubated, $^{15}NO_3^-$-injected cores, ^{15}N enrichment in the microbial biomass was determined by persulfate digestion of the extracts from fumigated and unfumigated soils, and diffusion was carried out by adding 2 ml of 10 M NaOH and Devarda's alloy to convert persulfate-N (in NO_3^- form) to NH_3 (Corre et al., 2007). Gross rates of N mineralization, NH_4^+ consumption and nitrification and NO_3^- immobilization rates were calculated using the equations provided by Davidson et al. (1991).

Statistical differences between measurement methods for each site were assessed using the Mann-Whitney U Test at $P \leq 0.05$ and correlation analysis using Spearman's rank correlation test, as assumptions for normal distribution and equality of variance were not met.

2.4 Results

Our results showed that NH_4^+ concentrations were slightly higher in in-situ than in stored-laboratory measurement except at one site (Figure 2-1A), but NO_3^- concentrations strongly increased in stored-laboratory measurement (Figure 2-1B). Gross N mineralization rates were higher in in-situ than in stored-laboratory measurement for all sites, although these differences were statistically significant only at two Ecuadorian sites (Figure 2-1C). These two Ecuadorian sites also showed significantly higher net N mineralization rates in in-situ than in stored-laboratory measurement. At all sites, gross nitrification (Figure 2-1D) and net nitrification rates were much lower in in-situ than in stored-laboratory measurement. Only 1–12% of the mineralized N was nitrified in-situ while 37->100% of the mineralized N was transformed to NO_3^- in stored-laboratory measurement. The increased gross nitrification rates in stored-laboratory measurement were paralleled with decreased NH_4^+ assimilation rates (NH_4^+ consumption - gross nitrification). NH_4^+ consumption rates decreased in stored-laboratory measurement (Figure 2-1E) whereas the converse was true for microbial immobilization of NO_3^- (Figure 2-1F) compared to in-situ measurement. Soil moisture contents did not differ between in-situ and stored-laboratory measurement in all but one site (Mindo). For the Mindo site, soil moisture was higher in in-situ than in stored-laboratory measurement and water-filled pore space was correlated with gross nitrification rates (r = -0.64; P = 0.05), indicating that for this site the increased gross nitrification rates in stored-laboratory measurement could be partly due to the change in soil aeration status.

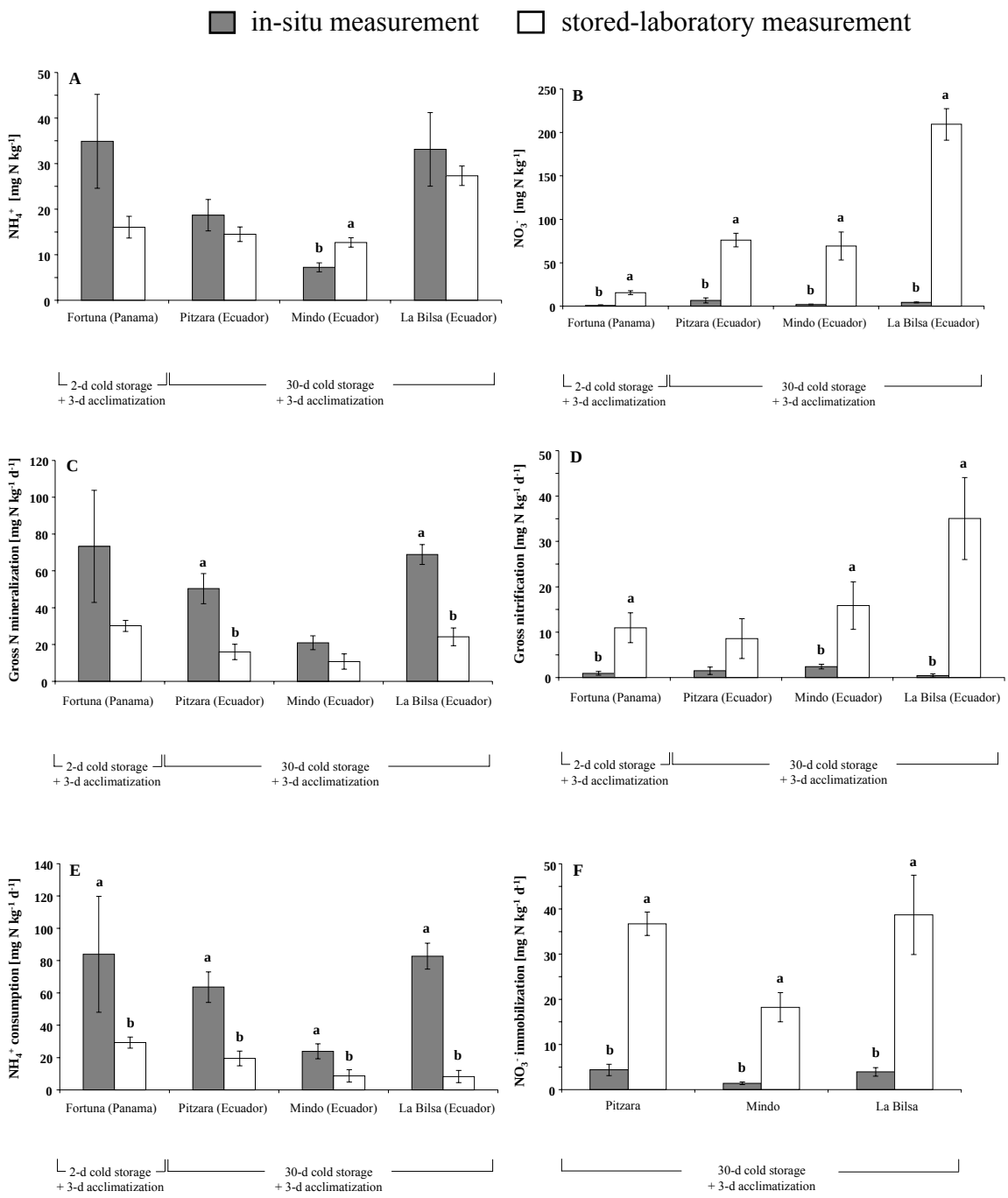

Figure 2-1: **A**) NH_4^+ concentrations, **B**) NO_3^- concentrations, **C**) gross N mineralization rates, **D**) gross nitrification rates, **E**) NH_4^+ consumption rates, and **F**) microbial NO_3^- immobilization rates from in-situ and stored-laboratory measurements. Means (±1 S.E.; n = 8 for Panama site and n = 5 for Ecuador sites) with different letters indicate significant difference between measurement methods for each site (Mann-Whitney U test at $P < 0.05$).

2.5 Discussion

The decrease in N mineralization in stored-laboratory measurement was probably due to decrease in easily mineralizable organic N with storage, especially in the long-term stored soil cores of the Ecuador sites. Reduced availability of organic matter with storage was reflected in the significant decrease of microbial biomass C in all Ecuador sites. The microbial C in stored-laboratory measurement was only 32-64% of those in in-situ measurement. With the absence of plant uptake during sample storage and laboratory incubation, the nitrifiers were probably able to compete more for available NH_4^+, resulting in increased nitrification rates. This was observed even in the shortly stored soil cores of the Panama site. In a study on effects of low temperatures on N transformation rates, Cookson et al. (2002) measured gross nitrification at 2 and 5 °C. The possible sustained nitrification activity during cold storage combined with favoured nitrification activity under laboratory incubation could have resulted to the dominance of NO_3^- over NH_4^+, with the lowest NH_4^+:NO_3^- ratio in the site (La Bilsa) with highest gross nitrification rates. The increased NO_3^- availability in stored-laboratory incubated soil cores consequently led to the enhanced uptake of NO_3^- by microbial biomass.

Verchot (1999) examined nitrification potential of Brazilian Oxisols under primary forests after 5-d cold storage of mixed soils, and although nitrification potential decreased in most of the sites, he observed a very high rate of NO_3^- production from stored soils of one primary forest site. To our knowledge, our present study is the first to report how storage and subsequent laboratory incubation of tropical soils under old-growth forests affect N transformation rates. Neill et al. (1999) reported 57-70% gross nitrification of gross N mineralization from mixed soils stored cold up to three weeks followed by laboratory incubation. Other studies on soil N cycling in old-growth tropical forests (e.g. Hall and Matson, 2003; Silver et al., 2005; Sotta et al., 2008) included storage of soils (mixed or intact cores) with subsequent laboratory incubation or at least transport of mixed soils to the laboratory from in-situ incubated cores prior to extraction, and all these studies showed higher ratios of gross nitrification to gross N mineralization rates than our in-situ measurement of the present sites. Although these data are from different soil types, climatic conditions, and extraction methods, relatively high ratios of gross nitrification to gross N mineralization rates in these studies support our present results.

2.6 Conclusions

We conclude that cold storage and laboratory incubation change the soils to such an extent that N cycling rates do not reflect field conditions. How large a problem for measuring tropical soil N dynamics arises from cold storage and subsequent laboratory incubation probably depends on the inherent rate of N cycling of a system. We suggest that the faster the inherent N transformation rates and the longer the storage (e.g. La Bilsa, Ecuador), the larger the discrepancy will turn out to be. The only reliable way to measure N transformation rates of tropical forest soils is in-situ incubation and mineral N extraction in the field. Our experience is that this is possible even at logistically-challenging sites because it does not involve any special or heavy equipment. Our findings should also be taken into consideration when making cross-site synthesis of N status indices of tropical forest soils because stimulation of NO_3^- production and repression of NH_4^+ production with soil storage and laboratory incubation will lead to erroneous evaluation when combined with measurements conducted in-situ.

Acknowledgments

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3 Soil N cycling in old-growth forests across an Andosol toposequence in Ecuador*

3.1 Abstract

Nitrogen (N) deposition in the tropics is predicted to increase drastically in the next decades. The sparse information on N cycling in tropical forests revealed that the soil N status of an ecosystem is the key to analyze its reactions to projected increase in N input. Our study was aimed at 1) comparing the soil N availability of forest sites across an Ecuadorian Andosol toposequence by quantifying gross rates of soil N cycling in-situ, and 2) determining the factors controlling the soil N cycling differences across sites. The toposequence was represented by five old-growth forest sites with elevations ranging from 300 m to 1500 m. Gross rates of N transformations, microbial N turnover time, and $\delta^{15}N$ signatures in soil and leaf litter decreased with increasing elevation, signifying a decreasing N availability across the toposequence. This was paralleled by a decreasing degree of soil development with increasing elevation, as indicated by declining clay contents, total C, total N, effective cation exchange capacity and increasing base saturation. Soil N cycling rates and $\delta^{15}N$ signatures were highly correlated with mean annual temperature but not with mean annual rainfall and soil moisture which did not systematically vary across the toposequence. Microbial immobilization was the largest fate of produced NH_4^+ across all sites, and nitrification activity was only 5%-11% of gross NH_4^+ production. We observed a fast reaction of NO_3^- to organic N and its role for N retention deserves further attention. If projected increase in N deposition will occur, the timing and magnitude of N losses may follow the pattern of N availability across this Andosol toposequence.

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* Soil N cycling in old-growth forests across an Andosol toposequence in Ecuador. Arnold, J., M.D. Corre, E. Veldkamp. Submitted to Forest Ecology and Management.

3.2 Introduction

Tropical regions are projected to receive the most dramatic increases in reactive N inputs over the next few decades due to continued increases in fertilizer use, legume cultivation, fossil fuel consumption (Galloway et al., 1994, 2008) and biomass burning (Crutzen and Andreae, 1990; Cochrane, 2003). Until recently, increased N deposition has been mainly a concern in industrialized temperate regions, but N deposition is projected to at least double in economically emerging tropical regions such as Southeast Asia and Latin America due to demands for food and energy by a growing population and by their evolving industries (Galloway et al., 1994, 2008).

The sparse information on deleterious effects of elevated N input in tropical forests suggests that the inherent soil N status (i.e. low or high N availability) of an ecosystem is the key to analyse the impact of augmented N depositions. A study in Hawaiian montane forests showed that N-additions resulted in large and immediate increases in NO and N_2O emissions from highly-weathered, N-rich forest soil compared to small and delayed increases in N-oxide emissions from young, N-limited forest soil (Hall and Matson, 2003). Also, the degree of soil development rather than N status determined the effects of N-additions on NO_3^- leaching losses (Lohse and Matson, 2005). At present, our knowledge on soil N status of tropical forests is based on net rates of soil N cycling, N-oxide emissions, NO_3^- leaching losses and ^{15}N isotope signatures in leaves and soils. None of these reveal the soil N retention processes which are important indicators of how a forest ecosystem reacts to future changes in N input. An alternative way to assess the soil N status of tropical forests is by quantifying gross rates of soil N cycling, revealing rates of mineral N production and immobilization. Information on gross rates of mineral N production and immobilization are largely missing for Ecuadorian forest soils.

Soil N status of old-growth tropical forests is generally influenced by forest type (lowland versus montane), soil development/age, legume abundance, degree of organic matter accumulation and climatic factors (temperature and rainfall). Lowland forests, commonly growing on heavily weathered soils, have higher net N mineralization rates (Marrs et al., 1988; Rhoades and Coleman, 1999), higher N-oxide emissions (Keller and Reiners, 1994; Davidson et al., 2000; Purbopuspito et al., 2006), higher NO_3^- leaching losses (Hedin et al., 2003; Klinge et al., 2004; Dechert et al., 2005; Schwendenmann and Veldkamp, 2005), and higher $\delta^{15}N$ signatures in leaves and soils (Martinelli et al.,

1999) than montane forests, which are likely to occur on less developed soils due to recurrence of substrate addition (volcanic ashes) and removal (erosion and slope processes) (Tanner et al., 1998). These observations support the speculation that lowland forests have high N availability while montane forests have low N availability. Furthermore, the abundance of legumes may also influence N availability. Erickson et al. (2001) reported that the presence of legumes was associated with high N transformation rates and N-oxide emissions. In a study conducted across an altitudinal gradient in Costa Rica, leguminous tree families were most abundant at the lowland forest sites (≤ 100 m above sea level (asl)), did not show clear pattern between 300 m and 1500 m asl, and were absent at >1750 m asl (Liebermann et al., 1996). Finally, the presence or absence of an organic layer may affect N availability of an ecosystem. Thick organic layers are common in tropical montane forests (Grieve et al., 1990; Tanner et al., 1998; Hafkenscheid, 2000) and altitude seems to control its thickness through differences in species composition (Burghouts et al., 1998) and/or differences in temperature and soil moisture (Wilcke et al., 2002). Although organic layers can be important nutrient sources in tropical montane forests (Wilcke et al., 2002), slow mineralization or immobilization of N may limit its availability (Vitousek and Matson, 1988; Bruijnzeel et al., 1993). As the release of nutrients stored in the organic layer depends on specific conditions (e.g. temperature and precipitation) in each site, it is impossible to make a general statement about nutrient availability (Tanner et al., 1998). Marrs et al. (1988) reported decreasing net N mineralization rates across an altitudinal gradient in Costa Rica. Hawaiian montane forests on soils of similar age and elevation showed decreasing N availability across a precipitation gradient, which was attributed to increased intensity and duration of anaerobic conditions with increased rainfall (Schuur and Matson, 2001). In summary, the effects of the aforementioned factors on soil N status cannot be separated but to some degree covaried with changes in altitude.

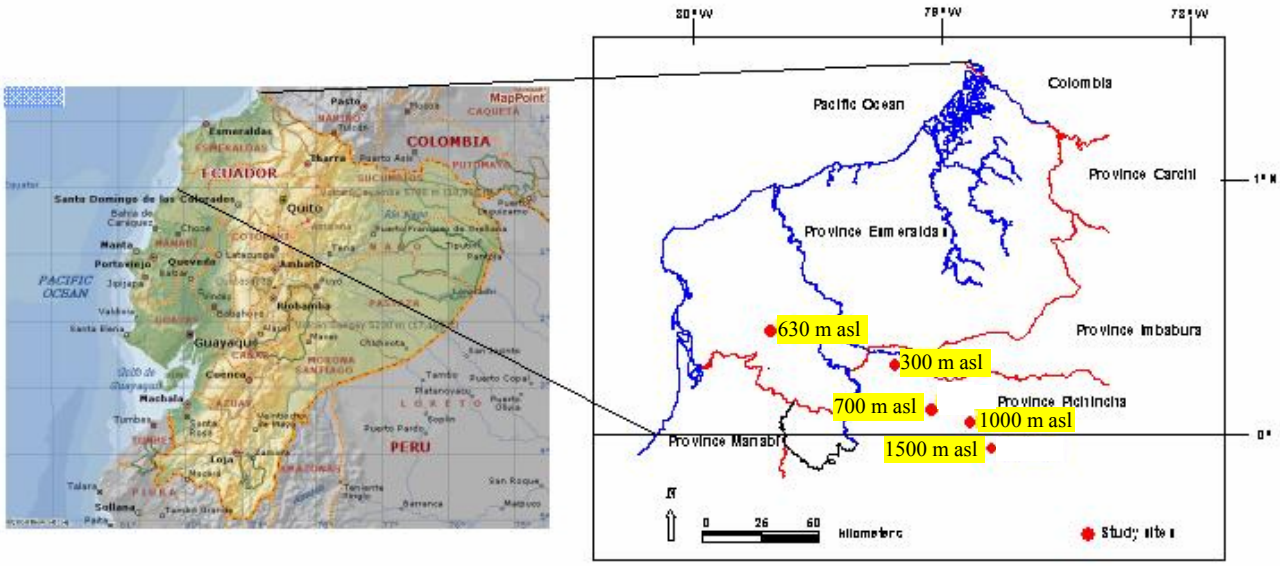
We studied an Andosol toposequence under old-growth forests in Ecuador. This represented a narrower range of soil development gradient compared to the chronosequence study in Hawaii (Hall and Matson, 2003). We hypothesized that differences in soil N availability across the toposequence are controlled by elevation-mediated factors (e.g. degree of soil development, organic layer accumulation, rainfall and temperature). Our objectives were 1) to compare the soil N availability forest sites across an Andosol toposequence by quantifying gross rates of soil N cycling in situ, and 2) to determine the factors controlling the soil N cycling differences across sites. Our

results provide the much-needed baseline information for Ecuadorian tropical forests, which may hint how such ecosystems will react to predicted increases in N deposition.

3.3 Materials and methods

3.3.1 Site description

Study sites were located in old growth forests of northwestern Ecuador, in the provinces of Esmeraldas and Pichincha (Map 3-1). These forests are situated in nature reserves and conservation areas of the Asociación de Industrias Forestales. We chose five study sites on volcanic ash soils (Andosols in FAO classification; Fulvudands and Hapludands in USDA classification) along an altitudinal gradient (Table 3-1): 300 m, 630 m, 700 m, 1000 m and 1500 m asl. These volcanic ash soils are characterized by having high allophane contents (implicating high water retention), low bulk densities, loamy to sandy textures, and are susceptible to erosion (López et al., 2002). Mean annual temperatures of these sites range from 18 - 23 °C and average annual precipitation varies from 2370 - 4860 mm yr^{-1} (Table 3-1). We measured the rates of soil N cycling within October and November 2006, which fall in the beginning of the rainy season.



Map 3-1: Map of Northwest Ecuador showing the study sites (modified after Microsoft Corp. and/or its suppliers, 2001 and de Koning et al., 2003).

