GÖTTINGER ZENTRUM

FÜR BIODIVERSITÄTSFORSCHUNG UND ÖKOLOGIE

– GÖTTINGEN CENTRE FOR BIODIVERSITY AND ECOLOGY –

Effects of increased nitrogen input on the net primary production of a tropical lower montane rain forest, Panama

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

vorgelegt von

Markus Adamek

aus

Lüdenscheid

Göttingen, Mai 2009

Referent: Dr. Marife D. Corre

Korreferent: Prof. Dr. Dirk Hölscher

Tag der mündlichen Prüfung:

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Summary

Increasing anthropogenic nitrogen (N) deposition from agricultural and industrial use, legume cultivation, combustion of fossil fuels, and biomass burning has until recently been a problem of industrialized countries in Europe, North America and East Asia. Consequently, most studies so far investigating the response of natural ecosystems to this threat originate from these temperate regions. With the ongoing development of economically-emerging countries, the most substantial increase in anthropogenic N deposition will occur in tropical regions of Asia and Latin America but knowledge about how tropical ecosystems will respond to this upcoming threat is greatly lacking. As net primary production (NPP) in many terrestrial ecosystems is N-limited and tropical rain forests – generating one-third of global terrestrial NPP – exert a considerable influence on the world's carbon (C) budget, human alterations of the N constraints on possibly N-limited NPP of some tropical ecosystems might have a drastic influence on the global C cycle.

The present thesis assessed how N fertilization affected different aspects of NPP in a tropical lower montane rain forest in western Panama with the objectives 1) to identify differences among components of above-ground net primary production (ANPP; stem growth, litterfall), 2) to determine the response of fine root productivity and turnover, and 3) to estimate the potential of the vegetation to serve as a sink for N and C. An N fertilization experiment was set up with four control and N-fertilized replicate plots of 40 × 40 m, the latter receiving 125 kg urea-N ha^{-1} $year^{-1}$ in four applications per year. Stem diameter growth was analyzed by diameter at breast height (DBH) classes and also for the three most abundant species (*Oreomunnea mexicana*, *Eschweilera panamensis*, *Vochysia guatemalensis*). Litterfall was collected every other week from four litter traps per plot. In three soil depths (organic layer, 0-10 cm and 10-20 cm mineral soil), fine root production and turnover were measured by sequential coring and fine root biomass allocation by the ingrowth core approach.

Analyses of the N and C content of different tissues (fresh leaves, wood and bark, leaf litter, and fine roots) were used to estimate N and C sequestration by NPP.

The responses of stem growth and litter production to N fertilization were highly variable as well within these components (DBH classes, species; litter categories) as in time, since the different ANPP components were not uniformly limited by N supply and subject to inter-annual climatic variation. N fertilization led to an increase in ANPP in the first year of the experiment driven by the response of its most important component which is litterfall. Total litterfall and leaf litterfall were higher under N fertilization also for the two years combined. Above-ground woody biomass was unresponsive to N addition as was stem growth of most DBH classes and species, the only exception being *E. panamensis* 10-30 cm DBH in the first year. The ability of a species to increase its stem growth in response to N addition seemed to depend on the N costs of stem growth expressed as wood C:N ratios. *E. panamensis* with a low wood C:N ratio was stronger limited by N than *O. mexicana* and *V. guatemalensis* with relatively high wood C:N ratios, and hence, a lower N demand for wood C sequestration.

Fine root production and turnover were not affected by N fertilization. Fine root biomass allocated to the 10-20 cm mineral soil in the N-fertilized plots increased two-fold compared to the control, probably because the changed vertical distribution of mineral N allows fine roots to forage for other limiting nutrients, e.g. phosphorus, in the mineral soil without being constrained by the low N availability of the unamended mineral soil.

N addition increased C sequestration in the first year. This increase can be attributed to an increased total NPP as tissue C concentrations did not change under N fertilization. Also the increase in C and N return to the forest floor with leaf litter is attributable to the increased leaf litter production. 16.5% of the added N were returned by this pathway. Leaf litter and fine root production were the most important C and N sinks. C and N sink strength of the

vegetation is dependent on whether an increase in NPP will occur and also on the C:N stoichiometry of the responsive NPP component(s).

Chapter

1

Introduction

1.1 The role of nitrogen in determining plant productivity

Nitrogen (N) occurs in large quantities in the atmosphere but the majority of it is biologically unavailable (N_2). The primary pathway of transformation of N_2 to biologically available N (ammonia [NH_4^+], nitrate [NO_3^-], and monomeric forms of organic N) is biological N fixation by microorganisms such as soil bacteria (many of which live in symbiosis with legumes; e.g. *Rhizobium*) and algae (Vitousek et al. 1997). A transformation process of minor importance is the creation of biologically available N through lightning. In contrast to the low biological availability of N is the high N demand of primary producers in many terrestrial ecosystems. N is a major constituent of amino acids, which are components of proteins, nucleic acids, chlorophyll, and enzymes that are crucial for photosynthesis (e.g. Ribulose 1,5-bisphosphate carboxylase) (Binkley et al. 2000, McGuire et al. 1995). A large fraction of plant N functions directly in capturing energy in photosynthesis and the photosynthetic capacity of leaves is closely related to their N content (Evans 1989, Evans & Seemann 1989). On the one hand N accumulates naturally in terrestrial ecosystems only at low rates through processes like biological N fixation and wet and dry deposition from the atmosphere, and on the other hand plants require N in larger quantities than other nutrients. Consequently, N limits net primary production (NPP) in many terrestrial ecosystems (Vitousek & Howarth 1991).

1.2 Net primary production

Net primary production (NPP) is the difference between total photosynthesis (gross primary production) and total plant respiration in an ecosystem. As it is not possible to measure NPP in terms of this difference in the field, NPP can be alternatively defined as the total new organic matter produced during a given interval. Hence, NPP comprises (1) the amount of new organic matter that is retained by live plants at the end of the interval, and (2) the amount of organic matter that was both produced and lost by the plants during the same interval. In the field, these components cannot be measured directly as they undergo a suite of transformations (consumption, decomposition, mortality, export) during the measurement interval. In forests, these materials are above-ground woody biomass increment, fine litterfall, aboveground losses to consumers, emissions of biogenic volatile organic compounds, aboveground losses of leached organic compounds, net increments in biomass of coarse and fine roots, dead coarse and fine roots, root losses to consumers, root exudates, carbohydrates exported by plants to their mycorrhizal or nodule symbionts and any net increases in stores of non-structural carbohydrates. For practical reasons, above-ground woody biomass increment and fine litterfall are the most frequently measured components, and their sum is equated with above-ground net primary production (ANPP). In contrast, below-ground net primary production (BNPP) is often ignored or estimated as a proportion of above-ground production (Clark et al. 2001). However, tropical moist forests may allocate as much as 50% of their annual net primary production into fine roots (diameter ≤ 2 mm) (Gill & Jackson 2000, Vogt et al. 1996).

1.3 The changing global N cycle

In the past 150 years human activities have become the dominant force in the transformation of N_2 to reactive N (Nr). Between 1860 and 1990 anthropogenic Nr creation increased ~10-fold and was by then ~1.5-fold the natural terrestrial Nr fixation, which has decreased by ~15% in the same time span (Galloway et al. 2004), mainly through the conversion of natural ecosystems into agricultural systems. Anthropogenic Nr creation is dominated by agricultural activities such as the cultivation of legumes (e.g. soy beans) and rice, intensive use of fertilizers created by the Haber-Bosch process, and biomass burning but also fossil fuel combustion plays an important role (Galloway et al. 2004, Galloway et al. 2008). Since human population as well as its per-capita food and energy consumption continue to increase, it is predicted that by 2050 anthropogenic Nr creation will be approximately triple the projected terrestrial biological N fixation (Galloway et al. 2004). As a consequence of the increasing anthropogenic Nr creation, average N deposition in large regions of the world now exceeds 10 kg N ha^{-1} year^{-1} and might double by 2050, while it is 0.5 kg N ha^{-1} year^{-1} or less in the absence of human influence (Dentener et al. 2006, Galloway et al. 2008). With the continuing development of economically-emerging countries, the most drastic increase in N deposition will shift from the industrialized regions of temperate Europe and North America to tropical regions in Latin America, Asia and Africa (Galloway et al. 2008, Matson et al. 1999, Phoenix et al. 2006).

Despite the fact that the most dramatic increase in anthropogenic N deposition is supposed to occur in the tropics, most of our knowledge on how ecosystems might respond to this threat originates from temperate ecosystems, most of which have an N-limited NPP (Galloway et al. 2008, Matson et al. 1999). The global cycles of carbon (C) and N are tightly coupled (Gruber & Galloway 2008) and an increase in N availability can alter the global C cycle by increasing NPP of formerly N-limited ecosystems, which might feed back on the rate of increase of carbon dioxide in the atmosphere (Matson et al. 1999, Vitousek et al. 1997). In contrast, elevated N input contributes to soil acidification and increases the formation of photochemical smog, which might decrease NPP of affected ecosystems (Vitousek et al. 1997). In addition, N pollution is known to change the dominance of species and to reduce plant species diversity of N-limited ecosystems (Tilman 1987). The deleterious effects of increasing N supply on N-limited ecosystems, the potential effects on global NPP and the fact that gaseous N derivates like nitrous oxide (N_2O) and nitric oxide (NO) are potent greenhouse gases, make the anthropogenic N pollution of the atmosphere one of the most threatening global change processes.