3.3.2 Soil sampling

For each site, 5 sampling points spaced between 25 m - 50 m were randomly selected on a generally level landscape such that these sampling points did not vary in topography. At each sampling point, 6 intact soil cores were taken within 0.3-m^2 area using stainless steel cores of 5 cm height and 8 cm diameter. The soil cores were taken after removing fresh and partially decomposed litter (Oi layer), and thus the soil samples encompassed the Oe+a layer down to 5-cm depth which included part of the mineral soil below it (hereafter referred to as topsoil). Additionally, we took random samples of decomposing leaf litter at each sampling point for analysis of other supporting parameters (see below). A soil profile (0-0.05, 0.05-0.10, 0.10-0.25 and 0.25-0.50-m depths) was also sampled for each site to determine general soil characteristics. Air-dried leaf litter and soil samples (for general characteristics) and frozen soil extracts (for N cycling measurements) were transported by air to University of Goettingen, Germany for analyses; all frozen samples remained frozen during transport and were stored immediately in a freezer upon arrival.

3.3.3 Gross rates of soil N cycling

We conducted the soil N cycling measurements in situ, including ^{15}N injection, incubation and mineral N extraction. We used the ^{15}N pool dilution technique and followed the procedures of ^{15}N injection into intact soil cores described by Davidson et al. (1991) and Hart et al. (1994a), which we also employed in our earlier studies of tropical forest soils (Corre et al., 2006; Sotta et al., 2008; Arnold et al., 2008). From four intact soil cores of each sampling point, two cores were injected with 5 ml $(^{15}NH_4)_2SO_4$ solution (containing 27 μg N ml^{-1} with 95% ^{15}N) for determination of gross N mineralization and NH_4^+ consumption rates while the other two cores were injected with 5 ml $K^{15}NO_3$ (containing 28 μg N ml^{-1} with 99% ^{15}N) for the measurement of gross nitrification and NO_3^- consumption rates. The rate of injected NH_4^+ or NO_3^- was on average 1.1 ± 0.1 μg N g^{-1}. The injected NH_4^+ was between 3% - 14% of the initial soil NH_4^+ concentrations of the sites, while the injected NO_3^- was between 7% - >100% of the extant soil NO_3^- concentrations.

One soil core of each labeled pair was broken up, mixed well in a plastic bag, and subsampled for 0.5 mol/L K_2SO_4 extraction 10 minutes after ^{15}N injection (T_0 cores). This was done in the field by bringing prepared bottles containing 150 ml K_2SO_4

solution and adding soil samples into the bottles; the average K_2SO_4 solution to soil dry mass ratio was 7. The other soil core of the labeled pair was put in a plastic bag, inserted back into the soil to incubate in situ for one day (T_1 cores), and extracted with K_2SO_4 in the field. The soil-K_2SO_4 bottles and the rest of the soil samples from the cores were put in a cooler during transport from the field to the laboratory. In the laboratory, extraction was continued by shaking the samples for 1 hour and filtering them through K_2SO_4-prewashed filter papers (4 µm nominal pore size). Extracts were frozen immediately after filtering and further analyses were conducted in Germany. Gravimetric moisture content from each soil core was measured as soon as the samples got in the laboratory. The T_0 cores were used to correct for the reactions that occur immediately after $^{15}NH_4^+$ and $^{15}NO_3^-$ injection and gross rates of N mineralization and nitrification were calculated according to Davidson et al. (1991) and Hart et al. (1994a).

3.3.4 Analysis of N concentration and ^{15}N recovery at 10 minutes (T_0)

NH_4^+ and NO_3^- concentrations were measured using continuous flow injection colorimetry (Cenco/Skalar Instuments, Breda, Netherlands) in which NH_4^+ is quantified using the Berthelot reaction method (Skalar Method 155-000) and NO_3^- is determined using the copper-cadmium reduction method (Skalar Method 461-000). Our standard method for NO_3^- analysis uses NH_4Cl as the buffer but without the complexing ligand, ethylenediamine tetraacetic acid (EDTA). Recently, Colman et al. (2007) claimed that the NH_4Cl+EDTA buffer method underestimates NO_3^- concentration due to its sensitivity to Fe interference. We addressed this concern in two ways. First, we compared our NH_4Cl buffer (without EDTA) against imidazole buffer (claimed by Colman et al. (2007) to be unaffected by Fe interference) for NO_3^- determination of our soil extracts and of standard solutions (0.5 mol/L K_2SO_4) containing 4.9 mg NO_3-N/L with increasing Fe^{2+} levels (Fe is dissolved in a 2% HCl solution to maintain the soluble Fe(II) oxidation state): 0, 5, 10, 25, and 30 mg Fe/L. Second, we measured total Fe concentrations in our soil extracts (using Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES); Spectro Analytical Instruments, Kleve, Germany) to determine if they were high enough to be worrisome.

We identified the fate of added ^{15}N at T_0 by measuring ^{15}N recoveries in K_2SO_4-extractable N pools. Part of the T_0 extracts was used for serial diffusion of NH_4^+ and NO_3^- (Corre and Lamersdorf, 2004) and part was used for persulfate digestion for determination of ^{15}N enrichment in extractable organic N pool (Corre et al., 2007). The

same diffusion procedure and blank correction were followed as described in our earlier works (Corre et al., 2003; Corre and Lamersdorf, 2004; Corre et al., 2007). The ^{15}N recovery in a particular N pool was calculated based on Hart et al. (1994a), in which the ^{15}N enrichment of an N pool is subtracted by the measured natural abundance ^{15}N of that pool divided by the amount of injected ^{15}N. ^{15}N recovery in the insoluble organic N pool was calculated as the difference between ^{15}N recoveries in the total N pool (analyzed from freeze-dried T_0 soil samples) and in K_2SO_4-extractable N pools. ^{15}N was analyzed using isotope ratio mass spectrometry (IRMS; Finigan MAT, Bremen, Germany).

3.3.5 Net N mineralization and nitrification and microbial biomass C and N

The other two intact soil cores from each sampling point were used for measurements of net N cycling rates. One soil core was extracted with 0.5 mol/L K_2SO_4 immediately in the field for determination of initial levels of NH_4^+ and NO_3^-. Part of the soil from this core was also used for microbial biomass determination and for determination of the natural abundance ^{15}N of different N pools. The other soil core was put in a plastic bag, reburied in the soil to incubate in situ for 7 days, and extracted in the field with 0.5 mol/L K_2SO_4. As described above, extraction (shaking and filtration) was continued upon arrival in the laboratory. All extracts were frozen until analyses in Germany. From each soil core, gravimetric water content was determined. Net rates of N mineralization were calculated by subtracting the initial $NH_4^+ + NO_3^-$ concentrations from those after incubation divided by the days of incubation. Net rates of nitrification were calculated similarly but only considering the NO_3^- concentrations.

Microbial biomass C and N were determined by fumigation-extraction method (Brookes et al., 1985; Davidson et al., 1989). Soils were fumigated with $CHCl_3$ for 5 days and extracted with 0.5 mol/L K_2SO_4 (volume to soil mass ratio of 5). Organic C from the extracts was analyzed by UV-enhanced persulfate oxidation using a Dohrmann DC-80 Carbon Analyzer with an infrared detector (Rosemount Analytical Division, CA, U. S. A.). Extractable organic N was determined using persulfate digestion described by Corre et al. (2007). Microbial biomass C and N were calculated as the difference in extractable organic C and persulfate-N between the fumigated and unfumigated soils divided by $k_C = 0.45$ and $k_N = 0.68$ for 5-day fumigated soils.

3.3.6 Mean residence time (MRT) of NH_4^+ and microbial N pools

MRT indicates the average length of time an N atom stays in a given pool. A lower MRT indicates a faster pool turnover rate and hence a more dynamic pool (Hart et al., 1994a). The calculation of MRT (N pool ÷ flux rate; e.g. MRT of NH_4^+ pool = NH_4^+ pool ÷ gross N mineralization rate) assumes that NH_4^+ or microbial N pool was at steady state and that the fluxes were equal to gross rates of N mineralization and N immobilization, respectively.

3.3.7 Other supporting parameters

Particle size distribution was analyzed using the pipette method with pyrophosphate as a dispersing agent (König and Fortmann, 1996). Total organic C and N were measured from air-dried, ground samples of decomposing leaf litter and soil using CNS Elemental Analyzer (Elementar Vario EL, Hanau, Germany). ^{15}N signatures were also measured from these samples using IRMS. Bulk density was determined using the soil core method (Blake and Hartge, 1986). Soil pH was measured from a saturated paste mixture (1:1 ratio of soil to H_2O). Effective cation exchange capacity (ECEC) was determined from air-dried, 2-mm sieved soil samples, percolated with unbuffered 1 mol/L NH_4Cl, and the percolates analyzed for exchangeable cations using ICP-EAS. Base saturation was calculated as the percentage base cations of the ECEC. Total P was determined from air-dried, ground samples, digested under high pressure in concentrated HNO_3 (König and Fortmann, 1996), and the digests were analyzed using ICP-EAS. Amorphous Al and Fe were determined using acid oxalate extraction (Buurman et al., 1996) followed by analysis of extracts using ICP-AES. Oxalate-extractable Al + ½ Fe is a criterion used for Andosol classification.

3.3.8 Statistical analyses

We first tested the sampling points of each site for spatial independence based on the data of gross N mineralization rates using the rank version of von Neumann's ratio test (Bartels, 1982). Our sampling points were spatially independent, and hence were considered replicates in the succeeding analyses. Tests for normality using

Kolmogorov-Smirnov D statistics and equality of variance using Levene statistic (Sokal and Rohlf, 1981) were conducted for each parameter. As the assumption of normality and equality of variance for parametric tests was not met, we used the nonparametric methods (Siegel and Castellan, 1988). Site differences were assessed using Kruskal-Wallis H test followed by multiple comparison extension test. Differences between two parameters measured in the same site were assessed using Paired-sample T test, and differences from 0 or 100% for ^{15}N recovery values for each site were assessed using One-sample T test. Correlation analyses were conducted on individual observations across sites using Spearman rank correlation test.

3.4 Results

3.4.1 Characteristics of decomposing leaf litter and soil

Total C concentrations of the decomposing leaf litter did not differ across sites; total N concentrations were highest at the 700-m site and lowest at the 630-m site while the converse was true for the total C:N ratios. The decomposing leaf litter in the three remaining sites (300-m, 1000-m and 1500-m sites) showed a tendency of decreasing total N concentrations and $\delta^{15}N$ signatures and increasing total C:N ratios with increasing elevation (Table 3-1).

Soil texture became coarser across the toposequence, ranging from loam (300-m and 630-m sites) to sandy loam (700-m and 1000-m sites) and loamy sand at the 1500-m site (Table 3-1). Total C, N and $\delta^{15}N$ signatures in the topsoil and 5-50 cm mineral soil also tended to decrease along the altitudinal gradient, except at the 630-m site where the lowest total C and N concentrations in the topsoil were observed (Table 3-1). Soil pH showed no trend across sites, ECEC of the 300-m and 630-m sites was slightly higher than those of ≥700-m sites, and base saturation tended to increase with increasing elevation (Table 3-1). Water filled pore space (WFPS) ranged from 62% - 89% across sites and showed no trend with increasing elevation.

Table 3-1: Characteristics of the study sites across an Andosol toposequence in Ecuador.

Location	Pitzara		Bilsa		Silanche		Los Bancos		Mindo	
Elevation [m above sea level]	300		630		700		1000		1500	
Mean annual rainfall [mm]	3467		2370		4860		3200		3485	
Mean annual temperature [ºC]	23		22		22		21		18	
FAO Soil Classification	Aluandic Andosol		Aluandic Andosol		Aluandic Andosol		Aluandic Andosol		Melanic Andosol	
USDA Soil Classification	Typic Fulvudand		Pachic Fulvudand		Typic Hapludand		Typic Hapludand		Typic Hapludand	
Texture (5-10 cm depth)	loam		loam		sandy loam		sandy loam		loamy sand	
Decomposing leaf litter										
C [mg C g^{-1}]	437.7 (6.4)		447.6 (7.0)		436.3 (7.9)		428.7 (4.5)		439.8 (8.2)	
N [mg N g^{-1}]	16.3 (0.8)	ab	14.1 (0.6)	b	17.3 (0.7)	a	15.1 (0.9)	ab	14.4 (0.7)	ab
C/N ratio	27.2 (1.5)	ab	31.9 (1.7)	a	25.4 (1.1)	b	28.8 (1.7)	ab	30.7 (1.2)	ab
$\delta^{15}N$ [‰]	3.0 (0.4)	a	0.5 (0.1)	b	3.4 (0.3)	a	0.8 (0.2)	b	-0.5 (0.2)	c
Top soil (Oe+a - 5-cm depth, which includes mineral soil)										
Bulk density [g cm^{-3}]	0.48 (0.05)	ab	0.31 (0.03)	b	0.60 (0.04)	ab	0.56 (0.02)	ab	0.69 (0.04)	a
pH (1:1 H_2O)	4.9		4.8		4.7		4.7		5.2	
Effective CEC [mmol(+) kg^{-1}]	100.4		112.8		92		82.2		92.1	
Base saturation [%]	11.8		50.7		45.1		48.6		80.3	
C [g C m^{-2}]	3384 (49)	a	2429 (222)	b	2958 (227)	ab	2861 (175)	ab	2627 (189)	ab
N [g N m^{-2}]	217.2 (8.7)	a	161.5 (8.5)	b	198.6 (10.7)	ab	197.0 (10.3)	ab	182.3 (9.9)	ab
C/N ratio	15.7 (0.6)		14.9 (0.6)		14.8 (0.6)		14.3 (0.3)		14.4 (0.4)	
$\delta^{15}N$ [‰]	6.4		4.4		5.2		3.9		2.3	
NH_4^+ [g m^{-2}]	0.4 (0.1)	a	0.5 (0.1)	a	0.4 (0.1)	a	0.2 (0.0)	b	0.2 (0.0)	b
NO_3^- [g m^{-2}]	0.2 (0.1)		0.1 (0.0)		0.2 (0.1)		0.1 (0.0)		0.1 (0.0)	
NH_4^+/NO_3^- ratio	10.7 (6.4)		8.5 (2.8)		2.2 (0.5)		2.0 (0.3)		2.0 (0.5)	
Total P [g P m^{-2}]	22.4		12.3		22.2		16.7		19.5	
Mineral soil (5-50 cm depth)										
pH (1:1 H_2O)	5.6		5.2		5.3		5.6		6.4	
Effective CEC [mmol(+)kg^{-1}]	11		34.9		15.8		9.5		18.5	
Base saturation [%]	54.8		45.6		39.8		51		66.3	
C [g C kg^{-1}]	43.7		38.3		32.4		25.4		15.9	
N [g N kg^{-1}]	3.4		3.1		2.8		1.9		1.3	
C/N ratio	12.4		11.8		11.2		12.9		12.3	
$\delta^{15}N$ [‰]	8.4		7.7		7.7		6.7		4.9	
Total P [g P kg^{-1}]	0.9		0.3		0.7		0.5		0.5	

Notes: Means (±1 S.E.; n = 5) within each row followed by different letter indicate significant differences among sites (Kruskal-Wallis H test with multiple comparison extension at $P \leq 0.05$). Soil characteristics without S.E. values were determined from one soil profile in each site. Element concentrations in the top soil (Oe+a-5-cm depth) are expressed on areal basis in order to be consistent with the units of soil N cycling rates, which were measured at the same depth.

3.4.2 Soil N cycling across the toposequence

Extractable NH_4^+ dominated over extractable NO_3^- in all sites (Table 3-1). NH_4^+ levels of the three lower sites were higher than those of the 1000-m and 1500-m sites. We measured consistently low NO_3^- concentrations across the elevation gradient with no difference among sites.

On average across sites, 81 ± 3% of the injected $^{15}NH_4^+$ was recovered in the form added when intact soil cores were extracted after ten minutes, and no difference was observed among sites. No ^{15}N was detected above the background levels in any of the other extractable N pools, and complete recovery of the injected $^{15}NH_4^+$ (92 ± 3%; not significantly different from 100%, One-sample T test at $P > 0.05$) was observed in the total N pool (analyzed from freeze-dried soil samples).

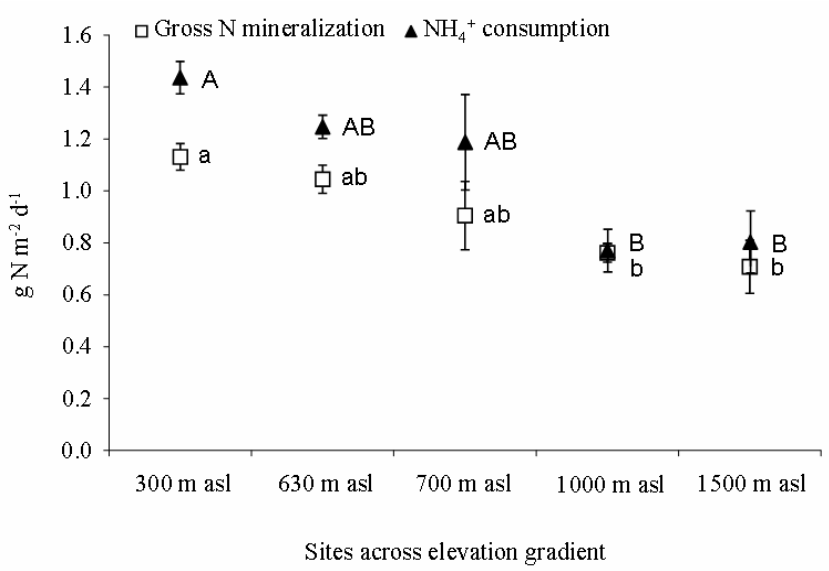


Figure 3-1: Gross N mineralization rates (open squares) and NH_4^+ consumption rates (filled triangles) across an Andosol toposequence in Ecuador. Means (± 1 standard error (S.E.); n = 5) with different letters indicate significant differences among sites (Kruskal-Wallis H test with multiple comparison extension at $P \leq 0.07$ for gross N mineralization and $P \leq 0.05$ for NH_4^+ consumption). Small letters for gross N mineralization rates; capital letters for NH_4^+ consumption rates.

Gross N mineralization decreased across the altitudinal gradient (Figure 3-1). The differences in microbial biomass N and microbial C:N ratio across sites did not follow the pattern of increasing elevation (Figure 3-2A). The lowest and highest

microbial biomass N were measured at the 630-m and 1000-m sites, respectively, while the rest of the sites showed intermediate levels. The average microbial C:N ratios varied between 14 and 21 across sites with the highest ratio at the 300-m site and the lowest ratio at the 630-m site. Specific gross N mineralization rates (i.e. gross N mineralization ÷ microbial N pool) declined across the elevation gradient (Figure 3-2B), while MRT of the microbial N pool indicated decreasing turnover rates of microbial N with increasing elevation (Figure 3-3). NH_4^+ consumption rates followed the same pattern as gross N mineralization rates and were significantly higher than gross N mineralization rates only at the lower elevation sites (300 m and 630 m; Figure 3-1). The MRT of NH_4^+ pool was on average 0.4 ± 0.0 day, and no difference was detected among sites.

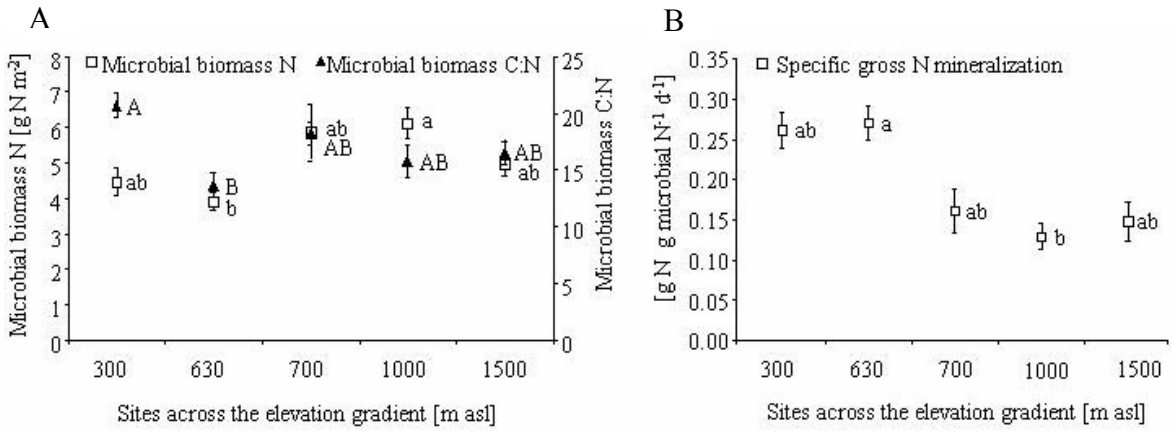


Figure 3-2: **A**) Microbial biomass N (open squares) and microbial C:N ratio (filled triangles) and **B**) Specific gross N mineralization across an Andosol toposequence in Ecuador. Means (± 1 S.E.; n = 5) with different letters indicate significant differences among sites (Kruskal-Wallis H test with multiple comparison extension at P ≤ 0.05). Small letters for microbial biomass N; capital letters for microbial C:N ratio.

Ten minutes after $^{15}NO_3^-$ injection into the intact soil cores, ^{15}N recoveries in the NO_3^- pool ranged from 33% to 78% across sites and no difference was observed among sites. A fast reaction of NO_3^- to organic N was detected after ten minutes: 8% - 18% of the injected $^{15}NO_3^-$ was recovered in the extractable organic N pool, and 30% - 47% was found in the insoluble organic N pool. No ^{15}N above the background level was detected in the NH_4^+ pool, and complete recovery of the injected $^{15}NO_3^-$ was obtained in the total N pool (96 ± 3%; not significantly different from 100%, One-sample T test at $P > 0.05$).

There was no detectable change in NO_3^- concentrations and in $^{15}NO_3^-$ atom percent excess after 1-day incubation from those at T_0 (Paired-sample T test at $P > 0.05$), and hence we were unable to calculate gross nitrification and NO_3^- consumption rates. Net nitrification rates were measurable after 7-day incubation period and showed a decrease along the toposequence (Figure 3-4).

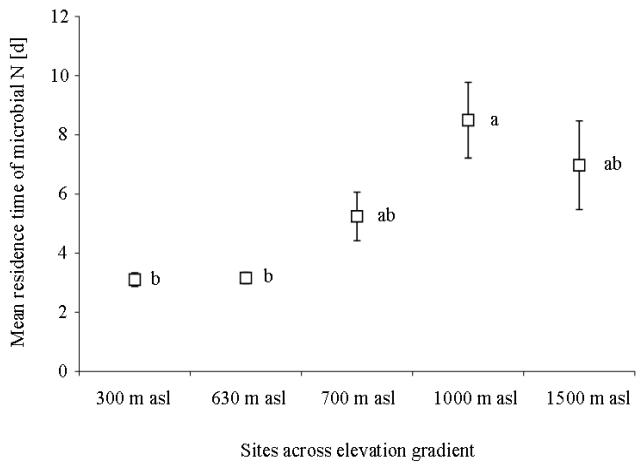


Figure 3-3: Mean residence time of the microbial biomass N across an Andosol toposequence in Ecuador. Means (± 1 S.E.; n = 5) with different letters indicate significant differences among sites (Kruskal-Wallis H test with multiple comparison extension at $P \leq 0.05$).

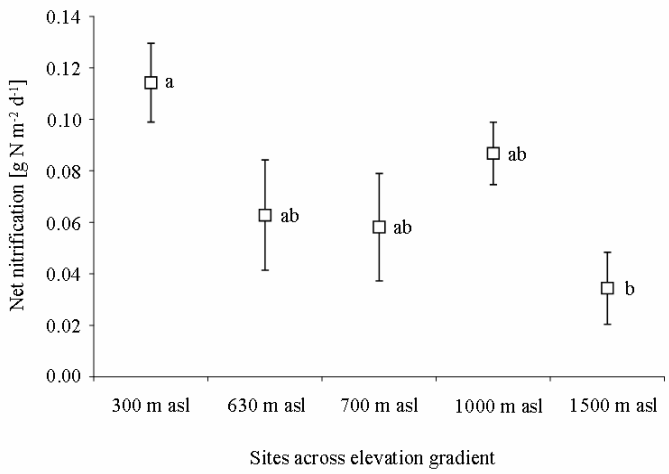


Figure 3-4: Net nitrification rates across an Andosol toposequence in Ecuador. Means (± 1 S.E.; n = 5) with different letters indicate significant differences among sites (Kruskal-Wallis H test with multiple comparison extension at $P \leq 0.05$).

Table 3-2: Spearman rank correlation coefficients among N cycling rates, microbial biomass, soil and climatic factors across an Andosol toposequence in Ecuador.

	Net nitrification [g N m^{-2} d^{-1}]	NH_4^+ consumption [g N m^{-2} d^{-1}]	Microbial biomass N [g N m^{-2}]	Microbial N mean residence time [d]	$\delta^{15}N$ [‰] (Oe+a-5 cm)	Mean annual rainfall [mm]	Mean annual temperature [°C]
Gross N mineralization [g N m^{-2} d^{-1}]	0.38†	0.90**	-0.29	-0.80**	0.59**	-0.22	0.66**
Net nitrification [g N m^{-2} d^{-1}]		0.44*	-0.05	-0.41*	0.45*	-0.22	0.47*
NH_4^+ consumption [g N m^{-2} d^{-1}]			-0.30	-0.86**	0.71**	-0.1	0.76**
Microbial biomass N [g N m^{-2}]				0.68**	-0.17	0.3	-0.28
Microbial N mean residence time [d]					-0.60**	0.29	-0.70**
$\delta^{15}N$ [‰] (top soil, Oe+a-5 cm)						0.10	0.98**
Mean annual rainfall [mm]							0.11

Notes: *n* (replicates) ≤ 25 across the altitudinal gradient; *, **, † - significant at $P \leq 0.05$, $P = \leq 0.01$, and $P = 0.08$, respectively.

3.4.3 Controlling factors

Across sites, gross N mineralization was not correlated with microbial N, microbial C:N ratios, total N, total C:N ratios of the top soil and decomposing leaf litter and with other chemical soil parameters (pH, ECEC, and base saturation). Gross N mineralization was correlated with net nitrification and NH_4^+ consumption, and because they covaried, similar correlations were observed for net nitrification and NH_4^+ consumption with the factors correlated with gross N mineralization (Table 3-2). Gross N mineralization was also correlated with microbial N MRT and $\delta^{15}N$ signatures of the topsoil (Table 3-2) and of the decomposing leaf litter ($R = 0.40$, $P \leq 0.05$, $n = 25$), and all were correlated with the mean annual air temperature (Table 3-2). Gross NH_4^+ consumption and microbial N are parameters for the calculation of microbial N MRT, and thus their correlations are methodological artefacts. Mean annual rainfall and WFPS were not correlated to gross N mineralization and net nitrification across sites.

3.5 Discussion

3.5.1 Soil characteristics indicate less developed soils with increasing elevation

The increasingly coarser soil texture across this Andosol elevation gradient can be explained by less weathered volcanic ashes at higher elevations, probably indicating contribution of relatively recent volcanic ash deposits at higher elevations. A support for this comes from a report that the area close to our 1500-m site had Andesitic volcanic ash deposited only 2500 years ago (Papale and Rossi, 1993). The generally decreasing total C and N concentrations in the topsoil and mineral soil across the toposequence suggest that the less developed soils at higher elevations constrained the accumulation of organic matter. While other old-growth montane forests at >1000-3000 m asl in southern Ecuador showed increasing accumulation of organic matter with increasing altitude (Wilcke et al., 2002; Leuschner et al., 2007), this was not the case in our Andosol toposequence at the northwestern Ecuador. The declining clay contents and organic matter (i.e. total C and N concentrations) in the topsoil contributed to the decreasing ECEC across the elevation gradient. On the other hand, the increasing base saturation across the toposequence signified an ample supply of substrate-derived cations in the less developed soils at higher elevations. An exception to these patterns

across the toposequence is the low total C and N concentrations in the 630-m site. One possible reason for this is the lower annual rainfall at this site, which may lead to low plant biomass production and subsequently low return of organic matter to the soil. The lower rainfall may also influence a different plant composition at this site which in turn might have resulted in its low leaf litter quality (i.e. lowest total N and highest C:N ratio); there are however no existing data on vegetation composition in our study sites to support this. In summary, the general trend of soil characteristics (texture, total C and N, ECEC, and base saturation) points to a decreasing degree of soil development across the elevation gradient. This Andosol toposequence depicted a relatively narrow range of soil development gradient, and in view of the general model of N availability with soil development (e.g. N which is derived primarily from the atmosphere gradually accumulates as soils develop while rock-derived nutrients become progressively unavailable as soils age; Walker and Syers, 1976), we investigated if mineral N production rates differ across sites and which factors are influencing their differences.

3.5.2 Soil N transformation rates indicate decreasing N availability with increasing elevation

There are only few measurements of gross N cycling rates in tropical soils under old-growth forests and methodologies vary considerably (particularly in sampling depth, soil incubation and mineral N extraction), making comparisons difficult. In our earlier study in some of these Ecuadorian forest sites, we found that cold storage and subsequent laboratory incubation of intact soil cores decreased gross NH_4^+ transformation rates and increased NO_3^- transformations rates compared to in-situ measurements (Arnold et al., 2008). Hence, we limit our comparison of gross NH_4^+ transformation rates only to those studies that employed in-situ incubation of intact soil cores. The gross N mineralization and NH_4^+ consumption rates of the 300-m site were 10 times higher than those reported for Ultisols in a lower montane forest in Puerto Rico (Silver et al., 2001) and exceeded those of Oxisols in lowland forests in Costa Rica by 6 times (Silver et al., 2005). The higher sites (630-m to 1500-m sites) showed gross N mineralization and NH_4^+ consumption rates which were 4 to 7 times higher than those of a montane cloud forest investigated in the same study of Silver et al. (2001). Aside from the obvious differences in soil and forest types, these studies also differed from our study in that soil N cycling was measured in the top 10-cm depth, which may have lower microbial activity than the topsoil (from Oa down to 5 cm) we measured.

There are three lines of evidence that show decreasing N availability with elevation across this Andosol toposequence. First, this was illustrated by the decreasing gross N mineralization rates with increasing elevation (Figure 3-1), which was mirrored by declining NH_4^+ concentrations (Table 3-1). Second, the decreasing turnover rates of microbial N pool across the toposequence (Figure 3-3) also implied a decline in C availability (or C mineralization) at higher elevations. Turnover rates of N pools are driven by C availability for microbial activity (Hart et al., 1994b; Corre et al., 2007; Sotta et al., 2008). Although we did not measure directly the bioavailable organic C, the pattern of available C could be inferred by the inverse relationship between gross N mineralization rates (reflecting C mineralization and hence its availability) and MRT of microbial N pool across the elevation gradient (Table 3-2). Third, the decreasing $\delta^{15}N$ signatures with increasing elevation (Table 3-1) also reflected the decreasing N cycling rates (Figures 3-1 and 3-4) with presumably declining N losses. On volcanic ash soils in Hawaii, $\delta^{15}N$ in mineral soil (0-15 cm depth) and leaves were higher at sites with old soil than at sites with younger soil, however there were no systematic changes with elevation (Vitousek et al., 1989). The $\delta^{15}N$ signatures reflect the long-term behavior of the soil N cycle of an ecosystem. Forest ecosystems with high N cycling rates have high $\delta^{15}N$ signatures because isotopically light N is lost from the ecosystem owing to fractionation during nitrification and denitrification, leaving isotopically enriched N behind (Robinson, 2001). The $\delta^{15}N$ signatures of litter and soil reflected the magnitude of soil N cycling rates of lowland forest in Brazilian Amazon (Sotta et al., 2008) and were also directly correlated with soil N-oxide emissions from a toposequence of montane forests in Indonesia (Purbopuspito et al., 2006). Putting all these lines of evidence together, our results signify that the decreasing degree of soil development with increasing elevation reflected lessening N availability.

On the other hand, both the rates of NH_4^+ consumption (Figure 3-1) and their strong correlation with gross rates of N mineralization (Table 3-2) indicated that microbial assimilation of NH_4^+ constituted the largest fate of produced NH_4^+. For this Andosol toposequence, this tightly-coupled NH_4^+ cycle limits the ^{15}N pool dilution technique to detect gross nitrification activity within one day of incubation. Similar cases were reported from montane forest soils in Indonesia (Corre et al., 2006) and Hawaii (Hall and Matson, 2003). We were able to measure net nitrification rates within 7-day incubation period, which indicated that the absence of competition from plant uptake during this longer incubation period enabled the nitrifiers to compete for

available substrate (NH_4^+ and/or organic N). This Andosol toposequence can thus be characterized as less nitrifying than the lowland Oxisols in Costa Rica (Silver et al., 2005) and Brazil (Sotta et al., 2008). The decreasing net nitrification rates across the elevation gradient (Figure 3-4) are also indicative for decreasing N availability at higher elevations.

The patterns of N cycling in this Andosol toposequence parallel those previously demonstrated for a much wider gradient of soil development (from Inceptisol, Andosol to Oxisol) in Hawaii (Hall and Matson, 2003). Just as actively cycling N accumulates as soils age over thousands and millions of years, actively cycling N increases with increasing degree of soil development (i.e. decreasing elevation) even within the Andosol order.

3.5.3 Factors controlling soil N transformation rates across the elevation gradient

We tried to identify which factors were controlling the decrease in N availability across this Andosol toposequence. Gross N mineralization rates reflect both the microbial biomass size (presumably active in mineralization, e.g. chemoheterotrophs) and the quantity and quality of substrate available for mineralization. Microbial biomass was not correlated with gross N mineralization rates across the toposequence, suggesting that microbial biomass size alone is not a good indicator of soil N cycling rates or N availability in these sites. Specific gross N mineralization rates cancels out the differences in microbial biomass size since it is expressed as N mineralization per unit microbial biomass. Thus, specific rates of gross N mineralization are a measure of quantity and quality of microbially-labile substrate for mineralization. The same index was used to reflect differences in substrate quantity and quality in different land use systems in Indonesian montane sites (Corre et al., 2006). The specific rates showed a similar pattern as gross N mineralization rates (Figures 3-1 and 3-2B), suggesting a decline in quantity and/or quality of substrate for mineralization across the elevation gradient. We however did not observe correlations between gross N mineralization rates and total N and C:N ratios both in the topsoil and decomposing leaf litter, signifying that total N concentrations and total C:N ratios do not necessarily reflect the actively cycling N fractions. Nonetheless, we cannot disregard the influence of the 630-m site on this correlation test, where its total N and C:N ratios did not follow the toposequence general trend. In this Andosol toposequence, rainfall and soil moisture did not vary systematically across the elevation gradient and were not correlated with any of the

measured N transformation rates. Instead, gross N mineralization, net nitrification, MRT of the microbial N pool, and $\delta^{15}N$ signatures were all highly correlated with temperature (Table 3-2). Unfortunately, there are no data on plant composition in our study sites to which we could possibly deduce the influence of leguminous tree species abundance. We can neither rule out nor support the influence of N fixation on N cycling rates in this Andosol toposequence.

Rates of N cycling were strongly correlated by the elevation-mediated factors: degree of soil development and temperature. Whether they both affect N cycling rates or one of them is a result of a spurious correlation, we were not able to deduct in the present study.

3.5.4 Implications of rapid reactions of injected $^{15}NO_3^-$ to organic N

The fast reaction of added $^{15}NO_3^-$ to organic N suggests abiotic NO_3^- immobilization, as also reported in other tropical forest soils (Corre et al., 2006, Sotta et al., 2008). Recently this fast reaction of NO_3^- to extractable organic N was disputed to be due to underestimation of NO_3^- caused by Fe interference, resulting in overestimation of extractable organic N (Colman et al. 2007). Our analytical method (NH_4Cl buffer without EDTA) showed a correlation of $R = 0.99$ with the imidazole method of Colman et al. (2007) for NO_3^- determination in our soil extracts, and both methods gave virtually the same NO_3^- values for the range of Fe concentrations tested. Also, the Fe concentrations (0 - 0.7 mg Fe/L) in our soil extracts were very low to cause analytical interference. Lastly, most of the injected $^{15}NO_3^-$ were recovered in the insoluble N pool, calculated as total ^{15}N recovery minus ^{15}N recovery in extractable N of which determination of extractable N by persulfate digestion is unaffected by Fe levels (Colman et al., 2007). The only hypothesis currently under consideration for the fast reaction of NO_3^- to organic N is that of Davidson et al. (2003, 2008): NO_3^- is reduced to NO_2^- in soil microsites by reduced metals in the soil (e.g. Fe(II)), followed by reaction of NO_2^- with organic matter to produce organic N and by regeneration of reducing microsites by heterotrophic activity in a C-rich medium. This abiotic NO_3^- reaction in forest soils deserves further attention as it may play an important role for N retention when projected increase of N deposition in tropical regions occurs.

3.6 Conclusions

Our study showed that N availability decreased across this Andosol toposequence. This was attributed to decreasing degree of soil development and mean annual temperature across the elevation gradient. In all sites, microbial assimilation of NH_4^+ was the main fate of produced NH_4^+. If predicted increase in N deposition occurs, the very low nitrification activity in this Andosol toposequence suggests for delayed and for the present low N losses. The timing and magnitude of N losses may follow the pattern of N availability across this Andosol elevation gradient. The fast reaction of NO_3^- to organic N remains controversial at present, although its occurrence has been reported from tropical and temperate forest soils. This process deserves further attention as it may play an important role for N retention in soils.

Acknowledgements

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4 Factors controlling the nitrogen status of contrasting forest soils along an elevation sequence in Costa Rica

4.1 Abstract

Nitrogen (N) deposition in the tropics is predicted to increase by several hundred percent in the next decades. The few studies on N cycling in tropical rain forests suggest that the nutrient status (i.e. low or high N availability) of soils is the key to investigate reactions on increasing N deposition. The main objective of our study was to quantify the soil internal N cycling rates across toposequences of Ferralsols and Andosols under old growth forests in Costa Rica and to investigate the factors controlling the N cycling. To determine gross rates of N transformation we used the ^{15}N pool dilution technique. In Ferralsols, gross N mineralization rates tended to decrease along the elevation gradient (110 m - 400 m above sea level (asl)) and were mainly controlled by the microbial biomass size which in turn was highly influenced by the quantity and quality of organic material in topsoil. Both quantity and quality of organic substrate available for mineralization strongly correlated with temperature and precipitation. Along the Andosol toposequence, gross N mineralization rates increased with elevation from 680 m to 1450 m (asl) and were also ruled by the size of microbial biomass. Here, microbial biomass was only controlled by the quality of organic matter in topsoil which in turn was mainly influenced by precipitation. In both toposequences, we also consider the abundance of legumes to be responsible for the changes in mineral N production rates. Over all sites, net N mineralization rates (an indicator for plant available N) in Ferralsols were lower than in Andosols. This was manifested in the comparison of a Ferralsol and an Andosol at two similar elevations and was not a matter of higher gross mineral N production rates in Andosols but of minor microbial NH_4^+ immobilization rates. Our results did not match the general presumption of lower N availability in young forest soils compared to more developed soils. If elevated N deposition occurs, N-losses (e.g. gaseous emissions and leaching) are predicted to follow the patterns of mineral N production rates across these two toposequences.