1.4 Tropical montane forests in an N-limited environment

Tropical rain forests decrease in stature and above-ground biomass (tree height, diameter at breast height, leaf area index [LAI], standing leaf biomass) as well as in ANPP (e.g. stem diameter growth, litterfall) with increasing elevation (Kitayama & Aiba 2002). Along with the decrease in above-ground biomass, goes an increase in below-ground biomass leading to increasing root:shoot ratios (Leuschner et al. 2007). As well as these changes in physiognomic characteristics are known, as great uncertainty exists about the constraints leading to these changes in forest structure and a variety of reasons has been discussed for more than three decades now (Bruijnzeel & Veneklaas 1998, Grubb 1977) with mineral nutrition, temperature and cloud cover being the most frequently considered amongst them.

In the humid tropics the weathering of rock-derived nutrients (e.g. phosphorous [P]) during pedogenesis is accelerated compared to the temperate zones and they become progressively unavailable, while N gradually accumulates from the atmosphere as soils age (Walker & Syers 1976). Hence, NPP in the majority of tropical old-growth lowland forests occurring on highly weathered soils, e.g. oxisols and ultisols, should be rather limited by P than by N (Herbert & Fownes 1995, Matson et al. 1999). In contrast, productivity of tropical montane forests is supposed to be mainly limited by N because they mostly grow on relatively young soils since soil development is repeatedly interrupted by erosion (Tanner et al. 1998). This geogenic shortage in N supply is further intensified by feedbacks with climatic conditions specific to the mountain environment. Frequent cloud cover could cause fewer photosynthetic radiation to reach the forest canopy lowering photosynthetic activity. The former as well as high precipitation cause water saturation and acidification of soils, which together with low temperatures reduces N mineralization. Low N availability decreases the production of leaves, leading to low LAI and low N content of leaves which reduce their photosynthetic capacity in an environment already low in radiation. The low N content of the foliage hampers the break-down of leaf litter and diminishes its decomposability, ultimately leading to an even tighter N cycling in tropical montane forests. To adjust to this imbalance in resource availability, the contribution of the below-ground compartment to forest NPP and biomass increases on tropical mountains, pointing to a growing relative importance of production limitation by soil nutrients at higher altitudes. A larger total root surface area might compensate for reduced specific nutrient uptake rates of fine roots in nutrient-poor soils (Röderstein et al. 2005). Adverse soil conditions are likely to shorten fine root lifespan and enhance fine root turnover and production resulting in higher root:shoot ratios and fine root biomass (Hertel & Leuschner, in press).

1.5 Topical forests in a changing global N cycle

Effects of the changing atmospheric chemistry on tropical forests are broadly discussed throughout scientific literature (Lewis et al. 2006, Matson et al. 1999, Phoenix et al. 2006, Singh & Tripathi 2000, Wright 2005, Wright 2006) but still little is known about the response of tropical forest productivity to an increase in anthropogenic N deposition (Phoenix et al. 2006). Although they are only 17% of potential vegetation by area (Melillo et al. 1993), tropical rain forests generate one-third of global terrestrial NPP (Field et al. 1998, Malhi et al. 2004). Hence, tropical forest ecosystems exert a disproportionately large influence over the world’s carbon budget. NPP in the majority of tropical old-growth lowland forests is supposed to be not limited by N supply and an increase in anthropogenic N deposition might therefore not directly affect their productivity (Matson et al. 1999). In contrast, elevated atmospheric N deposition may increase N-limited tropical montane forest NPP. The degree to which N limits productivity on tropical mountains depends on the degree of N depletion of the soils and on the structure and species composition of the ecosystem. Alleviation of N limitation to tropical forest productivity would have substantial impacts on global NPP and the global C cycle. The diversity of tropical forest ecosystems calls for more detailed investigations of the intensity to which N availability controls NPP in these ecosystems.

1.6 Objectives and working hypotheses

This study was conducted within the framework of the NITROF (Impact of elevated NItrogen input on the biogeochemistry and productivity of TROpical Forests) project, which investigates the impacts of elevated N input on the biogeochemistry and the productivity of tropical forests. The study area is located in a tropical lower montane rain forest in western Panama. The main hypothesis for the present work was that NPP in this forest would, at least in parts, be N-limited. As nutrient limitation to an ecosystem process is an interplay between nutrient availability and nutrient demand by this process, it can not be defined in an absolute way but can only be revealed if experimental addition of the respective nutrient increases the rate of this process (Tanner et al. 1998). Thus, an N fertilization experiment was set up in a paired-plots design with four 40 × 40 m replicate plots per treatment (control and N-fertilized). N-fertilized plots received 125 kg urea-N ha^{-1} $year^{-1}$ in four applications per year.

The objectives of this study were:

1.) to identify possible differences among components of above-ground net primary production (stem growth, litterfall) in the response to N fertilization, 2.) to determine whether fine root productivity and turnover respond to an increased N availability,

3.) to estimate the potential of the vegetation to serve as a sink for N and C.

The hypothesis for the first objective was that N addition would increase overall ANPP but that different components of ANPP (stem diameter growth, fine litter production) as well as different DBH classes and tree species might vary in the intensity of their response. For the second objective it was hypothesized that N fertilization would alleviate the necessity to invest biomass into fine roots foraging for N, thus leading to a decrease in fine root production and turnover.

The hypothesis for the third objective was that as plants would use a part of the added N to increase their NPP, N and also C storage in the biomass would increase. N and C concentrations of different plant tissues were measured in order to assess whether a change in N and C storage could be attributed to a change in element concentrations or plant productivity.

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Chapter

2

Early effect of elevated nitrogen input on above-ground net primary production of a lower montane rain forest, Panama

Markus Adamek, Marife D. Corre, Dirk Hölscher

Submitted manuscript

2.1 Abstract

To evaluate the effect of elevated N input on above-ground net primary production in a tropical lower montane rain forest an N fertilization experiment was conducted for two years. Stem diameter growth was analyzed by diameter at breast height classes and also for the three most abundant species. The response of stem growth and above-ground woody biomass production to N fertilization varied among diameter classes and species during the two-year N addition. N fertilization stimulated stem growth especially during a high-growth period. Stem growth of trees with 10-30 cm diameter increased quickly by N addition while trees >30 cm in diameter responded only in the second year of N addition. *Eschweilera panamensis* with 10-30 cm diameter had a higher stem growth in the N-fertilized than the control plots only in the first year of N addition, while *Oreomunnea mexicana* and *Vochysia guatemalensis* did not respond during the two-year N addition. Fine litterfall increased with N fertilization in the first year but not in the second year. Above-ground net primary production, of which fine litterfall constituted 68%, also increased only in the first year of N addition. The magnitude and timing of response of stem diameter growth and litterfall suggests that these aspects of above-ground productivity are not uniformly limited by N availability.

2.2 Introduction

Human activities have led to major increases in global emissions of nitrogen (N) to the atmosphere. At present, anthropogenic production rate of reactive N (Nr) from agricultural and industrial use, legume cultivation, combustion of fossil fuels, and biomass burning is twice that of the natural terrestrial Nr fixation (Galloway et al. 2004, Galloway et al. 2008). Continued increase in anthropogenic creation rate of Nr has led to increased N deposition in the industrialized temperate regions. However, the demand for a higher agricultural production and energy consumption by increasing human populations will shift the N deposition problem from the industrialized countries in Europe and North America to the economically-emerging countries in tropical regions (Galloway et al. 2008, Phoenix et al. 2006).

Forest ecosystems play an important role in the world's carbon budget with one-third of terrestrial net primary production generated by tropical rain forests alone (Field et al. 1998, Malhi et al. 2004). Although effects of the changing atmospheric chemistry on tropical forests are broadly discussed throughout scientific literature (Lewis et al. 2006, Matson et al. 1999, Phoenix et al. 2006, Singh & Tripathi 2000, Wright 2005, Wright 2006), little is known about the response of tropical forest productivity to an increase in N deposition (Phoenix et al. 2006). Earlier studies (e.g. Tanner et al. 1998) investigating the extent to which productivity of tropical forests is constraint by N availability seemed to confirm the soil-age nutrient limitation model by Walker & Syers (1976): N which is derived primarily from the atmosphere gradually accumulates as soils develop, while rock-derived nutrients (e.g. phosphorus, P) become progressively unavailable as soils age. This model suggests that productivity of forest ecosystems on young soils could be N-limited while ecosystems on highly weathered soils could be P-limited. The soil in our study area has developed on volcanic ash deposits and is classified as Alic Hapludand, which is less developed compared to highly weathered soils (e.g. Oxisol and Ultisol) but the actual age is unknown. We hypothesized that net primary productivity in our montane forest site is primarily N-limited.

Nutrient limitation to an ecosystem process can not be defined in an absolute way, e.g. nutrient concentration in soil, but is an interplay between nutrient availability on one hand and nutrient demand by an ecosystem process on the other hand. Thus, limitation by a nutrient can only be revealed if addition of that nutrient (i.e. experimentally) increases the rate of an ecosystem process (Tanner et al. 1998). We are aware of only eight studies that investigated N addition effects on aspects of primary productivity in natural tropical montane forests (see Appendices 1 and 2). Stem diameter growth increased under N addition in monospecific stands in Hawaii (Raich et al. 1996, Vitousek et al. 1993, Vitousek & Farrington 1997) on young to intermediate aged soils and in a mixed-species stand in Jamaica (Tanner et al. 1990) on soil of indeterminate age. However, N addition neither affected stem diameter growth in mixed-species stand in Columbia on a thin (young) soil (Cavelier et al. 2000) nor in monospecific stands in Hawaii on highly weathered Oxisol (Herbert & Fownes 1995). Litterfall was higher under N addition in Hawaiian sites located on young and highly weathered soils (Harrington et al. 2001, Herbert & Fownes 1995, Vitousek et al. 1993), while four years of N addition did not affect litterfall in a Venezuelan site on soil of indeterminate age (Tanner et al. 1992). Taken together, these results seem to generally support the soil-age N limitation model. However, this suggestion is strongly influenced by the well studied *Metrosideros polymorpha* forests on Hawaii and available results for species-rich tropical forests indicate a wider range of possible responses. Additionally, there is a deficiency in spatially and temporally concurrent data sets on the main components of above-ground net primary production of tropical forests (Shoo & VanDerWal 2008). Our study was aimed at (1) assessing changes in different components of above-ground net primary production (i.e. litterfall and stem diameter growth) in a tropical lower montane rain forest under increased N availability, and (2) identifying differences among tree diameter classes and tree species in the response of different components of above-ground net primary production of this mixed-species stand. Our results contribute to the scarce knowledge on possible responses of tropical montane forest productivity to increased anthropogenic N input.