4.2 Introduction

Nitrogen (N) deposition is projected to at least double in economically emerging tropical regions such as South-East Asia and Latin America due to demands for food and energy by a growing population with increasing per capita use of N and their evolving industries (Galloway et al., 2004). Impacts of altered N input to ecosystems have been studied mostly in temperate regions and investigations in tropical ecosystems are rare. The few studies which have been conducted in tropical forests (e.g. Vitousek and Farrington, 1997; Tanner et al., 1998; Hall and Matson, 2003) showed that the N status (i.e. high or low N availability) of ecosystems is the key to analyze reactions to elevated N input. Ecosystems in which N availability exceeds the biological demand will probably loose N through gaseous and leaching losses, while ecosystems in which N is a limiting nutrient may react with an increase in plant biomass. In Hawaii, Hall and Matson (2003) conducted an N fertilization experiment in montane forests on an N-limited young soil, on N- and phosphorus (P)-limited soil of intermediate age, and on P-limited (N-rich) old soil. The N-rich site reacted to N addition with immediate and large NO and N_2O emissions compared to delayed and small N-oxide emissions from the N-limited site, indicating that N retention and losses are dependent on the inherent N status of an ecosystem.

The sparse information on the N status of tropical forests generally depicts the conventional explanation that the degree of soil development primarily controls the N status: N which is derived largely from the atmosphere gradually accumulates as soils develop, while rock-derived nutrients (e.g. P) become progressively unavailable as soils age (Walker and Syers, 1976; Vitousek and Farrington, 1997). Lowland tropical forests located on old, heavily weathered soils are speculated to have N excess relative to nutrients derived from parent material. This is supported by high N contents in leaf and litterfall (Tanner et al., 1998), high net N mineralization rates (Davidson et al., 2000), high nitrate leaching (Klinge et al., 2004; Schwendenmann and Veldkamp, 2005), high N-oxide emissions (Keller and Reiners, 1994; Davidson et al., 2000), and high foliar and soil ^{15}N signatures (Martinelli et al., 1999) in lowland tropical forests. In contrast, montane tropical forests located on less developed soils are expected to be limited in N rather than in rock-derived nutrients which may be in sufficient supply due to weathering (Tanner et al., 1998).

Apart from the degree of soil development, other factors are suggested to influence the N status of tropical forests. Legume occurrence can affect N availability as was shown in Puerto Rico where the presence of legumes was correlated with increased N cycling and high N-oxide emissions (Erickson et al., 2001). Across an elevation gradient in Costa Rica, leguminous tree families were not present above 1250-1500 m above sea level (asl) (Liebermann et al., 1996). However, in non-N fixing trees in Hawaii, soil age rather than elevation controls foliar ^{15}N variation with increased foliar ^{15}N signatures in old soil (Vitousek et al., 1989). ^{15}N enrichment is indicative for the long-term behavior of the soil N cycle of an ecosystem. N-rich forest ecosystems have high ^{15}N signatures due to loss of isotopically light N owing to fractionation during nitrification and denitrification, leaving isotopically enriched N behind (Martinelli et al., 1999; Amundson et al., 2003; Purbopuspito et al., 2006). Also in Hawaii, montane forests at similar elevation and on the same old parent material showed a strong decline in soil N availability across a precipitation gradient, which was attributed to increased anaerobic conditions with increased rainfall causing slower decomposition rates (Schuur and Matson, 2001).

Many montane tropical forest soils contain a thick organic layer in which roots proliferate (Edwards and Grubb, 1977; Grieve et al., 1990; Purbopuspito et al., 2006), which has been interpreted as a nutrient conserving mechanism in infertile and highly leached soils (Stark and Jordan, 1978). Accumulation of organic matter in the soil means that at any one time an appreciable part of the total N will be locked up in the organic matter and unavailable to the plants (Edwards and Grubb, 1977). However, it has also been suggested that organic layers can be important in the nutrient supply of montane tropical forests (Wilcke et al., 2002), and that root proliferation is a response to a more readily available nutrient source rather than an adaptation to nutrient shortage (Sayer et al., 2006).

In our present study, our objective was to deduct the factors controlling the N status of soils along elevation sequences in the wet tropical forests of Costa Rica. We tested the following hypotheses:

1) Soil development is a major factor controlling N availability; heavily weathered soils are characterized by high N availability, while less developed soils are characterized by low available N.

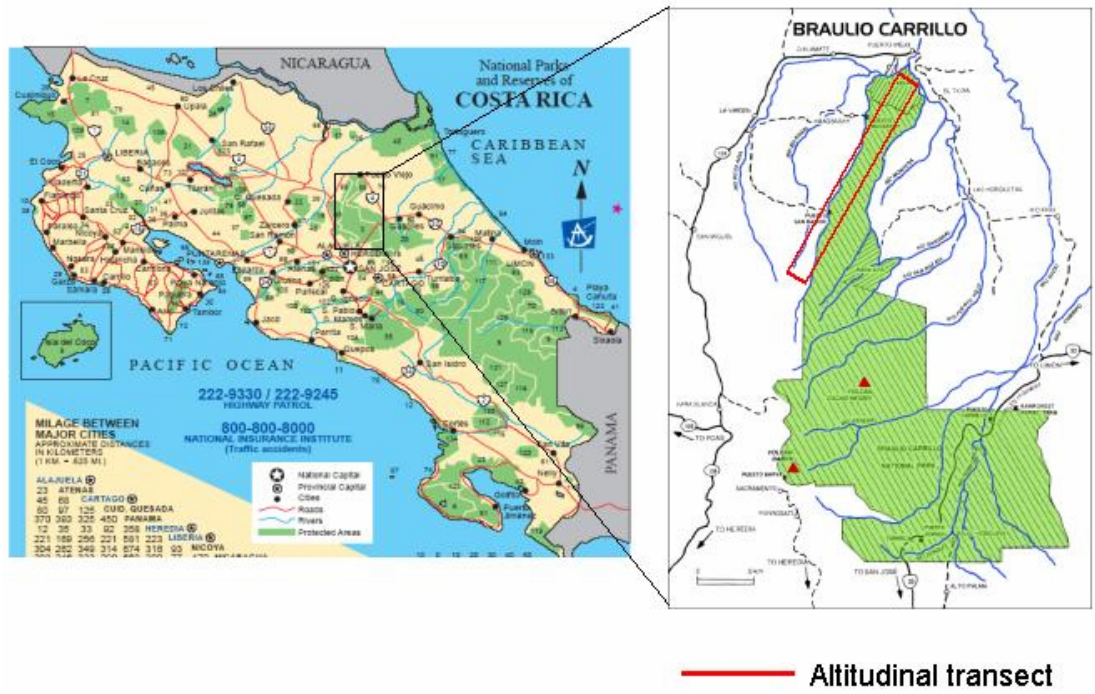
2) Elevation effects on N availability are controlled by legume abundance, climatic factors (precipitation and temperature), and presence of an organic layer.

We tested these hypotheses by measuring the soil internal N cycle using ^{15}N pool dilution along two toposequences on heavily weathered Ferralsols (Oxisols) and relatively young Andosols (Andisols) under old-growth forests in Costa Rica. We supported these measurements with ^{15}N natural abundance in litter and at different soil depths as an indicator of the long-term N cycling behavior.

4.3 Materials and methods

4.3.1 Site description and experimental design

All sites were situated on the Atlantic slopes of Volcán Barva within the Central Cordillera, Costa Rica (Map 4-1; Table 4-1). For the Ferralsol (FAO classification or Oxisol in USDA classification) toposequence, the two lower elevation sites are located in 'La Selva' Biological Station and the two upper elevations in the adjoining Braulio Carillo National Park. For the Andosol (FAO classification or Andisol in USDA classification) toposequence, the lowest elevation site is located in 'La Selva' and the higher elevations span from Braulio Carillo National Park to the border with Colonia Virgin del Socorro. The Ferralsols have developed on Andesitic lava flows (Alvarado, 1990). In the higher elevations these are heavily weathered and are called 'residual' soils by 'La Selva' convention (Sollins et al., 1994). In the lower elevations the weathered andesitic lava flows were later covered by alluvial and colluvial deposits of volcanic origin (termed as 'old alluvium' by 'La Selva' convention). These Ferralsols are heavily weathered (Kleber et al., 2007). The main difference of the Ferralsol at 400 m asl compared to the lower Ferralsol sites is the increasing influence of volcanic ash deposits (indicated by $Al_{oxalate}$ + 0.5 $Fe_{oxalate}$ of >1%) and the presence of an organic layer (indicated by the relative high C content in the top soil, Table 4-1). In the Andosol toposequence, the 40 m asl (termed as 'young alluvium' by 'La Selva' convention) contains a lot of river-deposited volcanic ash, which qualifies the soil as an Andosol ($Al_{oxalate}$ + 0.5 $Fe_{oxalate}$ >2 %). The higher elevation sites (≥680 m asl) of the Andosol toposequence showed high $Al_{oxalate}$ + 0.5 $Fe_{oxalate}$ concentrations in 5-50-cm depth as well as an increasing effective cation exchange capacity (ECEC) with altitude.



Map 4-1: Map of Costa Rica showing the Costa Rican altitudinal transect (modified after Conceptos Digitales C.R., S.A., 2002 and Organization for Tropical Studies, 2004).

4.3.2 Soil sampling

We measured the rates of soil N cycling in July and August 2006, which fall in the rainy season. For each site, 5 sampling points (considered as replicates) spaced between 25 m - 50 m were randomly selected. At each sampling point, 6 intact soil cores were taken within 0.3-m^2 area using stainless steel cores of 5 cm height and 8 cm diameter. The soil cores were taken after removing fresh and partially decomposed litter, and hence the soil samples encompassed the layer below this loose layer down to 5-cm depth (Oe+a - 5 cm). For the Ferralsols, the soil samples were largely mineral soil, but for the Andosols they included an organic layer with increasing elevation (as shown by high C contents in the top soils of higher elevation sites, Table 4-1). Additionally, we took random samples of decomposing leaf litter at each sampling point for analysis of other supporting parameters (Table 4-1). A soil profile (0-0.05, 0.05-0.10, 0.10-0.25 and 0.25-0.50 m depths) was also sampled for each site to determine general soil characteristics (Table 4-1). Air-dried plant and soil samples (for general characteristics) and frozen soil extracts (for N cycling measurements) were transported by air to

University of Goettingen, Germany for analyses; all frozen samples remained frozen during transport and were stored immediately in a freezer upon arrival.

4.3.3 Gross N mineralization, gross nitrification, and NH_4^+ immobilization rates

We conducted the N cycling measurements in-situ, including ^{15}N injection, incubation and extraction. Four of the soil cores from each sampling point were used for the determination of gross N cycling rates, using the ^{15}N pool dilution technique (Davidson et al., 1991; Hart et al., 1994a). Two cores were injected with $(^{15}NH_4)_2SO_4$ solution (containing 25 µg N ml^{-1} with 95% ^{15}N) for quantification of gross N mineralization and the other 2 cores with $K^{15}NO_3$ (containing 25 µg N ml^{-1} with 99% ^{15}N) for gross nitrification measurement. Each core received five 1-ml injections using a side-port needle and following the same injection procedures described by Davidson et al. (1991). The rate of injected NH_4^+ or NO_3^- was on average 0.5 ± 0.0 µg N g^{-1} soil for the sites up to 400 m asl and 1.1 ± 0.1 µg N g^{-1} soil for the sites ≥680 m asl that have low bulk densities. The injected NH_4^+ was between 1% and 5% of the initial soil NH_4^+ concentrations of the sites, while the injected NO_3^- was between 10% and 84% of the initial soil NO_3^- concentrations.

One soil core of each labeled pair was broken up, mixed well in a plastic bag, and subsampled for 0.5 mol/l K_2SO_4 extraction 10 minutes after ^{15}N injection (T_0 cores). This was done in the field by bringing prepared bottles containing 150 ml K_2SO_4 solution and adding soil samples into the bottles; the average K_2SO_4 solution to soil dry mass ratio was 5 for the sites up to 400 m asl and 15 for the for the sites ≥680 m asl. The other soil core of the labeled pair was put in a plastic bag, inserted back into the soil to incubate in situ for one day (T_1 cores), and extracted with K_2SO_4 in the field. The soil-K_2SO_4 bottles and the rest of the soil samples from the cores were brought to the laboratory within 2-6 hours. In the laboratory, extraction was continued by shaking the samples for 1 hour and filtering them through K_2SO_4-prewashed filter papers. Extracts were frozen immediately after filtering and further analyses were conducted in Germany. Gravimetric moisture content from each soil core was measured as soon as the samples got in the laboratory. The T_0 cores were used to correct for the reactions that occur immediately after $^{15}NH_4^+$ and $^{15}NO_3^-$ injection and gross rates of N mineralization and nitrification were calculated according to Davidson et al. (1991) and Hart et al. (1994a).

From the T_1 $^{15}NH_4^+$-labeled cores, microbial immobilization of NH_4^+ was determined upon arrival in the laboratory. About 25 g fresh soil was fumigated with $CHCl_3$ for 5 days and extracted with 0.5 mol/l K_2SO_4 (approx. 5:1 ratio of solution to dry mass soil). Extracts were frozen immediately and further analyses were conducted in Germany. From the fumigated T_1 extracts and the corresponding unfumigated T_1 extracts, extractable organic N and ^{15}N enrichment were determined using persulfate digestion and diffusion procedures are described in details by Corre et al. (2007). NH_4^+ immobilization rate was calculated using the non-linear model described by Davidson et al. (1991).

4.3.4 Analysis of N concentration and ^{15}N recovery at 10 minutes (T_0)

NH_4^+ and NO_3^- concentrations were measured using continuous flow injection colorimetry (Cenco/Skalar Instuments, Breda, Netherlands) in which NH_4^+ is quantified using the Berthelot reaction method (Skalar Method 155-000) and NO_3^- is determined using the copper-cadmium reduction method (Skalar Method 461-000). For the NO_3^- analytical method, NH_4Cl is used as the buffer but without the usual recommended chelating reagent, ethylenediamine tetraacetic acid (EDTA). Recently, Colman et al. (2007) claimed that NH_4Cl+EDTA buffer method underestimate NO_3^- concentration due to its sensitivity to Fe interference. We compared our buffer method (NH_4Cl without EDTA) against that of Colman (imidazole buffer) for NO_3^- determination of our soil extracts, and we obtained strong correlation (r = 0.99) with a slope of 0.9. The Fe levels in our soil extracts ranged from 0 to 0.7 mg Fe/l only. The organic N content of extracts was determined by persulfate digestion, described in detail by Corre et al. (2007).

We identified the fate of added ^{15}N at T_0 by measuring ^{15}N recoveries in K_2SO_4-extractable N pools. Part of the T_0 extracts was used for serial diffusion of NH_4^+ and NO_3^- (Corre and Lamersdorf, 2004) and part was used for persulfate digestion for determination of ^{15}N enrichment in extractable organic N pool (Corre et al., 2007). The same diffusion procedure and blank correction were followed as described in our earlier works (Corre et al., 2003; Corre and Lamersdorf, 2004). The ^{15}N recovery in a particular N pool was calculated based from Hart et al. (1994a), in which the ^{15}N enrichment of an N pool is subtracted by the measured natural ^{15}N abundance of that pool divided by the amount of injected ^{15}N. ^{15}N was analyzed using isotope ratio mass spectrometry (IRMS; Finigan MAT, Bremen, Germany).

4.3.5 Net N mineralization and nitrification and microbial biomass C and N

The other two cores from each sampling point were used for measurements of net N cycling rates. One core was extracted immediately in the field with 0.5 mol/l K_2SO_4 for determination of initial levels of NH_4^+ and NO_3^-. Part of the soil from this core was also used for microbial biomass determination, conducted in the laboratory after 2 – 6 h transport from the field. The other intact soil core was put in a plastic bag, reburied in the soil to incubate in-situ for 7 days, and was extracted in the field with 0.5 mol/l K_2SO_4. As described above, continuation of extraction (shaking and filtration) was done upon arrival in the laboratory. All extracts were frozen until analyses in Germany. From each soil core gravimetric water content was determined. Net rates of N mineralization were calculated by subtracting the initial NH_4^+ + NO_3^- concentrations from that after incubation divided by the days of incubation. Net rates of nitrification were calculated similarly but only considering the NO_3^- concentrations.

Microbial biomass C and N were determined by fumigation-extraction method (Brookes et al., 1985; Davidson et al., 1989). Soils were fumigated with $CHCl_3$ for 5 days and extracted with 0.5 mol/l K_2SO_4. Organic C from the extracts was analyzed by UV-enhanced persulfate oxidation using a Dohrmann DC-80 Carbon Analyzer with an infrared detector (Rosemount Analytical Division, CA, USA). Organic N was determined using persulfate digestion described earlier. Microbial biomass C and N were calculated as the difference in extractable organic C and persulfate-N between the fumigated and unfumigated soils divided by k_C = 0.45 and k_N = 0.68 for 5-day fumigated samples.

4.3.6 Other supporting parameters

Total organic C and N were measured from air-dried ground samples of decomposing leaf litter, and soil using CNS Elemental Analyzer (Elementar Vario EL, Hanau, Germany). $\delta^{15}N$ was also measured from these samples using IRMS. Bulk density was determined using the soil core method (Blake and Hartge, 1986). Soil texture was analyzed using the wet sieving and pipette method (Schlichting et al., 1995). Soil pH was measured from a saturated paste mixture (1:1 ratio of soil to H_2O). ECEC was determined from air-dried, 2-mm sieved samples, percolated with unbuffered 1 mol/l NH_4Cl, and the percolates analyzed for exchangeable cations using Flame-Atomic Absorption Spectrometer (Varian, Darmstadt, Germany). Base saturation was calculated as the percentage base cations of the ECEC. Total P was analyzed from

air-dried, ground samples, digested under high pressure in concentrated HNO_3, and the digests were analyzed using Inductively Coupled Plasma-Atomic Emission Spectrometer (Spectro Analytical Instruments, Kleve, Germany). Amorphous Fe and Al compounds were extracted in an acid oxalate solution (Del Campillo and Torrent, 1992; Buurman et al., 1996), and Fe and Al were measured using Flame-Atomic Absorption Spectrometer. Oxalate-extractable Al + ½ Fe is a criterion used for Andosol classification.

4.3.7 Statistical analyses

We first tested the sampling points of each site for spatial independence using the data of gross N mineralization rates based on the rank version of von Neumann's ratio test (Bartels, 1982). Our sampling points were spatially independent, and hence were considered replicates in the succeeding analyses. Tests for normality using Kolmogorov-Smirnov D statistics and equality of variance using Levene statistic (Sokal and Rohlf, 1981) were conducted for each parameter. As the assumption of normality and equality of variance for parametric tests was not met, we used the nonparametric methods (Siegel and Castellan, 1988). Site differences across each toposequence were assessed using Kruskal-Wallis H test followed by multiple comparison test. General comparisons between the two toposequences or specific comparisons between two sites of these toposequences (e.g. old alluvium Ferralsol and young alluvium Andosol at similar elevation) were assessed using Mann-Whitney U test. Correlation analyses were conducted on individual observations of each site using Spearman rank correlation test.

4.4 Results

4.4.1 Characteristics of decomposing leaf litter and soil along Ferralsol and Andosol toposequences

Across the Ferralsol toposequence, total C and N concentrations of the decomposing leaf litter did not differ while total C:N ratios increased (Table 4-1). $\delta^{15}N$ signatures in decomposing leaf litter constantly declined with elevation (Table 4-1). Soil texture was clay at all Ferralsol sites and total C concentrations of topsoil (0-5 cm) and deeper soil layers (5-50 cm) showed lowest values at the 310-m site and highest at the 400-m site while the two lower sites exhibited intermediate total C concentrations (Table 4-1). Nevertheless, these two lower sites showed highest total N concentrations

in the topsoil, and hence this resulted in narrower C:N ratios at the 110-m and 130-m sites than at the two upper sites (Table 4-1). As in the decomposing leaf litter, $\delta^{15}N$ in topsoil decreased across the elevation sequence (Table 4-1). In both soil depths, soil pH slightly increased across the Ferralsol toposequence (Table 4-1). No trend in ECEC and base saturation was observed for the topsoil whereas in deeper soil layers, ECEC tended to decline with elevation, and base saturation of the 310-m and 400-m sites exceeded those of the lower sites (Table 4-1). Water filled pore space (WFPS) ranged from 72% - 58% with no significant changes among sites.

Across the Andosol toposequence, total C concentrations of the decomposing leaf litter were lowest at the 40-m site and no differences were observed among the higher elevation sites (Table 4-1). Total N concentrations of the decomposing leaf litter at the 40-m site were comparable with the 1000-m and 1450-m sites while smallest total N contents were observed at 680 m altitude (Table 4-1). Consequently, we observed narrowest total C:N ratios at the 40-m site and from the 680-m site, which exhibited highest C:N values, ratios decreased again with rising altitude (Table 4-1). $\delta^{15}N$ signatures in decomposing leaf litter declined from 40 m - 1000 m asl; the 1450-m site showed signatures in between the two lowest sites (Table 4-1). Soil texture varied from silt loam (40-m and 1450-m sites) to sandy loam (680-m and 1000-m sites), and total C and N concentrations in the soil increased from the lowland up to the 1000-m site and declined again at 1450 m asl (Table 4-1). Alike in the decomposing leaf litter, ratios of C:N were narrowest at the 40-m site in both topsoil and deeper layers (Table 4-1). No pattern in $\delta^{15}N$ signatures in soils was detectable (Table 4-1). Soils became more acid across the altitudinal gradient and ECEC increased from 680 to 1450 m elevation while the 40-m site showed intermediate ECEC in topsoil and relatively high ECEC in deeper soil layers (Table 4-1). Base saturation was higher in the 40-m site than in higher elevation sites (Table 4-1). WFPS ranged from 84% - 69% with no significant differences across the gradient.

Table 4-1: General site characteristics of the Ferralsol and Andosol toposequences.

	Ferralsol toposequence				Andosol toposequence			
Elevation [m above sea level]	110	130	310	400	40	680	1000	1450
Location	La Selva Old Alluvium	La Selva Residual	Braulio Carillo	Braulio Carillo	La Selva Young Alluvium	Braulio Carillo	Virgin del Socorro	Virgin del Socorro
Mean annual precipitation [mm]	~4050	~4050	~4270	~4420	~4050	~4780	~5000	~4320
Mean annual temperature [ºC]	~23.8	~23.8	~22.7	~22.2	~23.8	~20.6	~19.1	~17.0
FAO Soil Classification	Vetic Ferralsol	Vetic Ferralsol	Vetic Ferralsol	Umbric Ferralsol	Aluandic Andosol	Aluandic Andosol	Aluandic Andosol	Aluandic Andosol
USDA Soil Classification	Haplic Haploperox	Haplic Haploperox	Haplic Haploperox	Andic Haploperox	Aquic Fulvudand	Eutric Fulvudand	Pachic Fulvudand	Pachic Fulvudand
Texture (5-10 cm depth)	Clay	Clay	Clay	Clay	Silt loam	Sandy loam	Sandy loam	Silt loam
Decomposing leaf litter								
C [mg C g^{-1}]	432.4 (21.3)	472.0 (9.7)	487.9 (9.3)	485.2 (3.6)	365.4 (19.4) b	501.5 (6.5) a	499.1 (11.2) a	499.6 (7.4) a
N [mg N g^{-1}]	16.7 (0.9)	17.4 (0.8)	17.6 (1.3)	14.0 (0.5)	18.7 (0.8) a	15.5 (0.6) b	18.0 (0.8) a	20.4 (0.6) a
C/N ratio	25.9 (0.9) b	27.1 (1.7) b	27.7 (2.8) b	34.6 (1.4) a	19.5 (0.6) d	32.4 (1.3) a	27.8 (1.5) b	24.4 (0.4) c
$\delta^{15}N$ [‰]	3.78 (0.21) a	2.83 (0.29) b	1.31 (0.30) c	0.94 (0.21) c	2.57 (0.14) a	1.38 (0.23) b	0.23 (0.2) c	2.06 (0.47) ab
Topsoil (Oe+a - 5 cm), which includes mineral soil								
Bulk density [g cm^{-3}]	0.58 (0.02)	0.52 (0.02)	0.49 (0.04)	0.45 (0.04)	0.61 (0.01) a	0.25 (0.02) b	0.24 (0.01) b	0.16 (0.02) c
pH-H_2O	4.1	4.1	4.4	4.8	6.0	4.7	4.7	3.8
Effective CEC [mmol(+)kg^{-1}]	71	78	55	88	159	71	129	319
Base saturation [%]	8.7	12.2	17.3	12.3	98.3	17.0	47.4	35.3

C [g C m^{-2}]	1886 (101)	ab	1742 (83)	ab	1526 (58)	b	1960 (55)	a	1495 (70)	b	2437(201)	ab	2649 (128)	a	2495 (174)	ab
N [g N m^{-2}]	139 (6)	a	134 (2)	a	101 (4)	b	125 (4)	ab	119 (4)		144 (13)		155 (7)		149 (10)	
C/N ratio	13.6 (0.2)	b	13.0 (0.6)	b	15.3 (0.6)	a	15.7 (0.4)	a	12.6 (0.2)	b	16.9 (0.3)	a	17.1 (0.2)	a	16.8 (0.6)	a
$\delta^{15}N$ [‰]	6.47		4.55		4.45		4.33		4.08		4.73		3.15		3.54	
NH_4^+ [g m^{-2}]	0.44 (0.06)		0.66 (0.05)		0.58 (0.05)		0.57 (0.05)		0.30 (0.03)		0.29 (0.03)		0.24 (0.03)		0.28 (0.03)	
NO_3^- [g m^{-2}]	0.07 (0.01)	a	0.06 (0.01)	a	0.03 (0.00)	b	0.03 (0.00)	b	0.06 (0.02)		0.03 (0.00)		0.03 (0.01)		0.08 (0.02)	
NH_4^+ : NO_3^- ratio	7.5 (1.6)	b	12.2 (2.7)	ab	19.8 (4.0)	a	17.6 (1.4)	a	6.7 (1.6)	ab	11.4 (1.4)	a	8.4 (2.1)	ab	4.1 (0.9)	b
Mineral soil (5-50 cm depth)																
pH-H_2O	4.3		4.3		4.7		4.8		6.0		4.9		4.9		5.0	
Effective CEC [mmol(+)kg^{-1}]	40		42		27		22		89		14		31		54	
Base saturation [%]	4.3		4.1		7.8		7.3		98.1		11.4		18.5		14.8	
C [mg C g^{-1}]	23.9		23.7		12.4		27.6		20.0		67.2		185.4		135.7	
N [mg N g^{-1}]	1.9		1.9		1.0		1.9		2.2		4.0		11.6		7.5	
C/N ratio	12.6		12.3		11.9		14.1		9.0		16.4		15.5		18.3	
$\delta^{15}N$ [‰]	6.8		6.9		6.9		7.1		6.7		7.0		5.5		6.6	

Notes: Means (±1 SE; n = 5) within each row followed by different letter indicate significant differences among sites within each toposequence (Kruskal-Wallis H test with multiple comparison extension at $P \leq 0.05$). Soil characteristics without SE values were determined from one soil profile in each site. Element concentrations in the topsoil (0-5 cm depth) are partly expressed on areal basis in order to be consistent with the units of soil N cycling rates, which were measured at the same depth.

Across all sites, total C contents in the decomposing leaf litter did not differ between the two soil types while total N concentrations were lower in Ferralsols than in Andosols. Nevertheless, no differences in C:N ratios were detected. The soils of the Ferralsol toposequence exhibited lesser concentrations of total C and N than those of the Andosol sequence, but with narrower C:N ratios. We measured higher $\delta^{15}N$ signatures in the old Ferralsols whereas pH, ECEC and base saturation of the old weathered soil sites were beyond those of Andosols.

Comparing the two alluvial soils at similar elevations (110 m and 40 m asl) but differing soil types (old alluvial Ferralsol and young alluvial Andosol), we found higher C concentrations and C:N ratios in the litter of the Ferralsol site and also C and N concentrations and C:N ratios of the old alluvial topsoil exceeded those of the Andosol. Alike in the comparison of soil types over all sites, the Ferralsol site exhibited higher $\delta^{15}N$ signatures in litter and soil while pH, ECEC and base saturation were higher at the 40-m Andosol site.

4.4.2 Soil N cycling rates and microbial biomass along Ferralsol and Andosol toposequences

As the soils in our study have bulk densities that vary from 0.16 - 0.61 g cm^{-3}, we present our N cycling rates on area base (g m^{-2} d^{-1}). Presentation on mass base would result in extremely high rates, especially in organic layers which have very low bulk densities. In the Ferralsol toposequence, gross N mineralization rates were highest at the lowest elevation and decreased at higher altitudes (Figure 4-1). NH_4^+ immobilization rates followed the same pattern, although the decrease was not significant, and accounted more than 100% of the N produced in gross mineralization at sites up to 310 m asl while both rates were similar at the 400-m site (Figure 4-1). Gross nitrification rates also decreased across the elevation gradient (Figure 4-2). Because of analytical problems, we were not able to measure gross nitrification at 130-m elevation. However, the net nitrification rates at this site were already higher than the gross nitrification rates at the 310-m and 400-m sites (Figure 4-4), hence we expect that gross nitrification at 130 m asl followed this pattern. Gross nitrification accounted 10% - 4% of gross N mineralization across sites. The pattern of gross N mineralization was also reflected in the decrease of microbial biomass N (Figure 4-3A) while there was no trend in mean residence time (MRT) of the microbial N pool (microbial N ÷ [NH_4^+ immobilization + NO_3^- consumption]) along this toposequence (Figure 4-3B). Net N transformation rates

did not differ across the elevation gradient and all of the mineralized N measured in net N mineralization was nitrified (Figure 4-4).

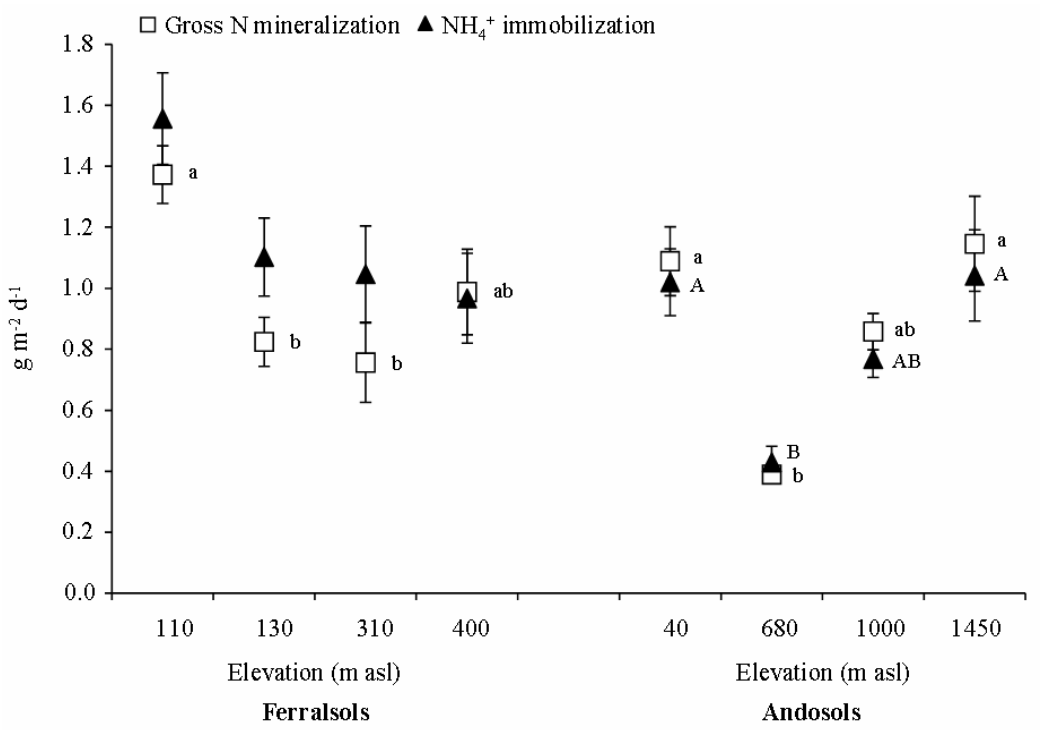


Figure 4-1: Gross N mineralization rates (open squares) and NH_4^+ immobilization rates (filled triangles) along Ferralsol and Andosol toposequences in Costa Rica. For each toposequence, means (± 1 S.E.; n = 5) with different letter indicate significant differences among sites (Kruskal-Wallis H test with multiple comparison extension at $P \leq 0.05$). Small letters for gross N mineralization rates; capital letters for NH_4^+ immobilization rates.

In the Andosol toposequence, gross N mineralization, NH_4^+ immobilization and gross nitrification rates were higher at 40 m than at 680 m asl, but increased thereafter with elevation, although not significant for gross nitrification (Figures 4-1 and 4-2). NH_4^+ immobilization comprised 90% - >100% of gross N mineralization while gross nitrification constituted 8% - 10% across the elevation gradient. Microbial biomass N tended to increase along the altitudinal gradient, although not significantly (Figure 4-3A). MRT of the microbial biomass N was shortest at the 40-m and highest at the 680-m site and thereafter showed a decrease with elevation indicating accelerated turnover rates with increasing altitude from 680 m - 1450 m asl (Figure 4-3B). Unlike in the Ferralsol toposequence, net nitrification rates at the upper three elevation sites were lower than net N mineralization rates (Figure 4-4).

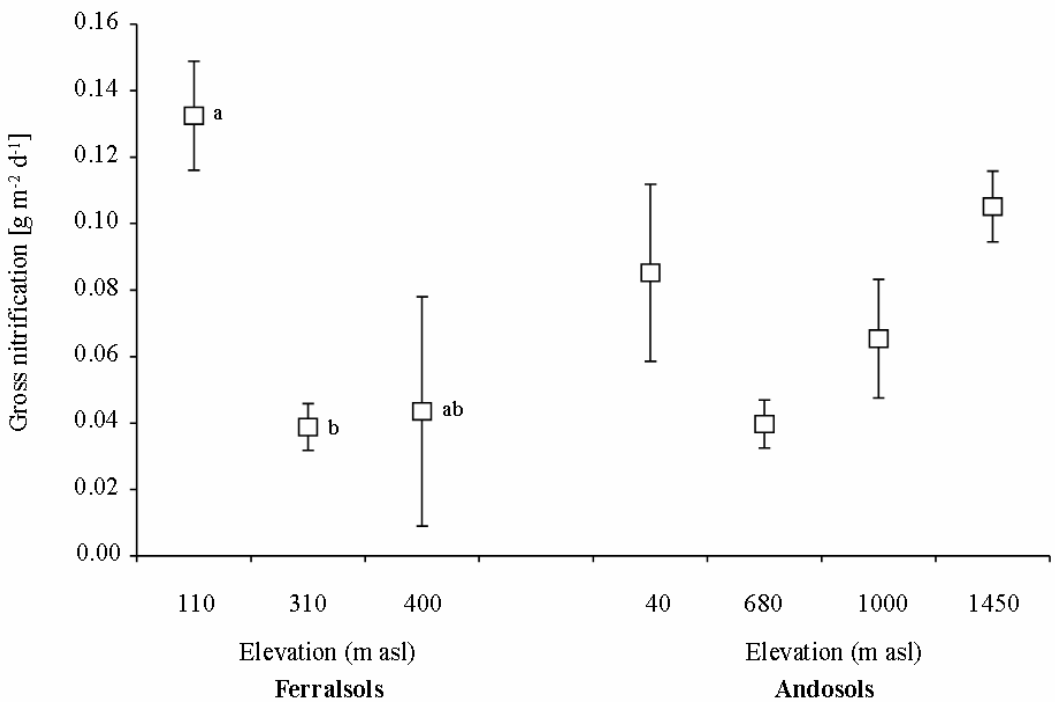


Figure 4-2: Gross N nitrification rates along Ferralsol and Andosol toposequences in Costa Rica. For each toposequence, means (± 1 S.E.; n = 5) with different letter indicate significant differences among sites (Kruskal-Wallis H test with multiple comparison extension at $P \leq 0.05$).

Comparing the two toposequences, no differences in gross N mineralization and nitrification rates could be detected between the two soil types but microbial NH_4^+ immobilization rates of Ferralsols (0.93 ± 0.12 g m^{-2} d^{-1}) exceeded those of Andosols (0.36 ± 0.06 g m^{-2} d^{-1}). Furthermore, microbial biomass N in Ferralsols (7.52 ± 0.44 g m^{-2}) was significantly higher than in Andosols (5.17 ± 0.27 g m^{-2}) whereas net N mineralization rates in Ferralsols (0.02 ± 0.01 g m^{-2} d^{-1}) were lower than in Andosols (0.09 ± 0.01 g m^{-2} d^{-1}). Comparing two soils at similar elevations but with different weathering status (i.e. old alluvium Ferralsol at 110 m and young alluvium Andosol at 40 m asl), the gross N mineralization and nitrification rates did not differ but NH_4^+ immobilization exceeded its production in the old alluvial Ferralsol while it was lesser than gross N mineralization in the young alluvial Andosol (Figure 4-1). Net N mineralization and nitrification rates were lower in the Ferralsol than in the Andosol (Figure 4-4). We also measured higher microbial biomass N (Figure 4-3A) but longer MRT of the microbial N pool (Figure 4-3B) in the old alluvial Ferralsol compared to the young alluvial Andosol.

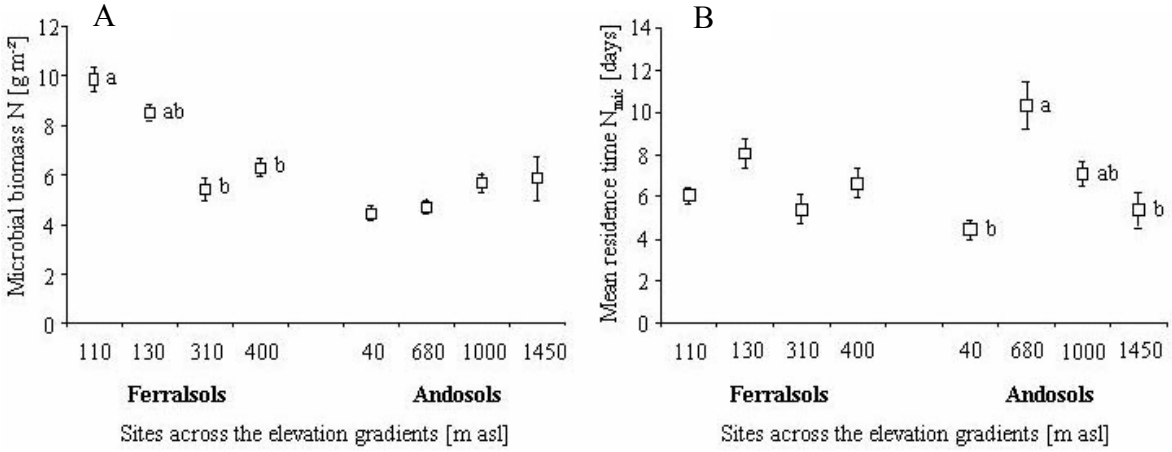


Figure 4-3: **A)** Microbial biomass N and **B)** mean residence time of the microbial biomass N along Ferralsol and Andosol toposequences in Costa Rica. For each toposequence, means (± 1 S.E.; n = 5) with different letter indicate significant differences among sites (Kruskal-Wallis H test with multiple comparison extension at $P \leq 0.05$).