2.3 Material and methods

2.3.1 Study area

The study area is situated in the western Panamanian province of Chiriquí. It lies within the Fortuna Forest Reserve which forms part of the La Amistad Biosphere Reserve. The Fortuna watershed forms a high valley in the Talamanca range. The study site is located at 1200-1300 m above sea level in the Quebrada Honda area (8°45' N, 82°15' W). The vegetation is a mature forest that can be classified as lower montane rain forest (*sensu* Grubb 1977, Holdridge et al. 1971). The site has a mean stem density of 1039 trees ha^{-1} and a mean basal area of 46.05 m² ha^{-1} (both for trees with ≥ 10 cm diameter at breast height (DBH)); trees over 10 cm DBH account for only 14% of all trees ≥ 1 cm DBH, but they make up for 86% of the total basal area. The mean canopy height is around 20 m; single trees (mostly individuals of *Oreomunnea mexicana*) can reach up to 40 m. The estimated number of tree species from two 1-ha plots in the Quebrada Honda area is approximately 90 (J. Dalling, personal communication). The most abundant species are: *Oreomunnea mexicana* (Standl.) Leroy (Juglandaceae), *Eschweilera panamensis* Pittier (Lecythidaceae), *Vochysia guatemalensis* Donn. Sm. (Vochysiaceae), *Cassipourea elliptica* (Sw.) Poir. (Rhizophoraceae), *Hedyosmum bonplandianum* Mart. (Chloranthaceae) and *Guarea glabra* Vahl (Meliaceae). Also common is the palm *Colpothrinax aphanopetala* R. Evans. Mean annual precipitation is 5545 ± 308 mm (1997-2007) without a clear dry season (no month < 100 mm precipitation); mean annual temperature is 20 ± 0.1 °C (1999-2007) (Figure 2-1A). Monthly precipitation and temperature for the period from January 2006 – March 2008 are shown in Figure 2-1B. Ambient N deposition from rainfall was 5 kg N ha^{-1} $year^{-1}$, measured every other week from 2006 to 2007 from a clearing near the study site; the rainfall chemical analyses are described in detail by Koehler et al. (2009).

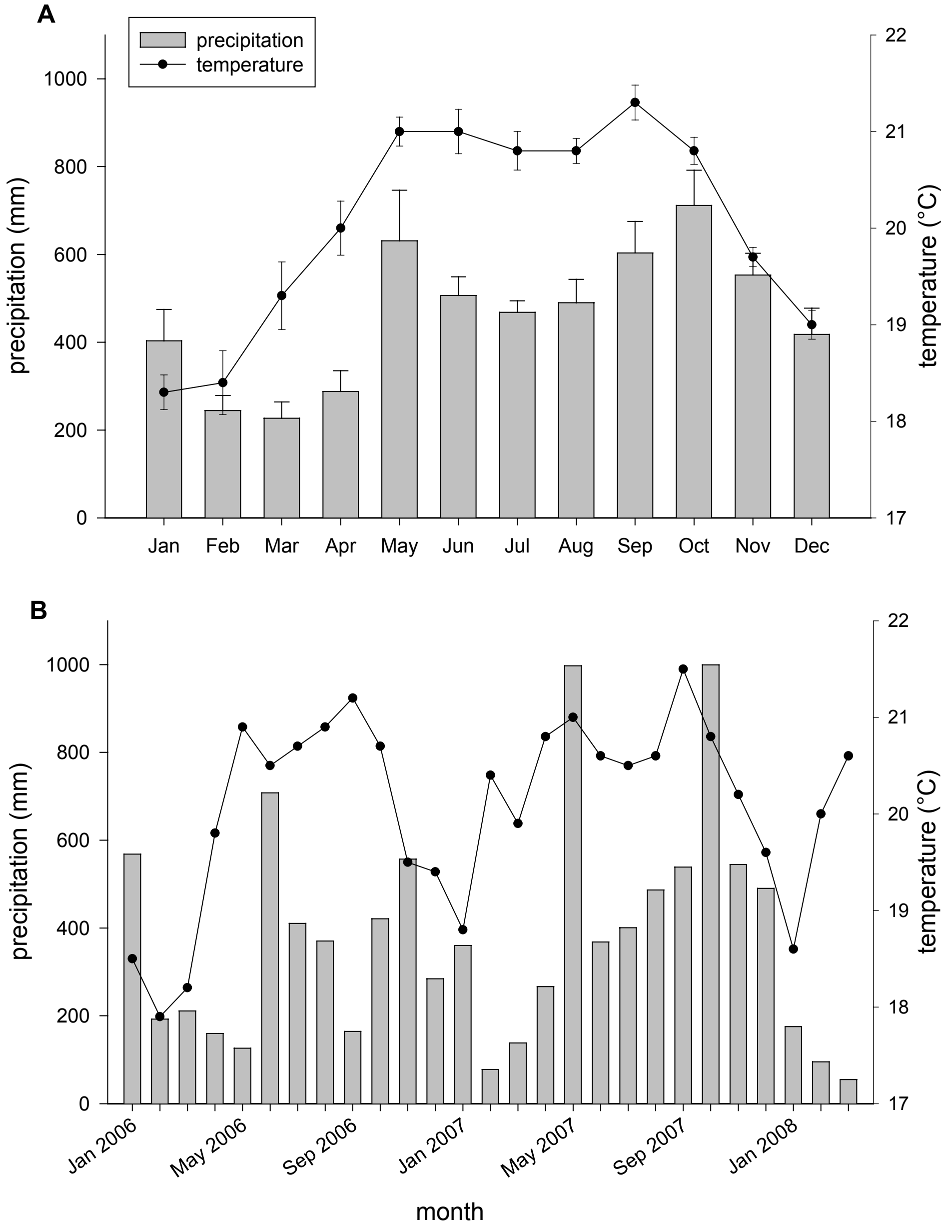

Figure 2-1. A) Monthly long-term average precipitation (1997-2007) and temperature (1999-2007), B) monthly precipitation and temperature during the study period (January 2006 – March 2008), taken from the Smithsonian Tropical Research Institute station (Casa Verde) 3.5 km from the study site, located at Quebrada Honda (Fortuna Forest Reserve), Chiriquí, Panama.

2.3.2 Experimental design and soil characteristics

The experiment was set up in a paired-plots design with four replicate plots. Each plot is 40 x 40 m, and plots are separated by at least 40 m. Plots lack gaps, swampy areas, streams or slopes steeper than 15°. Measurements of side lengths were corrected for inclination (Condit 1998). Each plot was divided into sixteen 10 x 10 m subplots. Control and N-fertilized treatments were randomly assigned to each pair of plots. The N-fertilized plots received 125 kg urea-N ha^{-1} $year^{-1}$ divided into four applications per year (February 2006, May 2006, July 2006, October 2006, February 2007, June 2007, August 2007, November 2007, March 2008). We chose urea for a practical reason: NH_4NO_3 is not sold in Panama due to security concerns. Urea was applied manually, walking back and forth across each subplot and changing directions (east-to-west and north-to-south) in subsequent applications.

Soil characteristics were determined in January 2006 prior to treatment application (for methodology see Koehler et al. 2009). These characteristics (Table 2-1) did not differ between plots which were later randomly assigned as control and N-fertilized. The soil is derived from volcanic ash deposits, has a sandy loam texture, and is classified as Aluandic Andosol (FAO classification) or Alic Hapludand (USDA) (Table 2-1).

Table 2-1. Soil characteristics of the lower montane rain forest in Quebrada Honda (Fortuna Forest Reserve), Chiriquí, Panama (means ± SE; n = 8 plots).