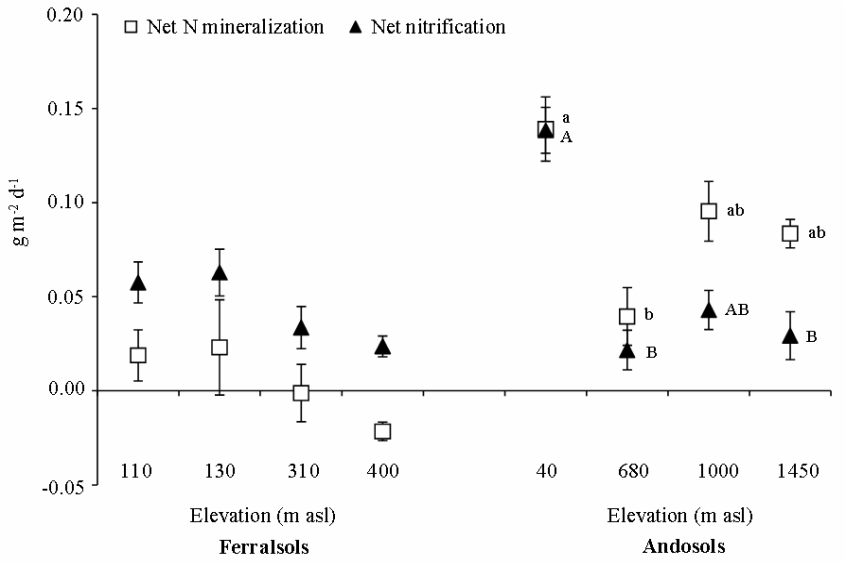


Figure 4-4: Net N mineralization rates (open squares) and net nitrification rates (filled triangles) along Ferralsol and Andosol toposequences in Costa Rica. For each toposequence, means (± 1 S.E.; n = 5) with different letter indicate significant differences among sites (Kruskal-Wallis H test with multiple comparison extension at $P \leq 0.05$). Small letters for net N mineralization rates; capital letters for net nitrification rates.

Table 4-2: Spearman correlation coefficients among N cycling rates, microbial biomass, soil and climatic factors across the Ferralsol toposequence.

	Gross nitrification [g N m^{-2} d^{-1}]	NH_4^+ immobilization [g N m^{-2} d^{-1}]	Microbial biomass N [g N m^{-2}]	Microbial N mean residence time [d]	Top soil (Oe+a - 5 cm) Total N [g N m^{-2}]	C:N ratio	δ15N [‰]	Precipitation [mm]	Temperature [° C]
Gross N mineralization [g N m^{-2} d^{-1}]	0.49†	0.81**	0.61**	-0.54**	0.53*	-0.27	0.38	-0.2	0.2
Gross nitrification [g N m^{-2} d^{-1}]		0.44	0.47†	-0.3	0.34	-0.59*	0.70**	-0.70**	0.70**
NH_4^+ immobilization [g N m^{-2} d^{-1}]			0.64**	-0.65**	0.52*	-0.47*	0.50**	-0.4	0.4
Microbial biomass N [g N m^{-2}]				0.08	0.85**	-0.76**	0.81**	-0.76**	0.76**
Microbial N mean residence time [d]					0.11	-0.13	0.03	-0.17	0.17
Total N (Oe+a - 5 cm) [g N m^{-2}]						-0.51*	0.53*	-0.51*	0.51*
C:N ratio (Oe+a - 5 cm)							-0.67**	0.75**	-0.75**
$\delta^{15}N$ (Oe+a - 5 cm) [‰]								-0.95**	0.95**
Precipitation [mm]									-1

*Notes: n (replicates) = 20 across the elevation gradient; †, *, ** - significant at P ≤0.08, P ≤0.05, and P = ≤0.01, respectively.*

Table 4-3: Spearman correlation coefficients among N cycling rates, microbial biomass, soil and climatic factors across the Andosol toposequence.

	Gross nitrification [g N m^{-2} d^{-1}]	NH_4^+ immobilization [g N m^{-2} d^{-1}]	Microbial biomass N [g N m^{-2}]	Microbial N mean residence time [d]	Top soil (Oe+a-5 cm) Total N [g N m^{-2}]	C:N ratio	δ15N [‰]	Precipitation [mm]	Temperature [° C]
Gross N mineralization [g N m^{-2} d^{-1}]	0.65**	0.95**	0.45†	-0.80**	0.10	-0.27	-0.45*	-0.43†	-0.20
Gross nitrification [g N m^{-2} d^{-1}]		0.59**	0.23	-0.61**	0.01	0.05	-0.29	-0.29	-0.29
NH_4^+ immobilization [g N m^{-2} d^{-1}]			0.56*	-0.84**	0.05	-0.35	-0.42†	-0.47*	-0.14
Microbial biomass N [g N m^{-2}]				-0.09	0.26	-0.69**	-0.42	0.04	-0.38
Microbial N mean residence time [d]					0.31	0.29	0.20	0.68**	-0.02
Total N (Oe+a - 5 cm) [g N m^{-2}]						0.33	-0.33	0.52*	-0.51*
C:N ratio (Oe+a - 5 cm)							-0.24	0.67**	-0.54*
$\delta^{15}N$ (Oe+a - 5 cm) [‰]								-0.40†	0.60**
Precipitation [mm]									-0.40†

*Notes: n (replicates) = 20 across the elevation gradient; †, *, ** - significant at P ≤0.09, P ≤0.05, and P = ≤0.01, respectively.*

4.4.3 Factors controlling soil N cycling in Ferralsols and Andosols

Across the Ferralsol toposequence, gross N mineralization was positively correlated with NH_4^+ immobilization and gross nitrification and covariation of gross N mineralization with NH_4^+ immobilization and gross nitrification led to similar correlations for these two factors with factors that were related to gross N mineralization (Table 4-2). The decrease in gross N mineralization across the gradient correlated with a decrease in microbial biomass N (Table 4-2). Because microbial biomass N and NH_4^+ immobilization strongly covaried, they showed similar correlations with the quality (expressed as total C:N ratio) and quantity (expressed in total N) of the soil organic matter (Table 4-2). Both soil organic matter quality and quantity were linked with climatic factors (precipitation and temperature, Table 4-2). Furthermore, precipitation and temperature strongly correlated with $\delta^{15}N$ signatures and with gross nitrification (Table 4-2). As precipitation and temperature strongly covaried, we were not able to distinguish which climatic factor was more important across the elevation sequence (Table 4-2).

Across the Andosol toposequence, gross N mineralization rates were strongly linked to NH_4^+ immobilization and gross nitrification rates and again, their covariation led to similar correlations for gross nitrification and NH_4^+ immobilization with the factors related to gross N mineralization (Table 4-3). Gross N mineralization correlated with microbial biomass N (Table 4-3). Microbial biomass N was related only to soil organic matter quality and unlike the Ferralsols not linked with the quantity of soil organic matter (Table 4-3). C:N ratios and $\delta^{15}N$ signatures of the topsoil were linked to both climatic factors (Table 4-3).

4.4.4 Ambient extractable N concentrations and ^{15}N recovery ten minutes (T_0) after ^{15}N addition

Along the Ferralsol toposequence, no differences in NH_4^+ concentrations were detected while NO_3^- concentrations decreased with elevation (Table 4-1). NH_4^+ concentrations dominated over NO_3^- concentrations at all sites with the tendency of increasing NH_4^+:NO_3^- ratios along the toposequence (Table 4-1). No clear pattern in extractable organic N concentrations was observed (data not shown). Recoveries of injected $^{15}NH_4^+$ in the NH_4^+ pool averaged 106.2 ± 2.7% and at none of the sites, recoveries of ^{15}N in the NH_4^+ pool were significantly lower than 100% (One-sample T

test, $P > 0.05$, Figure 4-5). 2.8 ± 0.6% of the ^{15}N added as NH_4^+ was recovered in the NO_3^- pool and recoveries were significantly higher than 0 at all sites except the 310-m site which showed high variations. ^{15}N that was injected as $^{15}NH_4^+$ and recovered in the extractable organic N pool did not differ from 0 at any of the sites. Recoveries of injected $^{15}NO_3^-$ in the NH_4^+ pool averaged 3.7 ± 0.9% and increased with elevation, differing from 0 at all sites (Figure 4-5). From the $^{15}NO_3^-$ added, we recovered an average of 11.8 ± 2.3% in the NO_3^- pool (Figure 4-5) and recoveries followed a similar pattern as the ambient NO_3^- concentrations ($R = 0.7$ at $P < 0.05$, Spearman's rho), hence sites that showed high NO_3^- concentrations also exhibited high $^{15}NO_3^-$ recoveries (Table 4-1). We detected high ^{15}N recoveries from the $^{15}NO_3^-$ injected in the extractable organic N pool (40 ± 3%) with no trend across the altitudinal gradient and no correlations with the ambient pool (Figure 4-5).

In Andosols, no trend in extractable NH_4^+, NO_3^- and organic N concentrations was observed across the elevation gradient (Table 4-1). NH_4^+ dominated over NO_3^- at all sites, and NH_4^+:NO_3^- ratios decreased from 680 m - 1450 m asl while the 40-m site exhibited an intermediate NH_4^+:NO_3^- ratio (Table 4-1). Recoveries of injected $^{15}NH_4^+$ in the NH_4^+ pool averaged 105.1 ± 4.1% and did not differ from 100% at all sites (One-sample T test, $P > 0.05$). ^{15}N recoveries in the NO_3^- pool, added as $^{15}NH_4^+$, averaged 4.3 ± 0.5% and were significantly higher than 0 at all sites. In the extractable organic N pool, ^{15}N recoveries from the injected $^{15}NH_4^+$ did not differ from 0 at any of the sites. Recoveries of injected $^{15}NO_3^-$ in the NH_4^+ pool averaged 1.6 ± 0.4% and recoveries at all sites besides the 1450-m site differed from 0 and declined along the toposequence (Figure 4-5). From the $^{15}NO_3^-$ added, we recovered 21.7 ± 4.8% in the NO_3^- pool (Figure 4-5). ^{15}N recoveries in the extractable organic N pool of the $^{15}NO_3^-$ added were high (56 ± 4%) with no trend across the altitudinal gradient and no correlations with the ambient pool (Figure 4-5).

Across all sites, extractable NH_4^+ and DON concentrations were higher in the Ferralsol sites while we did not discover differences in NO_3^- concentrations between the two soil types. Ratios of NH_4^+:NO_3^- were wider in Ferralsols. There was no consistent line of evidence that one of the two soil types generally allows higher ^{15}N recoveries than the other.

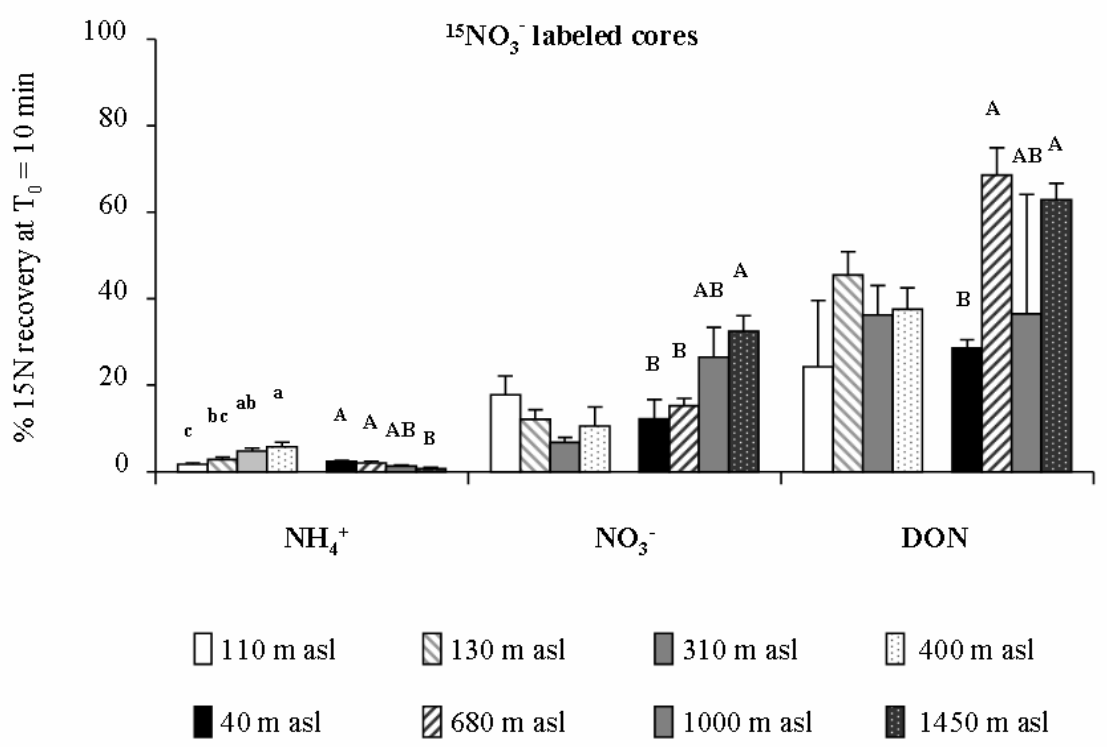


Figure 4-5: Percent recovery of injected ^{15}N (of the $^{15}NO_3^-$ injected core) in 0.5 mol/L K_2SO_4-extractable N pools after 10 minutes (T_0). For each N pool, means (± 1 S.E.; n = 5) with different letter indicate significant difference among sites for each toposequence (Kruskal-Wallis H test with multiple comparison extension at $P \leq 0.05$)). Small letters for the Ferralsol toposequence; capital letters for the Andosol toposequence.

4.5 Discussion

4.5.1 Soil N cycling and controlling factors across the Ferralsol toposequence

There are only few measurements of gross N cycling rates in tropical soils under old-growth forests and methodologies vary considerably (particularly in sampling depth, soil incubation and mineral N extraction). In our earlier study in Ecuadorian and Panamanian forest sites, we found that cold storage and subsequent laboratory incubation of intact soil cores decreased gross NH_4^+ transformation rates and increased NO_3^- transformations rates compared to in-situ measurements (Arnold et al., 2008). Hence, comparisons with other studies are difficult as most of them include cold storage and laboratory incubation.

Across the Ferralsol toposequence, decreasing gross N mineralization rates (Figure 4-1) and gross nitrification rates (Figure 4-2) together with increasing NH_4^+ : NO_3^- ratios (Table 4-1) suggest slower soil N cycling with increasing elevation (Davidson et al., 2007, 2000; Vitousek et al., 1982). This is also supported by lower microbial biomass N at higher elevations (Figure 4-3A). However, we found only limited evidence that plant available N changed from 110 m to 400 m elevation: net nitrification became negative from 310 m elevation on (net immobilization) resulting in lower NO_3^- concentrations, and C:N ratios in decomposing leaf litter were widest at the 400-m site. Other indicators of plant available N (net N mineralization and NH_4^+ concentrations) did not change significantly. As all four Ferralsols were heavily weathered, the reduction in N cycling with elevation cannot be explained by differences in weathering status. The lower quantity (lesser total N) and quality of soil organic matter (higher C:N ratio) at higher elevations were probably the direct cause of these slower rates of N cycling. Gross N mineralization rates decreased paralleled by decreasing microbial biomass N that in turn declined with lesser total N concentrations and wider C:N ratios of the soil organic matter. The correlations of total N concentrations and C:N ratios of soil organic matter with climatic factors suggest that varying precipitation and air temperature caused the changes of the organic layer, and hence the observed changes in N cycling across the Ferralsol toposequence. In addition, the link of gross nitrification to both climatic factors showed that temperature and precipitation had a direct control on changes in NO_3^- production rates. Across a mesic to wet precipitation gradient in Hawaiian montane forests, N availability declined which affected both net primary production (NPP) and nitrogen contents of the soil organic matter (N cycling was not investigated; Schuur and Matson, 2001). They attributed these changes to fluctuating anaerobic conditions that slowed down decomposition and N mineralization in the high precipitation sites. This possibly indicates that of the climatic factors temperature and rainfall (which were strongly correlated, Table 4-2) it was primarily the rainfall that affected N cycling across the Ferralsol toposequence. Alternatively, changes in the abundance of legumes with elevation may have affected N cycling along the Ferralsol toposequence. An inventory of tree species along the same elevation sequence of Volcán Barva showed that a considerable amount of legume trees occurred especially at lower elevations (Liebermann et al., 1996). The total number of leguminous trees (*Mimosaceae*, *Fabaceae* and *Caesalpiniaceae*) declined from a site at less than 100 m to a site at 300 m elevation and was again higher at 500 m asl

(Liebermann et al., 1996). This trend is similar to the trend we observed in our gross N mineralization rates (Figure 4-1). The potentially important role of legumes in the N-status of tropical forests has already been discussed by Vitousek (1984) and was illustrated in a study in Puerto Rico where a mid-successional forest with leguminous trees displayed the highest N cycling rates and corresponding N_2O + NO emissions among several secondary forest stands of different ages (Erickson et al., 2001).

The assumption of decreasing N dynamics and availability was supported by declining $\delta^{15}N$ signatures in the decomposing leaf litter and in soils across the elevation gradient. ^{15}N enrichment is an indicator for the long-term behavior of the soil N cycle of an ecosystem and N-rich forest ecosystems are reported to have high ^{15}N signatures due to the loss of isotopically light N owing to fractionation during nitrification and denitrification, leaving isotopically enriched N behind (Martinelli et al., 1999; Amundson et al., 2003; Purbopuspito et al., 2006). Purbopuspito et al. (2006) found a direct positive correlation of ^{15}N signals in litter and soil with gaseous losses along a toposequence in a montane forest in Indonesia, and hence we suspect declining gaseous N losses with increasing altitude across the Ferralsol toposequence.

4.5.2 Soil N cycling and controlling factors across the Andosol toposequence

Across the Andosol toposequence, increasing gross N mineralization (Figure 4-1) and gross nitrification rates (Figure 4-2), as well as declining ratios of NH_4^+ : NO_3^- from the 680-m to the 1450-m site indicate faster N cycling with higher altitude (Davidson et al., 2007, 2000; Vitousek et al., 1982). This was also supported by slightly increasing microbial N (Figure 4-3A) and decreasing mean residence time of the microbial biomass N at the three higher elevation sites (Figure 4-3B). Furthermore, decreasing C:N ratios in decomposing leaf litter between 680 m and 1450 m asl and the tendency of rising net N mineralization rates indicate that also N availability for plants increased with elevation. As was the case with the Ferralsol toposequence, the changing N cycling rates and N availability for plants across the toposequence could not be explained with differences in weathering stage. The correlation of gross N mineralization with microbial biomass which in turn was strongly linked to the C:N ratios of soil organic matter, and unlike in the Ferralsol toposequence not to its total N concentrations, let suspect that gross N cycling was mainly controlled by the quality of the available mineralizable substrate. Organic layers are common in montane tropical forest soils and it is generally assumed that they form as a result of reduced

decomposition rates caused by cool temperatures and high precipitation (Edwards and Grubb, 1977). The obtained results indicate that at sites providing thick organic layers, the effect of its quality overlaps the effect of its quantity for the mineral N production. The quality of organic layers is mainly governed by the composition of plant species (Burghouts et al., 1998), and hence this may have resulted in improving litter quality from the 680-m to the 1450-m site (Table 4-1). From a study on forest composition along an altitudinal transect on this volcano, we know that plant diversity decreased with altitude but the total amount of leguminous trees in this study increased from 36 to 56 individuals from a 750-m site to a 1250-m site and showed slightly lower abundance again (39 leguminous trees) at 1500 m asl (Lieberman et al., 1996). We suspect that a higher abundance of N-rich plants accounted for the better quality of decomposing leaf litter and thus improved the N cycling rates across the elevation gradient. The strong correlations between the C:N ratios of soil organic matter with climatic factors, particularly precipitation, suggest that the differing species composition was influenced by changing climatic conditions (Holdridge, 1967). Marrs et al. (1988) measured increasing total N concentrations in soils across a toposequence on Volcan Barva, but decreasing rates of net N mineralization and nitrification in soils incubated under in-situ conditions. Collateral they measured rates of mineral N production under laboratory conditions and obtained an inverse pattern for net N mineralization which they attributed to the drying and aeration of the samples measured under laboratory conditions. As our samples were collected from 0-5 cm depth and included great proportions of organic matter in contrast to those of Marrs et al. (1988) which were collected from 0-15 cm depth, the aeration of our samples was probably more similar to the aeration of samples prepared in the laboratory than of the in-situ samples. Hence, the net N mineralization we measured followed the pattern of samples which have been aerated (laboratory-incubated samples) and was similar to the pattern of total N concentrations in soils in the study of Marrs et al. (1988).