Characteristics	Organic layer	Mineral soil	
		0-5 cm	5-50 cm
Total C (g C kg^{-1})	443.0 (18.7)	73.0 (8.3)	30.7 (5.1)
Total N (g N kg^{-1})	22.4 (1.1)	5.0 (0.6)	1.9 (0.2)
C:N ratio	19.9 (0.4)	14.5 (0.5)	16.5 (0.6)
Total P (g P kg^{-1})	0.72 (0.07)	0.56 (0.05)	0.29 (0.07)
Effective cation exchange capacity ($mmol_c$ kg^{-1})	-	132 (25.0)	71 (18.0)
Base saturation (%)	-	21 (4.0)	11 (4.0)
Al saturation (%)	-	72 (6.0)	86 (4.0)
pH (H_2O)	4.1 (0.1)	4.1 (0.1)	4.6 (0.1)

2.3.3 Stem diameter increment

To monitor stem diameter increment rates, astralon dendrometer bands (D1, UMS, Munich, Germany) with an accuracy of 0.1 mm were attached to 40 trees with ≥ 10 cm DBH per replicate plot; dendrometer bands were installed at breast height (1.3 m) or at 0.5 m above buttresses on the few trees where they occurred at breast height (Condit 1998). Trees were categorized in three DBH classes: 10-30 cm, 30-50 cm, and > 50 cm with an average number of stems of 28, 10, and 2 trees per plot, respectively. This diameter distribution of trees monitored for stem diameter increment (hereafter dendrometer trees) was chosen because it represents the percentage diameter distribution of trees ≥ 10 cm DBH into these three DBH classes in the study area (J. Dalling, unpublished data). Dendrometer trees in the 30-50 cm and > 50 cm DBH classes often represented the only individuals of their class in a plot; trees in the 10-30 cm class were chosen by selecting two individuals in the inner subplots (inside 20 x 20 m area) and approximately one individual in the outer subplots (outside 20 x 20 m area), but all trees were chosen within the inner 30 x 30 m area of each plot so that a five meter border zone remained. Among the dendrometer trees, the growth rates of the 10-30 cm and 30-50 cm DBH classes were additionally analyzed for the three most abundant species (*O. mexicana*, *E. panamensis* and *V. guatemalensis*), as in these DBH classes they were present in at least two replicate plots per treatment. *O. mexicana* accounted for an average of 19% of dendrometer trees per plot, *E. panamensis* for 8%, and *V. guatemalensis* for 7%.

Dendrometer bands were installed in January 2006 and were allowed to stabilize for one month before measurements began. Monthly stem diameter growth was monitored for complete two years (February 2006 to March 2008) since the start of N manipulation. Readings were carried out always at the same time of day and in the same sequence to avoid bias from daily stem diameter fluctuations (Sheil 2003). Growth rates were calculated as the differences between consecutive monthly growth measurements for each tree, and monthly growth rates of the trees were averaged to represent each replicate plot; the monthly mean and standard error of the control and N-fertilized treatments were based on the four replicate plots per treatment.

2.3.4 Litter production

Fine litterfall was collected every other week beginning March 2006 using four 0.5 m² litter traps per plot. Litter traps were made of a PVC frame and a plastic mesh and were installed in the inner 20 x 20 m area of the plots to avoid litter from outside the plot to fall into the traps. The fine litter of every trap was separated into eight categories: leaves, twigs ≤ 2 cm, bark, flowers, fruits, epiphytes and unidentifiable material (rest). Dry mass of each category was determined by drying at 65°C for 72h. For each litterfall category, dry mass was calculated as a mean of the four traps per plot, and the mean and standard error were based on the four replicate plots per treatment.

Due to shortage of workforce from November 2006 to January 2007 and from April to June 2007, litter separation was only done every second litter collection for these periods. The amount of leaves during the collection dates when litter was not separated was calculated from the mean amount of leaves of the previous and following collection dates.

2.3.5 Hemispherical photographs

With the aim of comparing canopy closure and plant area index (PAI) between control and N-fertilized plots over a period of one year, hemispherical photographs of the canopy were taken in January through February 2006 and again in February 2007. Cover fraction was used as a measure of canopy closure. Photos were taken under overcast sky conditions from 11.00 to 14.00 hours. We used a Minolta Dimage Xt digital camera equipped with a 185° fisheye lens. The camera was mounted on a self-levelling device (HemiView, Delta-T, Cambridge, UK) and installed on a tripod 1.3 m above the ground facing skyward. Photographs in 2006 and 2007 were taken from the same sample points which were located at the fixed central points of the 10 x 10 m subplots of each plot, totalling to 16 photographs per plot. The photographs were analyzed with CAN_EYE Version 5 (INRA, Avignon, France). CAN_EYE includes all plant parts (stems, branches, leaves) into the analysis; thus, the calculated index is a PAI. As the increment in woody plant parts during the measurement period is assumed to be not detectable by the program, changes in PAI are attributed solely to changes in leaf area.

2.3.6 Above-ground net primary production (ANPP) estimation

Above-ground woody biomass production (AWBP) was estimated based on the growth rates of the dendrometer trees. The difference in above-ground woody biomass between succeeding years is reported here as the annual AWBP. The annual AWBP for each replicate plot was calculated by weighing the mean AWBP of the different DBH classes with the number of trees belonging to the respective DBH classes in each plot. Woody biomass was calculated based on the equation for wet forest stands given by Chave et al. (2005):

$$(AGB)_{est} = 0.0776 \times (\rho D^2 H)^{0.940}$$

where ρ is the wood density (or wood specific gravity defined as oven-dry wood mass over green volume), D is the diameter at breast height, and H is the height of the tree. We chose their formula because formulas that include three parameters (i.e. DBH, tree height and wood density) are usually considered more accurate than formulas that require a smaller set of parameters.

We measured the height of the 320 dendrometer trees at the beginning of the study using a Vertex III Ultrasonic Hypsometer with Transponder T3 (Haglöf, Sweden). Wood density was determined based on wood cores of the most important species. Wood cores were sampled by driving an increment borer (Haglöf, Sweden) with an inner diameter of 5.1 mm into the wood towards the center of the trunk to a maximum depth of 30 cm. Wood density was calculated by dividing the oven-dry mass (65°C, 72h) of a bark-free wood core with its fresh volume. Fresh volume was measured by sinking a wood core into a water-filled cylinder that was placed on a balance and the measured weight of displaced water gave the volume of the wood core (Chave 2005). A total of 96 trees from 16 species were sampled for wood density measurements with a higher number of samples (13-18 trees) in the five most abundant species. For species for which wood density was not measured, a weighted mean wood density was calculated per plot based on the measured wood densities. ANPP was calculated for each plot as the sum of annual AWBP and annual total fine litterfall.

2.3.7 Statistical analyses

Mann-Whitney U test was used to test significant differences between control and N-fertilized treatments as it is suitable for comparison of non-normally distributed data sets. Means and standard errors, determined from 4 replicate plots, are reported as measures of central tendency and dispersion. Statistical differences in canopy closure were tested using Paired T test for photo-by-photo comparison between years at each plot and Independent T test to compare the changes in canopy closure between treatments. $P \leq 0.05$ indicates significant difference.

2.4 Results

2.4.1 Stem diameter increment

Overall monthly stem diameter increment rates in the first year of N addition followed a seasonal pattern. Low increment rates were observed from February to May 2006, increasing from June to December 2006 and starting to decline in January 2007 (Figure 2-2A). This pattern was modestly correlated with monthly rainfall ($r^2 = 0.38$, $P = 0.033$, and $r^2 = 0.34$, $P = 0.047$, for control and N-fertilized plots, respectively) but not with mean monthly temperature. In the second year of N addition, monthly stem diameter increment rates showed no seasonal pattern and were neither correlated with monthly rainfall nor with mean monthly temperature. Similar seasonal patterns were observed for separate DBH classes (Figures 2-2B – 2-2D).

Trees in the 10-30 cm DBH class responded quickly to N addition; higher diameter increment rates in N-fertilized than the control plots were observed already six months after N manipulation began (Figure 2-2B). This reaction was observed during the high-growth period (August to January in both years). There was generally no difference between the control and N-fertilized plots during the low-growth period (February to May in both years). The reaction of trees in 30-50 cm DBH class was seen after 18 months (Figure 2-2C), and the higher diameter increment rates in the N-fertilized than the control plots was only observed during the high-growth period. Trees in > 50 cm DBH class generally did not respond to N addition during the two years of study (Figure 2-2D). Annual diameter increment of the overall DBH class and of single DBH classes did not differ between treatments during the two-year measurement (Table 2-2).

Of the three most abundant species, *E. panamensis* responded quickly to N addition. Diameter increment of the N-fertilized *E. panamensis* in the 10-30 cm DBH class was significantly higher after eight months (Figure 2-3A). Again, the response of *E. panamensis* to N addition was exhibited during the high-growth period. The *E. panamensis* in the 30-50 cm DBH class did not respond to N addition (Figure 2-3B). Annual diameter increment of *E. panamensis* was fivefold higher in the N-fertilized than in the control plots for the 10-30 cm DBH class in the first year (Table 2-2). In addition, *V. guatemalensis* in the 30-50 cm DBH class responded to N addition (Table 2-2). However, this has to be seen with caution, because this increased annual diameter increment in N-fertilized *V. guatemalensis* was mostly due to the extraordinarily high growth rate of one fast growing individual; exclusion of this individual annulled the difference. Diameter increment of *O. mexicana* did not respond to N addition during the two-year measurement (Table 2-2).

Table 2-2. Stem diameter growth for different diameter at breast height (DBH) classes and species. Within each column, means (SE; n = 4 plots) followed by a different letter indicate significant differences between treatments within years (Mann-Whitney U test at $P \leq 0.05$).

Treatment	DBH classes (cm)				*E. panamensis* *DBH (cm)*		*O. mexicana* *DBH (cm)*		*V. guatemalensis* *DBH (cm)*	
	overall	10-30	30-50	> 50	10-30	30-50	10-30	30-50	10-30	30-50
					1st year (mm y^{-1})					
Control	1.68^{a}	1.06^{a}	2.90^{a}	3.77^{a}	0.15^{a}	0.10^{a}	2.79^{a}	5.95^{a}	4.05^{a}	2.81^{a}
	(0.17)	(0.14)	(0.42)	(0.89)	(0.06)	(0.00)	(0.96)	(1.10)	(1.05)	(1.32)
N-fertilized	2.04^{a}	1.50^{a}	3.61^{a}	2.26^{a}	0.84^{b}	2.31^{a}	2.69^{a}	5.24^{a}	3.52^{a}	9.6^{b}
	(0.21)	(0.08)	(0.25)	(1.42)	(0.34)	(0.79)	(0.29)	(0.60)	(2.83)	(3.11)
					2nd year (mm y^{-1})					
Control	1.54^{a}	1.09^{a}	2.07^{a}	4.62^{a}	0.29^{a}	0.00^{a}	3.22^{a}	4.85^{a}	1.58^{a}	1.18^{a}
	(0.17)	(0.13)	(0.34)	(1.15)	(0.15)	(0.10)	(1.51)	(0.95)	(0.73)	(0.43)
N-fertilized	1.81^{a}	1.36^{a}	3.18^{a}	2.89^{a}	0.79^{a}	2.10^{a}	2.57^{a}	4.37^{a}	1.33^{a}	4.10^{a}
	(0.17)	(0.12)	(0.13)	(0.93)	(0.35)	(1.60)	(0.17)	(0.81)	(0.12)	(1.27)
					Combined 2 years (mm)					
Control	3.23^{a}	1.92^{a}	4.97^{a}	8.38^{a}	0.44^{a}	0.10^{a}	6.01^{a}	10.81^{a}	5.63^{a}	3.99^{a}
	(0.34)	(0.37)	(0.73)	(2.03)	(0.19)	(0.10)	(2.47)	(2.03)	(1.78)	(1.70)
N-fertilized	3.85^{a}	2.85^{a}	6.79^{a}	5.14^{a}	1.63^{a}	4.41^{a}	5.26^{a}	9.61^{a}	4.85^{a}	13.70^{b}
	(0.38)	(0.18)	(0.29)	(2.17)	(0.57)	(2.39)	(0.46)	(0.82)	(2.95)	(3.95)

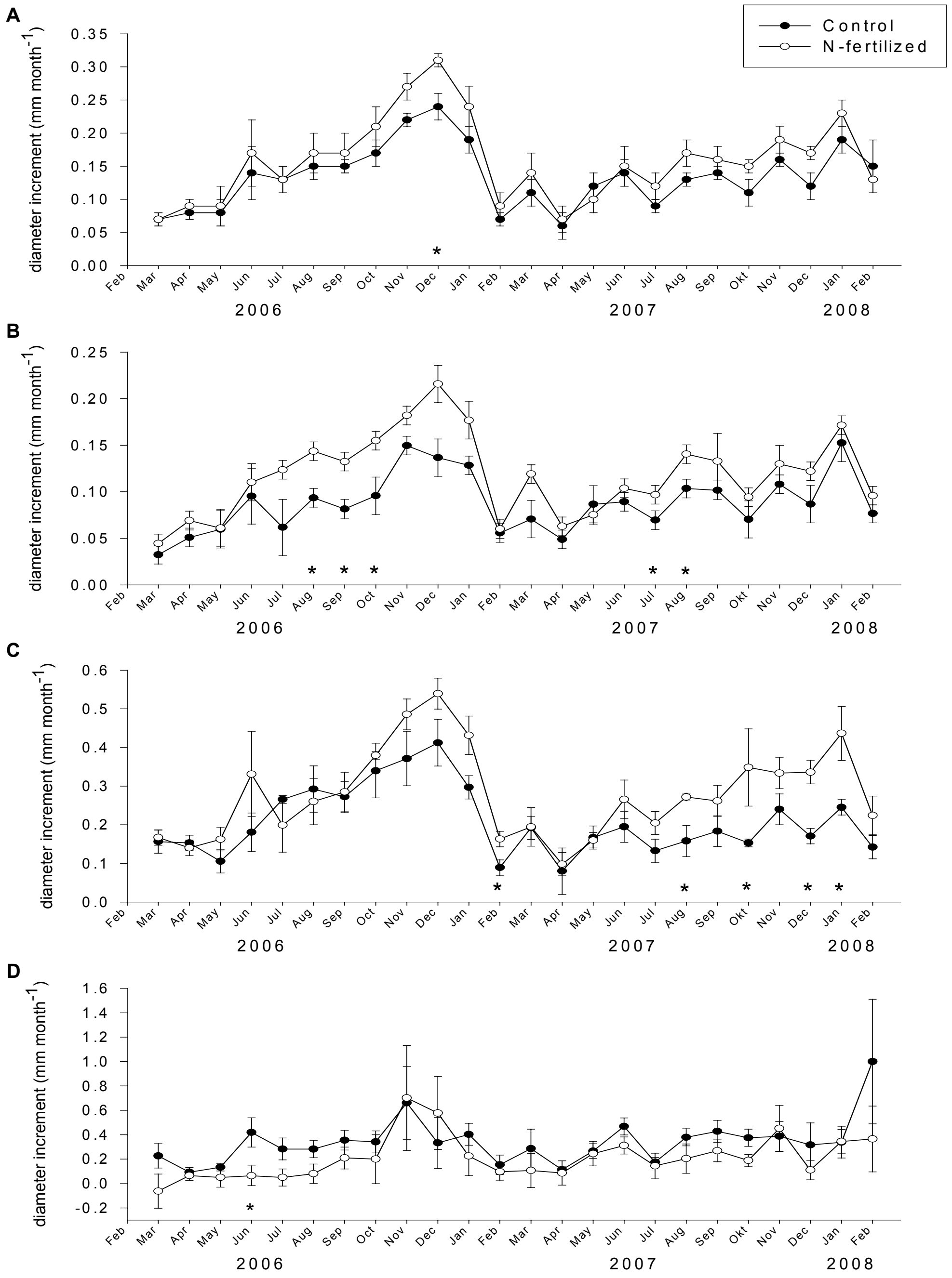

Figure 2-2. Monthly stem diameter increment rates (mm month^{-1}) of A) overall DBH classes, B) trees 10-30 cm DBH, C) trees 30-50 cm DBH, D) trees > 50 cm DBH. Each replicate plot was represented on average by 28 trees for 10-30 cm DBH, 10 trees for 30-50 cm DBH and 2 trees for > 50 cm DBH. N-fertilized plots received 125 kg urea-N ha^{-1} y^{-1} split in four applications (Feb, May, Jul and Oct in 2006; Feb, Jun, Aug and Nov in 2007; Mar in 2008). Monthly means (SE bars; n = 4 plots) with * indicate significant differences between treatments (Mann-Whitney U test at *P* ≤ 0.05).

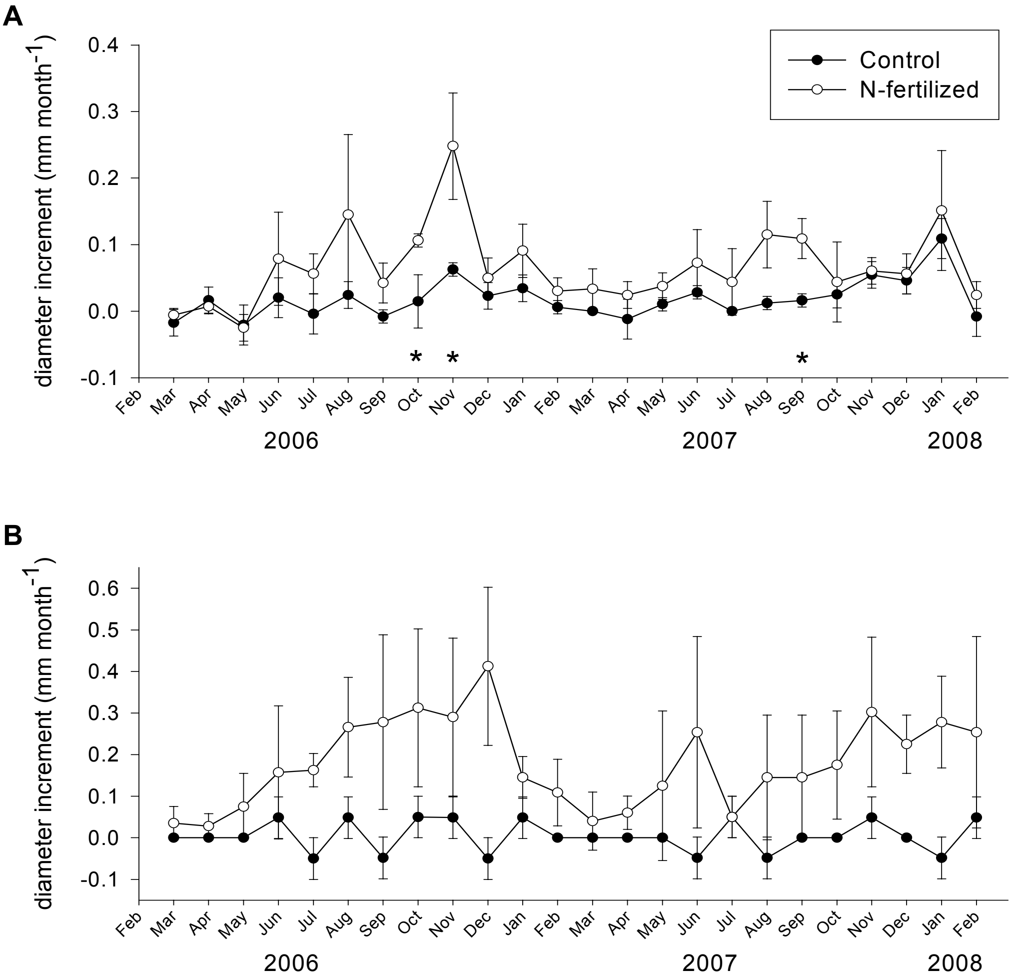

Figure 2-3. Monthly stem diameter increment rates (mm month^{-1}) of *Eschweilera panamensis* in two DBH classes: A) 10-30 cm, B) 30-50 cm. Each replicate plot was represented on average by 3 trees for 10-30 cm DBH and 2 trees for 30-50 cm DBH. N-fertilized plots received 125 kg urea-N ha^{-1} y^{-1} split in four applications (Feb, May, Jul and Oct in 2006; Feb, Jun, Aug and Nov in 2007; Mar in 2008). Monthly means (SE bars; n = 4 plots) with * indicate significant differences between treatments (Mann-Whitney U test at $P \leq 0.05$).