Delta ^{15}N signals of litter and soil do not reflect the pattern we received from N cycling across the toposequence and may be attributed to more recent slope processes and erosions in Andosols. Vitousek et al. (1989) detected varying foliar delta ^{15}N values in plants growing on soils of different age, with higher ^{15}N enrichment in plants growing on older soils. Hence, although we could not observe a defined difference in weathering stage across this toposequence, low delta ^{15}N values in soils and litter of the

two higher Andosol sites are probably a matter of soil age and do not reflect the present N dynamics.

The 40-m Andosol site does not fit in the pattern of increasing N cycling and plant available N with elevation which may be attributed to two factors: first, this area is flooded from a nearby creek approximately once a year which may bring an additional N input ([1]) and second, the study on forest composition of Lieberman et al. (1996) revealed that the legume abundance was the highest of all sites in this lowland area ('La Selva'). This site did not show a thick organic layer (expressed in low C contents in the topsoil) but comparably high total N concentrations resulting in extremely low C:N ratios in soil. The same trend was observed in the decomposing leaf litter, having low C and relatively high N concentrations. N cycling rates were comparable to the highest Andosol site and turnover rates of the microbial biomass were short; probably a consequence of high temperatures and high quality of leaf litter. Net rates of N mineralization and nitrification manifest the evidence of high N availability for plants at this site.

4.5.3 Comparison of Ferralsol and Andosol toposequences

Contrariwise to our expectations, gross mineral N production rates of the heavily weathered Ferralsols did not exceed those of the young Andosols. Nevertheless, N dynamics differed in that the microbial immobilization of the mineralized N in Ferralsols exceeded that in Andosols which was supported by higher microbial biomass N in Ferralsols. Overall Ferralsol sites, the ratios of NH_4^+ immobilization:gross N mineralization were > 1 in contrast to the Andosols where ratios of NH_4^+ immobilization:gross N mineralization were < 1. These microbial NH_4^+ immobilization rates were probably responsible for the difference observed in net N mineralization rates. Net N mineralization is commonly used as an indicator for plant available N in soils. Lower microbial N retention led to higher availability of mineralized N for plants in the Andosols which was also supported by higher N concentrations in the decomposing leaf litter and its narrower C:N ratios. Nevertheless, extractable mineral N concentrations of Ferralsols exceeded those of Andosols revealing that mineralized N is quickly absorbed by plants in Andosols (which we excluded within the 7 days incubation for measuring net rates). Organic layers play an important role in the nutrient supply of montane tropical forests (Wilcke et al., 2002) and roots proliferate the organic

[1] Clark, Organization for Tropical Studies, Costa Rica, personal communication

layer as a response to a readily available nutrient source (Sayer et al., 2006). We found higher fine root densities in the topsoil of sites with thick organic layers (680 - 1450 m asl) and that might have been responsible for the fast uptake of mineralized N by plants as roots in Andosols were able to compete with the microbial biomass. We obtained the same pattern in N cycling and availability in comparing only the old alluvial Ferralsol with the young alluvial Andosol and plant available N (measured as net mineralization rates) differed even more drastically. The superior N supply to plants at the young alluvial site was also manifested by extremely low C:N ratios in the decomposing leaf litter and soil. In comparing only the old alluvium and the young alluvial soil, these general differences between Ferralsols and Andosols were probably amplified by the flooding occurrences of the young alluvial soil (see page 64). However, these variations support the differences in N cycling we measured across all sites.

We know from a study concentrated on gas fluxes in old forest soils at 'La Selva' that high emissions of N_2O and moderate fluxes of NO were detected (Keller and Reiners, 1994). This brings up the assumption that great proportions of the microbially immobilized NH_4^+ is assimilated by nitrifiers and then lost as N-oxides during nitrification. Either way, our findings give great evidence that the large microbial immobilization of N is responsible for the lower N availability for plants in these Ferralsols. This does not support the general theory in that heavily weathered Ferralsols have been characterized as P-limited for plant growth and relatively rich in N, while young volcanic ash soils have been characterized as being N limited for plant growth (Vitousek and Farrington, 1997; Hall and Matson, 2003). General soil characteristics like base saturation and $\delta^{15}N$ signatures underline the difference in age between these two soil types, but even so there was much more time for N to accumulate in Ferralsols, they provided less plant available N than the Andosols. We support that soil development controls N availability in soils but against our expectations, this was not a matter of differing N production rates but of mineral N retention by the microbial biomass.

4.5.4 Ambient extractable N concentrations and ^{15}N recovery ten minutes (T_0) after ^{15}N addition

Previous studies conducted in a lower montane forest in Indonesia (Corre et al., 2006), in a montane forest on the Hawaiian Islands (Hall and Matson, 1999; Lohse and Matson, 2005) and in a lower montane forest in Puerto Rico (Silver et al., 2001) correspond with our data in finding greater amounts of NH_4^+ than NO_3^- in soils. Silver

et al. (2005) measured NH_4^+ concentrations under old growth forest at 'La Selva' Biological Station which were much lower than our findings (0.4 ± 0.1 mg kg^{-1} in a residual soil and 0.9 ± 0.5 mg kg^{-1} in an alluvial soil) but they sampled from 0-10 cm depth and extracted with 2 mol/l KCL while our results display the status in the upper 5 cm, determined with 0.5 mol/l K_2SO_4. As we only removed leaves and partly-decomposed litter before sampling, but included organic layer within the 5 cm, it is self-evident that we measured far higher concentrations of mineralized N since mineralization mainly takes place in the upper soil. NO_3^- concentrations were comparable to ours (1.7 ± 0.3 mg kg^{-1} in the residual soil and 2.2 ± 0.3 mg kg^{-1} in the alluvial soil).

Our ^{15}N recoveries in the $^{15}NH_4^+$ labeled cores at T_0 were similar to those reported for an eastern Amazonian rainforest in Brazil (Sotta et al., 2008) and a high-N-availability montane forest in Hawaii (Hall and Matson, 1999). In contrast, $^{15}NH_4^+$ recoveries in a Hawaiian N-limited site described in the same study of Hall and Matson were lower. Findings in N-limited sites in Indonesia (Corre et al., 2006) also showed a far greater fade of $^{15}NH_4^+$ added, militating against an extreme N-limitation of our Costa Rican sites. Recovery rates of more than 100% were probably due to analytical errors or an amendment rate somewhat greater than the target of 5 ml solution per core (Dail et al., 2001). Even if we consider an overestimation of $^{15}NH_4^+$ recoveries, our results indicate low abiotic NH_4^+ immobilization through fixation to clay minerals (Davidson et al., 1991) or physical condensation reactions with phenolic compounds (Nömmik, 1970; Nömmik and Vahtras, 1982; Johnson et al., 2000) which normally occurs a few minutes after injection. The conversion of $^{15}NH_4^+$ to $^{15}NO_3^-$ within 10 minutes after injection points to fast rates of N transformation at all of the sites.

In the $^{15}NO_3^-$ labeled samples, recoveries of ^{15}N in the NH_4^+ pool were high compared to the study conducted in a tropical forest in Brazil (Sotta et al., 2008), linking to fast dissimilatory nitrate reduction to ammonium (DNRA). DNRA is an anaerobic microbial process that transforms NO_3^- first to NO_2^- and then on to NH_4^+ (Silver et al., 2001). In Ferralsols and in Andosols, DNRA followed the inverse pattern of NO_3^- concentrations. Silver et al. (2001) reported of a rapid turn over of small NO_3^- pools in a tropical lower montane forest in Puerto Rico, leading to limited NO_3^- availability for denitrification and leaching. Silver et al. suggest that DNRA is sensitive to the amount of C. They found lower DNRA in forests with high C contents what would explain low ^{15}N recoveries in the NH_4^+ pool at our highest sites providing large C

concentrations. Recoveries of ^{15}N in the NO_3^- fraction were low in contrast to results from the mentioned study conducted in Brazil (Sotta et al., 2008) but higher than those reported from an N-limited forest in Indonesia (Corre et al., 2006). There are no other results from the tropics, but from temperate forests we know that forests which are richer in N allow higher $^{15}NO_3^-$ recoveries, confirming the assumed N enrichment gradient along the Andosol toposequence. The fast disappearance of injected $^{15}NO_3^-$ from the NO_3^- pool might be due to abiotic NO_3^- immobilization (Berntson and Aber, 2000; Dail et al., 2001). ^{15}N recoveries in the extractable organic N pool are high compared to the study of Sotta et al. (2008) and indicate rapid rates of transformation. Davidson et al. (2003) hypothesized that immobilized NO_3^- is converted to organic N driven by DOC. Although there was no clear pattern along the toposequences, relative high ^{15}N recoveries in the extractable organic N pool show that abiotic NO_3^- immobilization has to be taken into account when dealing with NO_3^- retention capacity of forest soils.

4.6 Conclusions

Across both toposequences, microbial biomass size largely influenced the elevation effects on N cycling rates. Decreasing gross N mineralization rates in Ferralsols and increasing rates in Andosols were controlled by the microbial biomass size which in turn was ruled weather by the quantity and quality of organic matter in topsoil (Ferralsols) or only by its quality (Andosols). Both quantity and quality of organic matter were controlled by climatic factors, particularly precipitation. Differences in trends of mineral N production rates across these two altitudinal gradients, as well as in the factors regulating these changes show that soil development is a major control for N cycling in tropical forest soils. In the strongly weathered Ferralsols, mineralized N was largely assimilated by the microbial biomass, while in the less developed Andosols, produced mineral N was more available to plants. In both soil types, we additionally consider the N cycling to be influenced by the abundance of N-fixing legumes which could be supported by a previous study on plant composition. In case of elevated N deposition, we predict N-losses, such as gaseous emissions and leaching, to follow the pattern of N availability (gross N mineralization) in soils. Thus,

we expect decreasing N-losses across the Ferralsol toposequence and increasing N-losses from 680 m to 1450 m asl in Andosols while the lowland Andosol site may show comparably high losses as we consider for the 1450-m site. The fast reaction of NO_3^- to organic N, which has been also reported in previous studies, deserves further attention as this may constitute an important retention process in soils.

5 Impacts of elevated nitrogen input on nitrogen production and retention processes in a deeply-weathered lowland forest soil in Panama*

5.1 Abstract

Nitrogen (N) deposition in the tropics is predicted to increase drastically within the next decades. This work investigated changes in mineral N production and retention processes after 9-years of N-addition to a tropical lowland forest soil, using the ^{15}N pool dilution technique. Long-term N amendment increased gross N mineralization rates whereas microbial NH_4^+ immobilization declined. Consequently, nitrifiers were able to compete for more NH_4^+ resulting in increased NO_3^- concentrations in plots that received N-addition. Ratios of NH_4^+:NO_3^- in N-treated plots were lower than in controls. Our results give strong evidence that increasing N deposition will generate environmental harm for tropical lowland forest ecosystems that show a similar N status as the tested site (i. e. relative high N availability). We expect elevated N input to result in increasing N-oxide emissions and NO_3^- leaching to ground and surface waters, since mineral N production rates and NO_3^- pools are a strong predictor for these N-losses.

*Data are included in: Impacts of elevated N input on N cycling and retention of soils under old-growth lowland and montane forests in Panama. Corre, M.D., Veldkamp, E., Arnold, J., and Wright, S.J. In preparation.

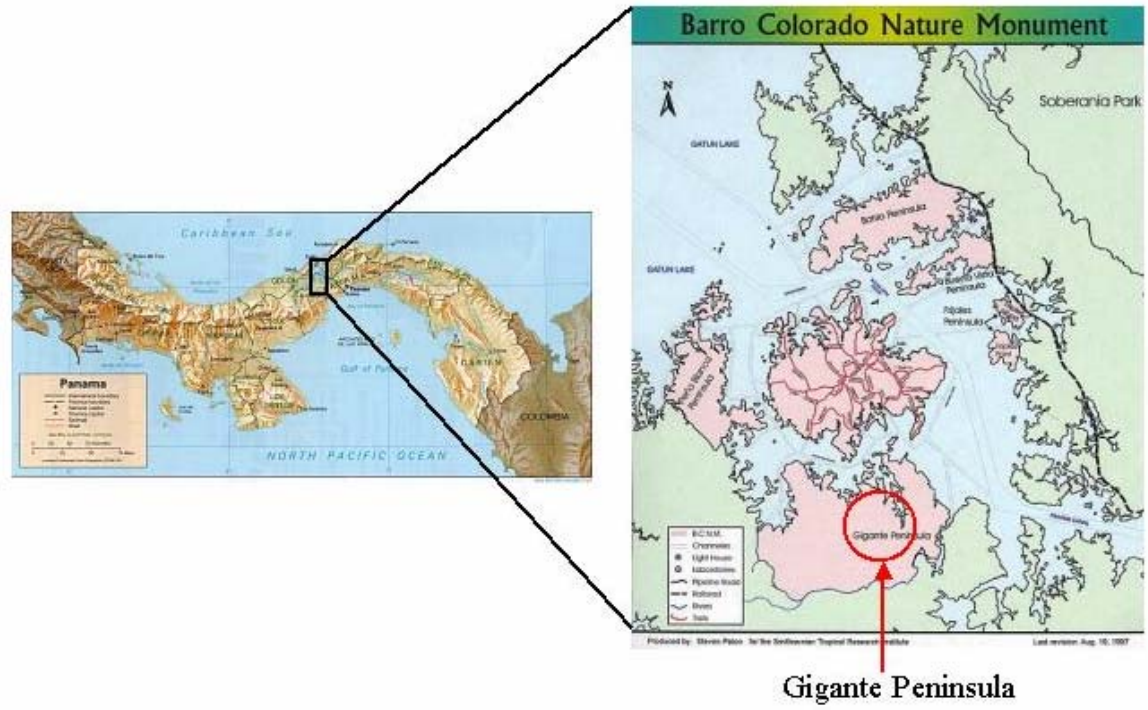
5.2 Introduction

Growing human population and activities such as the continued increase in fossil fuel consumption (Galloway et al., 1994), fertilizer use (Matthews, 1994), and biomass burning (Crutzen and Andreae, 1990; Cochrane, 2003) have increased the amount of nitrogen (N) deposition considerably. Until recently, augmented N deposition has been mainly a concern in temperate, highly industrialized regions but deposition of N increasingly occurs in fast-developing and industrializing tropical regions like Latin America and South-East Asia (Galloway and Cowling, 2002; Galloway et al., 1994; Lamarque et al., 2005). The most harmful impacts of elevated N deposition are increases in N_2O (a potent green house gas) and NO emissions (important for the formation of tropospheric ozone and acid rain) as well as NO_3^- leaching to ground and surface waters. A fertilization experiment conducted in a montane forest in Hawaii suggested that N-oxide emissions are largely influenced by mineral N production rates (Hall and Matson, 2003). Moreover, this study revealed that the abundance of nitrifiers and the NO_3^- pool of soils permit predictions on N-oxide emissions after N-addition. In an experiment undertaken in Puerto Rico, increasing net N mineralization and nitrification rates were strongly correlated with increasing N-oxide emissions (Erickson et al., 2001). Furthermore, NO_3^- leaching from the soil was partly controlled by N transformation rates in soils in Hawaii (Lohse and Matson, 2005). Hence, to estimate the consequences of the projected increase in N deposition to tropical ecosystems, data on the internal N cycling of soils and its changes after elevated N input are required.

The objectives of the present study are to assess the changes in mineral N production and retention processes after chronic N-addition in a tropical lowland forest. Since N transformation processes control the occurrence of gaseous and leaching losses, this study gives an insight into how increased N deposition will affect these forest ecosystems. We measured N transformation rates in soils using the ^{15}N pool dilution technique and supported our N cycling measurements with ^{15}N natural abundance analysis in litter and in soil of different depths.

5.3 Material and methods

The present study was conducted on the Peninsula Gigante (9° 06’ N, 79° 50” W) in the Republic of Panama (Map 5-1). The investigated site was under old-growth forest in the Barro Colorado Nature Monument, administered by the Smithsonian Tropical Institute (STRI). The soil was classified as Nitisol and previous studies revealed that plant growth is not limited by N in this soil. This study site is part of the only on-going long-term nutrient manipulation experiment in tropical lowland forests. Each treatment is represented by a 40 m x 40 m plot with four replicates, randomly distributed over a 26.6 ha area. We sampled in 4 control plots and in 4 plots that received N-additions at a rate of 125 kg urea-N ha^{-1} $year^{-1}$ since 1998. N-applications were conducted four times a year during the rainy season. Sampling took place in January 2006 in the dry season.



Map 5-1: Map of Panama showing the study site (modified after Microsoft®Encarta®Professional, 2002 and Palon, 1997).

In each plot, 6 intact soil cores were taken within a 0.3-m^2 area using stainless steel cores of 5-cm height and 8-cm diameter. Soil cores were taken after removing the litter, and hence the soil samples encompassed only mineral soil since no organic layer was abundant. Four of the 6 soil cores were used for the determination of gross rates of

N cycling using the ^{15}N pool dilution technique (Davidson et al., 1991; Hart et al., 1994a). Two cores were each injected with 125 µg N-$(NH_4)_2SO_4$ (95 % ^{15}N) contained in 5 ml solution (for gross N mineralization and NH_4^+ immobilization), and each of the other two cores with 125 µg N-KNO_3 (99 % ^{15}N) in 5 ml (for gross nitrification and NO_3^- consumption). These are equivalent to a rate of 0.9 µg N g^{-1} mineral soil. One core of each labelled pair was extracted with 0.5M K_2SO_4 after 10 minutes and the other core was put in a plastic bag and incubated for 1 day followed by extraction. The cores were incubated as intact soil cores in-situ and extracted for mineral N right in the field by bringing prepared bottles of 150 ml 0.5M K_2SO_4 solution to which well-mixed soil samples (approximate solution to dry mass soil ratio of 5) were added. The soil-K_2SO_4 bottles were brought from the field to the laboratory, shaken for 1 hour, filtered, and the extracts were frozen immediately. The T_0 cores were used to correct for the reactions that occur immediately after $^{15}NH_4^+$ and $^{15}NO_3^-$ injection and gross rates of N mineralization, nitrification, microbial NH_4^+ immobilization and NO_3^- consumption were calculated according to Davidson et al. (1991) and Hart et al. (1994a). The two remaining cores were used for measurements of initial mineral N concentrations and net N transformation rates with 7-d incubation period. Gravimetric moisture content was measured from each soil sample by oven drying at 105° C for 1 day. Detailed analytical descriptions can be found in chapter 3.3.4.

Microbial biomass N was determined by 5-days $CHCl_3$-fumigation-extraction method (Brookes et al., 1985; Davidson et al., 1989). Extractable organic N was determined using persulfate digestion described by Corre et al. (2007). Microbial biomass C and N were calculated as the difference in extractable organic C and persulfate-N between the fumigated and unfumigated soils divided by k_C = 0.45 and k_N = 0.68. Mean residence time (MRT) indicates the average length of time an N atom stays in a given pool. A lower MRT indicates a faster pool turnover rate and hence a more dynamic pool (Hart et al., 1994a). The calculation of MRT (e.g. MRT of NH_4^+ pool = NH_4^+ pool ÷ gross N mineralization rate) assumes that NH_4^+ was at steady state and that the fluxes were equal to gross rates of N mineralization and N immobilization, respectively.