2.4.2 Litter production and hemispherical photographs

Total litterfall showed a seasonal pattern: low amounts from May to August, and high amounts from November to February (Figure 2-4A). Leaves dominated the total litterfall (Table 2-3) and followed a similar seasonal pattern as total litterfall (Figure 2-4B). Total litterfall and leaf litter started to increase in the N-fertilized plots in June 2006 (Figure 2-4A-

B) and remained higher than in the control plots throughout the low-litterfall period (until October 2006). There was no difference between treatments during the high-litterfall period, indicating that litterfall was at its maximum during this time and was not increased further by N fertilization.

In the first-year N addition, annual total litterfall was higher in the N-fertilized than in the control plots. For the two-year measurement period, total litterfall and leaf litter were higher in the N-fertilized than in the control plots (Table 2-3). The amounts in other litterfall categories (twigs ≤ 2 cm, bark, flowers, fruits, epiphytes, rest) did not differ between treatments.

The comparison of canopy closure and PAI at the beginning of N fertilization and after one year showed no difference between control and N-fertilized plots. Canopy closure was 87% and 83% in 2006 and 85% and 83% in 2007 in control and N-fertilized plots, respectively. PAI was 5.02 m^{-2} and 5.16 m^{-2} in 2006, and 4.68 m^{-2} and 4.82 m^{-2} in 2007 in control and N-fertilized plots, respectively.

Table 2-3. Amounts of fine litterfall. Within each column, means (SE; n = 4 plots) followed by a different letter indicate significant differences between treatments within years (Mann-Whitney U test at *P* ≤ 0.05).

Treatment	total litterfall	leaves	twigs ≤ 2 cm	bark	flowers	fruits	epiphytes	rest
			1st year (Mg ha^{-1} y^{-1})					
Control	8.46^{a}	5.07^{a}	1.01^{a}	0.42^{a}	0.29^{a}	0.27^{a}	0.37^{a}	0.61^{a}
	(0.22)	(0.22)	(0.14)	(0.16)	(0.07)	(0.22)	(0.11)	(0.04)
N-fertilized	9.40^{b}	6.06^{a}	1.11^{a}	0.26^{a}	0.30^{a}	0.35^{a}	0.42^{a}	0.65^{a}
	(0.22)	(0.29)	(0.21)	(0.21)	(0.06)	(0.17)	(0.07)	(0.08)
			2nd year (Mg ha^{-1} y^{-1})					
Control	9.18^{a}	5.44^{a}	1.14^{a}	0.67^{a}	0.23^{a}	0.32^{a}	0.61^{a}	0.63^{a}
	(0.46)	(0.16)	(0.11)	(0.13)	(0.08)	(0.12)	(0.16)	(0.07)
N-fertilized	10.25^{a}	6.21^{a}	1.40^{a}	0.38^{a}	0.30^{a}	0.50^{a}	0.60^{a}	0.56^{a}
	(0.27)	(0.20)	(0.13)	(0.24)	(0.04)	(0.11)	(0.14)	(0.08)
			Combined 2 years (Mg ha^{-1})					
Control	17.64^{a}	10.51^{a}	2.15^{a}	1.11^{a}	0.52^{a}	0.59^{a}	0.98^{a}	1.24^{a}
	(0.47)	(0.26)	(0.18)	(0.28)	(0.12)	(0.34)	(0.26)	(0.11)
N-fertilized	19.65^{b}	12.27^{b}	2.52^{a}	0.64^{a}	0.60^{a}	0.85^{a}	1.01^{a}	1.21^{a}
	(0.23)	(0.37)	(0.33)	(0.45)	(0.09)	(0.21)	(0.22)	(0.16)

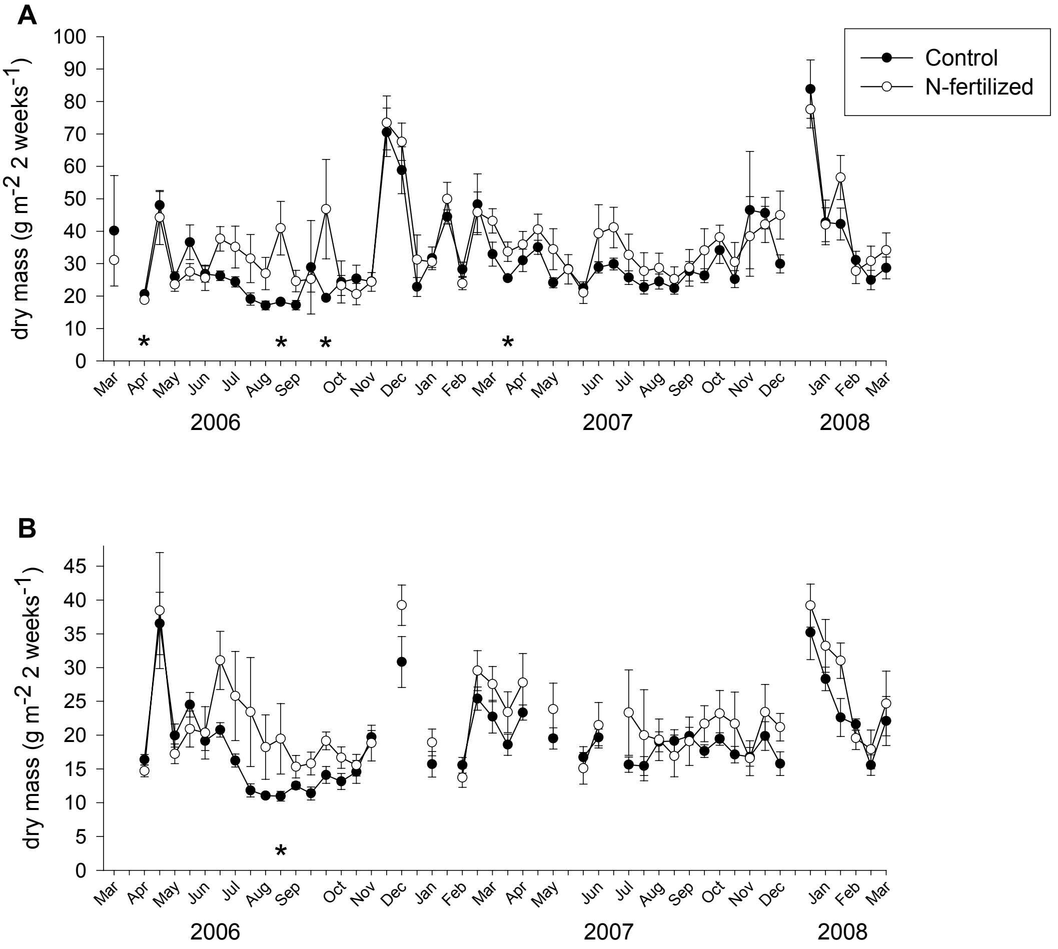

Figure 2-4. A) Total fine litterfall and B) leaves (g m^{-2} 2 $weeks^{-1}$) collected every other week. Each replicate plot was represented by four litter traps of 0.5 m^2 area each. N-fertilized plots received 125 kg urea-N ha^{-1} y^{-1} split in four applications (Feb, May, Jul and Oct in 2006; Feb, Jun, Aug and Nov in 2007; Mar in 2008). During Nov 2006 to Jan 2007 and Apr to Jun 2007, litter was separated only every second collection. Means (SE bars; n = 4 plots) with * indicate significant differences between treatments (Mann-Whitney U test at $P \leq 0.05$).

2.4.3 Above-ground net primary production (ANPP)

Above-ground woody biomass prior to treatment application was 345.75 ± 16.84 and 385.58 ± 32.04 Mg ha^{-1} in the plots that were later assigned as control and N-fertilized, respectively. Neither overall AWBP nor AWBP in the different DBH classes differed between treatments in both years (Table 2-4). ANPP (AWBP + total litterfall) in the N-fertilized plots exceeded ANPP in the control plots by 14.6% in the first year (Table 2-4), while there was no difference in the second year. Total litterfall contributed 69% and 68% to ANPP in the control and N-fertilized plots, respectively.

Table 2-4. Above-ground woody biomass production (AWBP) and above-ground net primary production (ANPP). Within each column, means (SE; n = plots) followed by a different letter indicate significant differences between treatments within years (Mann-Whitney U test at *P* ≤ 0.05).

	AWBP				ANPP
Treatment	overall	10-30 cm DBH	30-50 cm DBH	> 50 cm DBH	
1st year (Mg ha^{-1} y^{-1})					
Control	4.23^{a} (0.37)	1.46^{a} (0.19)	1.57^{a} (0.25)	1.20^{a} (0.57)	12.68^{a} (0.27)
N-fertilized	5.14^{a} (0.41)	2.77^{a} (0.49)	1.96^{a} (0.27)	0.41^{a} (0.28)	14.54^{b} (0.24)
2nd year (Mg ha^{-1} y^{-1})					
Control	3.88^{a} (0.51)	1.43^{a} (0.17)	1.05^{a} (0.25)	1.39^{a} (0.63)	13.06^{a} (0.92)
N-fertilized	4.36^{a} (0.42)	2.20^{a} (0.39)	1.69^{a} (0.08)	0.47^{a} (0.21)	14.61^{a} (0.67)
Combined 2 years (Mg ha^{-1})					
Control	8.12^{a} (0.89)	2.90^{a} (0.32)	2.62^{a} (0.49)	2.59^{a} (1.20)	25.76^{a} (1.18)
N-fertilized	9.34^{a} (0.74)	4.81^{a} (0.77)	3.65^{a} (0.35)	0.88^{a} (0.46)	28.99^{a} (0.85)

2.5 Discussion

2.5.1 Stem diameter growth response to nitrogen addition

The observed seasonal pattern of stem growth rates over the two years was not correlated with rainfall and temperature and only modestly correlated with monthly rainfall in the first year. However, in our site with 5500 mm annual rainfall and no clear dry season it can be assumed that an increase in monthly precipitation is not a main cause of increased tree growth. In a review article, Baker et al. (2003) highlighted irradiance as the most important factor influencing temporal growth patterns in aseasonal tropical forests, having a clear, positive effect on tree growth. Inter-annual variation of tree growth rates in a tropical wet forest at La Selva, Costa Rica was also attributed to variation in irradiance (Clark & Clark 1994). As there are no irradiance data for our study area available it remains unknown whether and if so which meteorological factor(s) drive(s) the intra-annual pattern and inter-annual differences in stem growth rates.

Stem growth rates in control plots were very strongly and positively correlated with the difference between stem growth rates in control and N-fertilized plots in the first year (r^2 = 0.81; P = 0.000), but not correlated in the second year resulting in a modest correlation for both years combined (r^2 = 0.35; P = 0.002). This correlation suggests that N fertilization stimulated stem growth especially during the high-growth period.

In our mixed-species study site, the varied responses of different DBH classes and/or species to N fertilization influenced the overall stem growth response. There was only a weak response of stem growth to N fertilization in trees 10-30 cm DBH and 30-50 cm DBH in the first and second year, respectively (Figure 2-2B and 2-2C), resulting in statistically insignificant effect of N addition on overall stem growth rates (Figure 2-2A). Among the three most common species, *E. panamensis* with 10-30 cm DBH increased stem growth rates in response to N addition, while *O. mexicana* did not respond to N addition. From fertilization experiments in Jamaica (Tanner et al. 1990) and Colombia (Cavelier et al. 2000) (Appendix 2-1), also different intensities of stem growth response of tree species to N addition were observed. These fertilization experiments in tropical montane forests have also shown that stem growth is often co-limited by N and P. Fertilization with N and P separately resulted in an increase in stem growth in a montane forest in Jamaica (Tanner et al. 1990), while only a combined fertilization of N and P increased stem growth in an elfin forest in Colombia (Cavelier et al. 2000). In our N fertilization experiment, we can not test whether stem diameter growth is only limited by N or co-limited by P or other nutrients. Our results show that stem growth of some species (e.g. *O. mexicana* and *V. guatemalensis*) may not be limited by N alone on the short term and their high stem growth rates (compared to other species in the stand; Table 2-2) indicate that they may not be strongly constrained by other limiting factors. On the other hand, the low stem growth rates of *E. panamensis* and its response (at least during the first year) to N fertilization suggest that this species may be mainly limited by N availability. Overall stem growth did not significantly change with N addition because the ecosystem is dominated by species that are not constrained by the N availability of the study site (26% of the trees in the study were *O. mexicana* and *V. guatemalensis).*

2.5.2 Response in fine litter production and plant area index (PAI) to nitrogen addition

The observed seasonal pattern of fine litterfall did not correspond with the seasonal pattern of rainfall or temperature. Most authors related the litterfall maximum of a year to the drier time of the year (Brasell et al. 1980, Kunkel-Westphal & Kunkel 1979, Röderstein et al. 2005, Tanner et al. 1992), but premontane and montane rain forests may also have a second litterfall peak in the wet season (Nadkarni & Matelson 1991, Priess et al. 1999, Röderstein et al. 2005). The latter authors suggested that this second maximum may be due to strong winds occurring during this time. At our site, strong winds occur from November to February and during this windy period higher fine litterfall was observed.

Total fine litterfall lies within the range reported for neotropical montane rain forests: 3.1 – 12.9 Mg ha^{-1} year^{-1} (Köhler et al. 2008, Vitousek 1984). The proportion of leaves in total fine litterfall was similar to the average of 70% calculated by Vitousek (1984) for 62 tropical forests. To our knowledge there are only five studies (Appendix 2-2) that investigated the effect of N addition on fine litterfall in tropical montane forests. From Hawaiian N fertilization experiments, Herbert & Fownes (1995) measured a higher total litterfall and leaf litter after 1.5 years of N addition and Vitousek et al. (1993) reported an N fertilization effect on total litterfall in the second year of their study. The investigated forest stands in Hawaii are nearly exclusively composed of *Metrosideros polymorpha*, a species that seems to be responsive to N fertilization. From a mixed-species stand in Venezuela, Tanner et al. (1992) found no effect of N addition on fine litterfall during four years of N fertilization. In our study, we found increased total fine litterfall already in the first year of N fertilization. Despite increased leaf production, PAI was not increased in N-fertilized plots, indicating that leaf turnover increased through decreased leaf life span under N addition. Similar results were found in Hawaiian N fertilization experiments (Herbert & Fownes 1995, Vitousek et al. 1993). In ecosystems with a low N availability, e.g. tropical montane forests, build-up costs for leaf material are very high and thus leaves are maintained in the canopy over a long time (Chapin 1980, Cordell et al. 2001, Reich et al. 1992). As N limitation to leaf production is reduced under N addition, leaves may be shed faster and leaf turnover times may become shorter.

2.5.3 Response of above-ground net primary production (ANPP) to nitrogen addition

AWBP at our study site is within the range reported for tropical montane forests: 3.5 – 9.1 Mg ha^{-1} year^{-1} (Harrington et al. 2001, Herbert & Fownes 1995, Tanner 1980 a,b). Trees ≥ 50 cm DBH had the highest stem growth rates of the three DBH classes (in control plots; in N-fertilized plots this order is changed due to the N fertilization effect; Table 2-2), a finding consistent with the studies of Clark & Clark (1999) and da Silva et al. (2002). Nevertheless, they contributed a minor proportion to the overall AWBP (Table 2-4) because they only account for 3% of the stem density and 21% of the basal area of trees ≥ 10 cm DBH.

ANPP at our site is at the upper end of the range reported for tropical montane forests: 5.8 – 13.9 Mg ha^{-1} year^{-1} (Harrington et al. 2001, Herbert & Fownes 1995, Raich 1998, Tanner 1980 a,b, Weaver & Murphy 1990). ANPP in the N-fertilized plots increased only in the first year due to a higher litter production (Table 2-3). As in other neotropical forests

(Clark et al. 2001, Malhi et al. 2004), litterfall dominated ANPP in our site. Neotropical forests differ from the mature lowland forest in Kalimantan, Indonesia, where ANPP was strongly related to AWBP of large trees (> 60 cm DBH; Paoli & Curran 2007). As maximum net photosynthesis and above-ground production efficiency (ANPP/foliar biomass) decrease with increasing leaf life-span (Reich et al. 1992), the production of short-lived leaves due to higher N availability should result in an increased ANPP. Since leaves are the primary assimilatory organs of plants that enable all other life processes, leaf production (resulting in higher leaf litter) should have a higher priority than stem growth (Malhi et al. 2004, Tanner et al. 1998). In our study, litter production not only contributes a higher proportion to ANPP but also reacts stronger to N fertilization than stem diameter growth (and therefore AWBP), and is the most important component driving the response of ANPP to N fertilization.

2.6 Conclusions

Our DBH class-stratified and species-specific approach has highlighted that production limitation by N does not uniformly affect aspects of above-ground productivity (e.g. AWBP, litterfall). N fertilization did not result to an increase in overall stem diameter growth and AWBP, while total fine litterfall and leaf litter increased. Due to the response of total fine litterfall, which is the dominant component of ANPP, N addition increased ANPP in the first year. N fertilization resulted in a quick increase in stem diameter growth of *E. panamensis* during the first year of N addition, but caused no reaction of stem diameter growth of the fast-growing species *O. mexicana* and *V. guatemalensis*. On the other hand, the N limitation to stem diameter growth of *E. panamensis* signified that this species is not growing optimally under the intrinsic N availability of the study site. The magnitude and timing of response of stem diameter growth, at least for *E. panamensis*, and fine litterfall to N fertilization suggest that above-ground productivity at our lower montane rain forest site was partly limited by N availability.

If projected increase in N deposition to this natural ecosystem will occur, species with N-limited productivity may gain an advantage, while the relative importance of other species might decelerate. The increasing importance of species with different productivity traits (AWBP, litter production) might affect the overall NPP of the ecosystem. Investigations of tropical forest productivity should account for the structure of an ecosystem as well as for its species composition. The peculiarities of tropical montane forests should be considered in predictions of NPP response to changes in atmospheric N deposition.

Acknowledgements

The Smithsonian Tropical Research Institute provided administrative and technical support. We highly appreciate the help of the research assistants Arturo Morris, Rodolfo Rojas, Evidelio Garcia and Ignacio Delcid. We are grateful to Inga Krämer for the introduction into the CAN_EYE program. Dr. James Dalling provided background information and assisted in the site selection. This study is under the NITROF project funded by the Robert Bosch Foundation (Germany) and awarded to M. D. Corre as independent research group leader.

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Appendix 2-1. Stem diameter increment rates of N fertilization experiments in tropical montane forests.