Additionally, we took random samples of decomposing leaf litter at each sampling point for analysis of other supporting parameters. A soil profile (0-0.05, 0.05-0.10, 0.10-0.25 and 0.25-0.50-m depths) was also sampled to determine general soil characteristics. Air-dried leaf litter, soil samples (for general characteristics) and frozen soil extracts (for N cycling measurements) were transported by air to the University of

Goettingen, Germany for analyses; all frozen samples remained frozen during transport and were stored immediately in a freezer upon arrival.

Statistical differences between measurement methods for each site were assessed using the Mann-Whitney U Test at $P \leq 0.05$ and correlation analysis using Spearman's rank correlation test, as assumptions for normal distribution and equality of variance were not met.

5.4 Results

Initial NH_4^+ concentrations did not differ between the N-addition and control plots, whereas initial NO_3^- concentrations of plots that received chronic N-addition exceeded those of controls (Table 5-1). Consequently, ratios of NH_4^+:NO_3^- were lower in the plots obtaining N amendments. No differences in extractable organic N concentrations were detected between the two treatments (data not shown).

Gross N mineralization rates in N-fertilized plots exceeded those in control plots while the microbial immobilization of NH_4^+ decreased after 9 years of chronic N-addition (Table 5-1). In both treatments, microbial NH_4^+ immobilization comprised a much greater consumption process for NH_4^+ than nitrification. We observed the tendency of increasing gross nitrification paralleled by increasing NO_3^- consumption rates in the plots underlying chronic N-addition, but due to high variations in these N-fertilized plots this was not statistically significant (Table 5-1). Shorter mean residence times for NH_4^+ indicated faster turn over rates in the N-fertilization plots (Table 5-1), and were negatively linked to gross nitrification rates ($R = -0.88$ at $P = 0.01$). No significant differences in net N mineralization and net nitrification were observed (Table 5-1).

Focusing on the microbial biomass, concentrations of microbial biomass N and microbial biomass C:N ratios tended to decline after elevated N input (Table 5-1), due to high variation this was not significant. Water filled pore space did not differ between treatments whereas pH of soil declined after chronic N-addition. We observed higher delta ^{15}N signals in leaf litter collected in N-treated plots. The same trend was visible in delta ^{15}N signals in soil, although not significant.

Table 5-1: General characteristics of decomposing leaf litter and soil, mineral N concentrations, microbial biomass N, microbial biomass C:N, and N transformation rates.

	Control		9-year N-fertilized	
Decomposing leaf litter				
C:N ratio	36.6 (1.9)		36.6 (1.4)	
δ15N [‰]	0.05 (0.25)	b*	0.90 (0.35)	a*
Mineral soil (0-5 cm)				
pH	5.3 (0.2)	a	4.5 (0.1)	b
Water filled pore space [%]	0.5 (0.1)		0.5 (0.1)	
NH_4^+ concentration [mg kg^{-1}]	18.5 (2.5)		14.6 (2.2)	
NO_3^- concentration [mg kg^{-1}]	0.6 (0.6)	b	7.0 (1.6)	a
δ15N in 0-5 cm mineral soil [‰]	4.9 (0.5)		5.6 (0.2)	
Microbial biomass N [mg kg^{-1}]	193 (14)		135 (34)	
Microbial C:N ratio	6.6 (0.5)		4.8 (1.1)	
Gross N mineralization [mg kg^{-1} d^{-1}]	36 (2)	b	46 (3)	a
NH_4^+ immobilization [mg kg^{-1} d^{-1}]	28 (4)	a	18 (3)	b
Mean residence time for NH_4^+ [d]	0.6 (0.1)	b	0.3 (0.0)	a
Gross nitrification [mg kg^{-1} d^{-1}]	0.2 (0.1)		3.1 (2.1)	
NO_3^- consumption [mg kg^{-1} d^{-1}]	0.0 (0.0)		2.1 (1.2)	
Net N mineralization [mg kg^{-1} d^{-1}]	1.2 (0.4)		2.1 (1.1)	
Net nitrification [mg kg^{-1} d^{-1}]	1.7 (0.3)		2.3 (0.8)	

Notes: Means (±1 S.E.; n = 5) within each row followed by; different letter indicate differences between treatments (Mann-Whitney U Test at P = 0.05); significant at P ≤0.08, respectively.*

5.5 Discussion

At the present time, there are only two other studies on gross N cycling rates in tropical forest soils that are based on data obtained from intact soil cores that were incubated in-situ (Silver et al., 2001, 2005). Even so, these data are hardly comparable with ours as gross rates of N transformation were measured in 0-10 cm depth while the present study delivers data from 0-5 cm depth and N mineralization rates are highest in the topsoil (Neill et al., 1995; Piccolo et al., 1994). Hence, it is not astonishing that our rates of gross N mineralization are up to 7-fold higher than those reported from Silver et

al. (2001, 2005). In a study conducted in Hawaii, gross rates of N mineralization did not increase after long-term N-addition to a montane forest site having N in relative excess (Hall and Matson, 2003). Nevertheless, net rates of N mineralization and nitrification as well as NH_4^+ and NO_3^- concentrations rose after the amendment of N. In contrast to this, we observed altered gross N mineralization rates after long-term N-fertilization while changes in net N transformations rates were not significant.

The present study provides three signs that chronic N-addition led to increased N cycling which altered the risk of N losses. 1) Gross N mineralization rates rose with long-term N-fertilization. This increase in mineral N availability resulted from improved quality of incoming organic matter. Although, we could not measure differences in C:N ratios during the dry season in January 2006 (Table 5-1), we know from a long-term observation (1998 - 2005) that the C:N ratios of litter declined with chronic N amendments (Corre et al., in preparation). 2) Microbial NH_4^+ immobilization declined and did not keep pace with the increased production of NH_4^+. Hence, nitrifiers were able to compete for more of the produced NH_4^+, reflected by the increase of NO_3^- concentrations and declining NH_4^+:NO_3^- ratios. Davidson et al. (2000, 2007) and Vitousek et al. (1982) found that NO_3^- pools increased with the N-enrichment of ecosystems which consequently led to a decline in NH_4^+:NO_3^- ratios. 3) Highly enriched delta ^{15}N signals of leaf litter manifested that the long-term N-addition increased N cycling. ^{15}N enrichment is indicative for the long-term behavior of the soil N cycle of an ecosystem. Forest ecosystems providing fast N cycling are reported to have high ^{15}N signatures due to the loss of isotopically light N owing to fractionation during nitrification and denitrification, leaving isotopically enriched N behind (Martinelli et al., 1999; Amundson et al., 2003; Purbopuspito et al., 2006). Hence, these delta ^{15}N signatures of leaf litter (the same trend was observed in soil) manifested the assumption of accelerated N cycling paralleled by higher N-oxide losses, like observed in the Hawaiian study of Hall and Matson (2003). Additionally, we expect that NO_3^- leaching losses increased following chronic N-addition since the NO_3^- consumption did not keep pace with the increasingly available NO_3^- (Table 5-1).

These changes in N cycling rates and hence higher mineral N availability after chronic N-addition were paralleled by the tendency of decreasing microbial biomass. Declines in microbial biomass and changes in microbial communities are reported to occur when pH of soils decline, often emerging after elevated N input (Compton et al., 2004; Frey et al., 2004). But although the microbial biomass tended to decrease after

chronic N-addition, the tendency of increasing turn over rates in the microbial N pool mirrored the general trend of fastening N cycling due to N-fertilization. Narrower C:N ratios in the microbial biomass of the N-fertilization plots indicated a higher N availability for micro organisms and suggest a shift to a more bacterial dominated microbial population in these plots.

5.6 Conclusions

Long-term N-addition increased gross N mineralization due to the improvement of incoming litter quality. Microbial NH_4^+ retention declined and hence this enabled nitrifiers to compete for more NH_4^+. This was reflected in higher NO_3^- concentrations and lower ratios of NH_4^+:NO_3^- in the plots that received chronic N amendments. Since the NO_3^- pool of a system is a strong predictor of the extent of N-oxide emissions following N-additions, we assume that the changes in N cycling were paralleled by increasing N losses. Our study gives great evidence that the projected increase in N deposition will contribute to increasing emissions of N-oxides and NO_3^- leaching from ecosystems that are relatively rich in N.

6 Summarizing synthesis and conclusions of the thesis

In the first experiment of this thesis, two ways of measuring gross N cycling rates of soil were contrasted, one based on in-situ processing and incubation of soil samples and the other one based on laboratory processing and incubation. Since this comparison revealed significant differences between the two methodologies, the subsequent analyses were based on the former approach, to ensure that the data obtained represent the in-situ soil N cycling rates and not a methodological artifact. Second, we examined the soil N status (i.e. low or high N availability) of forest sites across toposequences of different soil types in Ecuador and Costa Rica by assessing the gross rates of soil N cycling. Third, this work explored the factors controlling the N availability (indicated by gross N mineralization) of each toposequence. Finally, it was studied how N cycling change after elevated N input by simulating increased N deposition in a tropical lowland forest in Panama.

6.1 Necessity of testing the methodology beforehand

Testing the differences between data obtained from samples that were processed in-situ and those that were stored before processing and incubated in the laboratory was fundamental for this thesis. This investigation revealed that reliable data of gross N cycling rates in tropical forest soils can only be obtained from samples that were processed (injected, extracted and incubated) in-situ. In view of decreasing gross N mineralization rates and increasing gross nitrification rates occurring from soil storage and laboratory incubation before analysis, we became aware of the difficulties of comparing our results with previous studies based on laboratory processing. In this thesis, the measurements of gross and net N transformation rates were conducted following the same methodology (in-situ processing) at each site, thus guaranteeing the highest degree of comparability across the individual results.

6.2 N status of soils differing in elevation and soil types

The study sites were chosen across three altitudinal gradients and included both relatively young Andosols and old, heavily weathered Ferralsols. Thus, besides the comparison of N cycling in one soil type at varying elevations, the experimental design allows the comparison of N cycling in soils at different stages of development. Gross N mineralization rates in soils did not follow a uniform pattern across the three tested toposequences. The two Andosol toposequences in Ecuador and Costa Rica showed opposite trends in gross and net mineral N production rates, although these toposequences consisted of the same soil type at similar elevations. While these parameters decreased across the altitudinal gradient of Andosols in Ecuador, they increased with elevation in the Costa Rican Andosols (disregarding the lowest site, see chapter 4.5.2). Since net N mineralization rates are used as an indicator for plant available N, this was a sign for decreasing plant available N across the Ecuadorian gradient whereas plant available N seemed to increase with elevation in Costa Rica. For the Ferralsol toposequence, we observed a decreasing trend in both gross and net mineral N production rates. These different patterns show that gross and net mineral N production rates change with elevation, but they also make clear that no uniform conclusions on changes with rising altitude are possible, nor across toposequences of the same soil type.

In comparing soils at different stages of development, this thesis tests the theory of Walker and Syers (1976). Old soils are expected to contain N, which accumulates from the atmosphere over time, in relative excess as opposed to nutrients derived from parent material that get progressively unavailable during pedogenesis. In contrast, young soils are constituted to be richer in rock derived nutrients than in N. The results of the present thesis contradict this theory as our data show that Ferralsols and Andosols, despite their difference in soil age, exhibited similar gross N mineralization and thus N availability (largely for micro organisms and plants). Even more inconsistent with Walker's and Syers' theory is the exceeding net N mineralization in Andosols compared to Ferralsols. This suggests that the plant available N was greater in the young volcanic ash soils than in the old Ferralsols. Hence, the present results suggest reconsidering the theory of Walker and Syers (1976). This is particularly the case as time progresses, given that increasing atmospheric N deposition will lead to rapid N accumulation even in young soils.

6.3 Factors controlling the N status of soils

In Ecuador, changes in gross N mineralization were paralleled by a declining degree of soil development which we did not observe along the Costa Rican toposequences. Where significant differences in soil development were found in Costa Rica, namely between Ferralsols and Andosols, the development stage did not affect gross N mineralization. However, soil age was responsible for the differing microbial immobilization of mineralized N and therefore it controlled the plant available mineral N. Also the trend observed from the Ecuador toposequence suggests that the relation of microbial NH_4^+ immobilization to its production may be partly determined by the age of soils. Ratios of NH_4^+ consumption (which largely consists of microbial NH_4^+ immobilization) to gross N mineralization tended to decrease in lesser developed soils. Nevertheless, this did not affect the net N mineralization rates across the Ecuadorian toposequence; the gradient of soil age was probably too narrow. Our results make clear that N circulates differently in soils of different ages. Microbial biomass seems to be higher (shown in the Costa Rican study) or/and more dynamic (shown by increasing MRT for the microbial N pool in the Ecuadorian study) in older soils. This would also explain the substantial gaseous emissions reported from old, strongly weathered soils, as gaseous losses occur during microbial N transformation processes. Consequently this work suggests that the stronger microbial retention of mineralized N in old soils may lead to amplified N_2O and NO emissions and on the other hand may decrease the mineral N availability for plants.

In all of the tested toposequences, mean annual air temperature influenced the gross N mineralization rates in soils either directly (Ecuador) or indirectly by controlling the thickness of organic layers (Costa Rica). In Ecuador, temperature was the only climatic factor behind the gross N mineralization rates across the Andosol toposequence. In Costa Rica, the mean annual precipitation appeared to be an additional factor influencing the mineral N production rates, by controlling the organic layer across both altitudinal gradients. Temperatures showed a similar range at the test sites in Ecuador and Costa Rica and hence their significance for N dynamics was similar. Annual rainfall, however, was considerably lower in Ecuador than in Costa Rica across all sites, which may have reduced its role in controlling the N cycling. Thus, temperature has proven to be a highly influential factor for the availability of mineral N. In contrast, it seems that precipitation does not have a substantial effect as long as it lies

in a medium range in which it does not limit mineral N production by anaerobic conditions or drought.

Again, in all of the tested toposequences, the organic layers of sites were partly responsible for the mineral N production in soils. This was manifested in the Costa Rican sites, where both quantity and quality of the organic material in topsoil controlled the gross N mineralization rates by regulating the microbial biomass in the old Ferralsols. In Andosols, it was only the quality of the organic substrate that was responsible for changes in gross N mineralization rates by controlling the microbial biomass. This evidence suggests that in ecosystems in which the accumulation of organic layers is high and mineralizable substrate is in great supply, often associated with relatively low temperatures, the effect of substrate quality gains in importance. In Ecuador, the influence of organic layers could neither be confirmed by correlations between mineral N production with quantity and quality of the organic material in topsoil, nor by correlations of substrate's quantity and quality with microbial biomass N. But specific gross N mineralization rates, which reflect the mineralizable substrate in canceling out the difference in microbial biomass size, indicated a declining quantity and/or quality of the microbially-labile substrate for mineralization (according to the pattern of gross N mineralization). We conclude that organic layers are a strong influential factor for mineral N availability. Our results suggest that the thicker the organic layer and/or the lower the temperatures, the greater the impact of the substrate's quality.

We also consider the abundance of N fixing legumes to be responsible for the N availability observed at all sites. In Costa Rica, our results could be related to a previous study on the plant diversity on an altitudinal transect in the same region. The abundance of legumes showed a similar pattern as the production rates of mineral N. Unfortunately, there is no study on the plant inventory of the tested sites in Ecuador which could be used to confirm this conjecture. However, similarities in patterns of legume abundance and gross N mineralization in Costa Rica suggest that legumes have been partly responsible for our results.

We conclude that the N status of soils is controlled by 1) the development stage of soil, 2) climatic factors, 3) the quantity and quality of the organic layer, and 4) the abundance of legumes. No doubt, theses factors are interdependent to some extent, thus making it impossible to separate their meanings. However, the fact that these factors controlling the N status in soils varied among the three toposequences suggests that the

effect of one factor may counteract and possibly outweigh one another dependent on the soil characteristics and locations.

6.4 Impacts of increasing N deposition

The experimental chronic N-addition to the Panamanian lowland forest resulted in augmented gross N mineralization. These increasing rates of mineral N production were induced by the improving quality of organic substrate available for mineralization. The chronic N-addition decreased pH and tended to reduce the microbial biomass. This was reflected in lower microbial NH_4^+ immobilization rates in the N-treated plots. Due to these changes, more NH_4^+ was available for nitrification, which was manifested in higher NO_3^- concentrations.

According to this, we expect gross N mineralization rates to increase with elevated N deposition in the tested toposequences. The extent may vary dependent on the factors that ruled gross N mineralization at each of the three gradients. Across the Ecuadorian Andosol toposequence, the increase of gross N mineralization may be weakened in elevated altitudes since temperatures and/or the development stage of soils may limit its increase. In Andosols of Costa Rica, the quality of substrate was a strong influential factor for the microbial biomass which in turn controlled gross N mineralization. Hence, we expect significant changes to occur upon the improvement of mineralizable substrate. In the upper elevations, extended rainfall may counteract the increase in mineral N production, as correlations showed that high precipitation inhibited gross N mineralization. In the Ferralsols, we also expect increasing gross N mineralization and there is no evidence that climatic conditions will inhibit these increases. In the long term, enhanced availability of N in soil may also increase above-ground plant growth, at least in the two upper Ferralsol sites which showed signs of relatively low N availability for plants (negative net rates of mineral N production). This would also enhance the quantity of organic material available for mineralization and may additionally raise the gross N mineralization rates in these two Ferralsol sites.

On the other hand, plant composition may change with elevated N deposition and the abundance of legumes could be affected. If high N availability reduces the legume incidence, this might balance the effect of the deposited N up to a certain level.

The increase of gross N mineralization rates may be paralleled by increasing N losses through gaseous emission and/or leaching. As patterns of N availability were resembled by nitrification rates and N losses are reported to be strongly connected with nitrification activities, we expect that possible N losses follow the patterns of N availability across the investigated toposequences. Microbial assimilation of NH_4^+ constituted a great sink for the produced mineral N at all sites, and the quantity of immobilized NH_4^+ was close to its production or even higher. Therefore we suspect that increasing mineral N availability will also increase the mineral N assimilation by the microbial biomass. At a point when N deposition will lead to a decline in microbial biomass size, the nitrifying population of microbial biomass may be benefited, as observed in the Panamanian experiment. This would result in increasing nitrification rates and elevated losses through N_2O and NO emission and/or NO_3^- leaching are expected. Finally, it has to be mentioned that the fast reaction of NO_3^- to organic N, also observed in studies of others, needs further investigation as this process may play an important role for N retention in times of increasing N deposition.

In conclusion, the Panamanian experiment strengthens the concern that increasing N deposition may imply strong environmental harm due to gaseous and leaching losses of N. These reactions may differ in response time and intensity according to the present N status of these tropical forest ecosystems.

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Sprachen

Deutsch	Muttersprache
Englisch	fließend in Wort und Schrift
Spanisch	fließend in Wort und Schrift
Französisch	Grundkenntnisse

Veröffentlichungen

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Beiträge zu Tagungen

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Arnold, J., Corre, M.D., Veldkamp, E. Nitrogen cycling along toposequences in two different tropical forest soils and effects of elevated N input. International NCCR Climate Summer School. 2007 in Grindelwald, Schweiz

Arnold, J., Tato, A., Domínguez, J., Zaller, J.G.: Effects of compost and vermicompost on the growth of tomato (*Lycopersicon esculentum*) and on the suppression of plant disease caused by *Phytophthora infestans*. XIV th International Colloquium on Soil Zoology and Ecology
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Erklärung

Ich versichere, dass ich die vorliegende Dissertation selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe.

Ich versichere, dass ich nicht bereits anderweitig eine Dissertation eingereicht habe oder versucht habe, mich einer Doktorprüfung zu unterziehen.

Göttingen, den 05.11.2008

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(Julia Elisabeth Arnold)