Study	Location	Altitude (m above sea level)	Mean annual precipitation and air temperature	Forest description	Soil type/description	N source, rate, application frequency	Years of N addition and of stem diameter growth measurement	Diameter increment: N-fertilized vs Control and significance	Trees studied
Tanner et al. 1990	Blue Mountains, Jamaica	1600	2500 mm; 19 °C	Ridge top forest	Organic, very low pH (3.5)	Urea; 150 kg N ha^{-1} y^{-1}; applied once a year	1983-1986; stems measured in 1987 after 3.5 years	Overall: 1.38 vs 0.76-0.78 mm (significant), *Clethra occidentalis*: 1.21 vs 0.34 mm (significant), *Podocarpus urbanii*: 2.60 vs 1.80 mm (not significant)	Trees >25 cm DBH; *Clethra occidentalis; Podocarpus urbanii*
Vitousek et al. 1993	Volcanoes National Park, Hawaii	1190	2500 mm; 16 °C	Dominated by *Metrosideros polymorpha* 14-16 m high	Hydric Lithic Dystrandept	Urea & $(NH_4)_2SO_4$; 100 kg N ha^{-1} in 1985; thereafter 50 kg N ha^{-1} y^{-1}, split in 2 applications per year	1985-1987; stems measured in 1985-1987 at bimonthly interval	1.2 vs 0.5 mm y^{-1} for second year (significant)	*Metrosideros polymorpha*
Herbert & Fownes 1995	Kauai, Hawaii	1134	2500 mm; 16 °C	Dominated by *Metrosideros*	Acrudox	Urea & NH_4NO_3; 100 kg N ha^{-1} in 1991;	1991-1992; stems measured in	2.6 vs 2.5 mm y^{-1} (not significant)	*Metrosideros polymorpha*

				polymorpha		thereafter 100 kg N ha^{-1} y^{-1}, split in 2 applications per year	1991-1992 every 6 months		
Raich et al. 1996	Mauna Loa Volcano, Hawaii	1130	4300 mm; 15.5 °C	Primary successional dominated by *Metrosideros polymorpha* ≈ 4 m high	Lithic Tropofolists on young lava	Urea & NH_4NO_3; 100 kg N ha^{-1} in 1991; thereafter 100 kg N ha^{-1} y^{-1}, split in 2 applications per year	1991-1993; stems remeasured in 1992 and 1993	3.2 vs 0.7 mm y^{-1} in ‘a‘ā site (significant), 1.8 vs 0.5 mm y^{-1} in Pāhoehoe site (significant)	*Metrosideros polymorpha* ≥ 3.2 cm DBH
Vitousek & Farrington 1997	Laupahoehoe Forest Reserve, Hawaii	1200	2500 mm; 16 °C	Primary, dominated by *Metrosideros polymorpha*	Typic Hydrandept on thick volcanic ash	Urea & NH_4NO_3; 100 kg N ha^{-1} in 1993; thereafter 100 kg N ha^{-1} y^{-1}, split in 2 applications per year	1993-1995; stems measured in 1994-1995	2.8 vs 1.6 mm y^{-1} (significant)	*Metrosideros polymorpha* 60-140 cm DBH
Cavelier et al. 2000	Serranía de Macuira, Colombia	865	853 mm (+796 mm from mist and fog)	Elfin cloud forest, trees 5-8 m high	Thin A horizon on 2C gravel and 3C saprolite horizon	Urea; 300 kg N ha^{-1} applied once	1985; stems measured in 1985 and remeasured in 1989	*Guapira fragrans* 2 vs 1.3 mm, *Rapanea guianensis* 2.8 vs 2 mm, *Myrcianthes fragrans* 1.9 vs 1 mm (all not significant)	Trees ≥10 cm DBH of 3 species: *Guapira fragrans*, *Rapanea guianensis*, and *Myrcianthes fragrans*
This study	Fortuna	1200-	5545 mm;	mixed-	Aluandic	Urea; 125 kg N	2006-2008;	Overall:	Trees ≥ 10 cm

	watershed, Panama	1300	20 °C	species, old growth forest	Andosol (FAO) or Alic Hapludand (USDA)	ha^{-1} y^{-1}, split in 4 applications per year	stems measured in 2006-2008 at monthly interval	1.92 vs 1.62 mm y^{-1} (not significant), *Eschweilera panamensis* 0.82 vs. 0.22 mm y^{-1} (significant only in the first year)	DBH and also analyzed for 3 species: *Eschweilera panamensis*, *Oreomunnea mexicana*, and *Vochysia guatemalensis*

Appendix 2-2. Litterfall amounts of N fertilization experiments in tropical montane forests.

Study	Location	Altitude (m above sea level)	Mean annual precipitation and air temperature	Forest description	Soil type/description	N source, rate, application frequency	Years of N addition and of litter measurement	Litterfall: N-fertilized vs Control and significance	Frequency of litter collection
Tanner et al. 1992	Sierra Nevada National Park, Venezuela	2500	2500 mm; 13 °C	Ridge top forest	Humitropept, very low pH (3.5)	Urea; 225 kg N ha^{-1} y^{-1} for the first 2 years, split in 6 applications per year; thereafter 150 kg ha^{-1} y^{-1} applied once a year	1986 – mid 1991; litter measured 1986-1991	Total: 4.3 Mg ha^{-1} y^{-1} in Control plots (no significant difference to N-fertilized plots)	monthly
Vitousek et al. 1993	Volcanoes National Park, Hawaii	1190	2500 mm; 16 °C	Dominated by *Metrosideros polymorpha* 14-16 m high	Hydric Lithic Dystrandept	Urea & $(NH_4)_2SO_4$; 100 kg N ha^{-1} in 1985; thereafter 50 kg N ha^{-1} y^{-1}, split in 2 applications per year	1985-1987; litter measurement reported for 1986-1987	Total: 6.20 vs 5.37 Mg ha^{-1} y^{-1} (significant)	monthly
Herbert & Fownes 1995	Kauai, Hawaii	1134	2500 mm; 16 °C	Dominated by *Metrosideros polymorpha*	Acrudox	Urea & NH_4NO_3;100 kg N ha^{-1} in 1991; thereafter 100 kg N ha^{-1} y^{-1}, split in 2 applications per year	1991-1992; litter measured in 1991-1992	Total: 5.80 vs. 4.84 Mg ha^{-1} y^{-1} (significant); Leaves: 4.55 vs. 3.76 Mg ha^{-1} y^{-1} (significant)	monthly
Harrington et al. 2001[1]	Volcanoes National Park & Kauai, Hawaii	1190 & 1134	2500 mm; 16 °C	Dominated by *Metrosideros polymorpha*	Hydric Lithic Dystrandept & Acrudox	Urea & NH_4NO_3; 100 kg N ha^{-1} y^{-1} in 1985 and 1991, split in 2 applications per year	1985-1996 and 1991-1997; litter measured in 1985-1996	Leaves: 3.13 vs 2.07 Mg ha^{-1} y^{-1} from site on Dystrandept	monthly

							and in 1991-1997	(significant); leaves: 4.65 vs 3.60 Mg ha^{-1} y^{-1} from site on Acrudox (significant)	
This study	Fortuna watershed, Panama	1200-1300	5545 mm; 20 °C	mixed-species, old growth forest	Aluandic Andosol (FAO) or Hapludand (USDA)	Urea; 125 kg N ha^{-1} y^{-1}, split in 4 application per year	2006-2008; litter measured in 2006-2008	Total: 9.83 vs 8.82 Mg ha^{-1} y^{-1} (significant); Leaves: 6.14 vs 5.26 Mg ha^{-1} y^{-1} (significant)	every other week

[1] **long-term effect study using the the same sites as Vitousek et al. (1993) and Herbert & Fownes (1995).**

Chapter

3

Fine root responses to elevated nitrogen input in a tropical lower montane rain forest, Panama

Markus Adamek, Marife D. Corre, Dirk Hölscher

Submitted manuscript

3.1 Abstract

Anthropogenic nitrogen deposition in tropical regions is projected to double by 2020 but large uncertainty exists about possible consequences of increased N input on ecosystem processes that are potentially sensitive to changes in environmental conditions such as fine root production and turnover in tropical montane forests. We established an N fertilization experiment (125 kg urea-N ha^{-1} y^{-1}) with four replicate plots per treatment (N-fertilized and control) in a lower montane rain forest with the aim to analyze possible changes in fine root production and turnover. In three soil depths (organic layer, 0-10 cm and 10-20 cm mineral soil), fine root production and turnover were measured by sequential coring and fine root biomass allocation by the ingrowth core approach during 1.5 years of treatment. Production and turnover of fine roots were not affected by N fertilization. Fine root production was 320 ± 21 and 396 ± 52 g m^{-2} y^{-1}, representing 20% of total (above- and below-ground) net primary production, and fine root turnover was 72 ± 6 and 93 ± 20 % per year in control and N-fertilized plots, respectively. Fine root biomass allocated to the 10-20 cm mineral soil in the N-fertilized plots increased two-fold compared to the control. We hypothesize that the changed fine root allocation allows access to other limiting nutrients, e.g. phosphorus, in deeper mineral soil.

3.2 Introduction

Tropical moist forests might allocate 50% of their annual net primary production into fine roots (diameter ≤ 2 mm) (Gill & Jackson 2000, Vogt 1996), and organic matter inputs to soil from dead roots can equal or surpass the return from leaf litter (Röderstein et al. 2005). Thus, fine root production and turnover serve as an important pathway of organic carbon input into soil, which stores the largest pool of terrestrial carbon (Jobbágy & Jackson 2000, Matamala et al. 2003).

Nitrogen (N) availability is one of the major controls on fine root growth variables (Vogt et al. 1996). Anthropogenic N deposition to tropical regions is projected to at least double by 2020 because of increases in agricultural and industrial use of N, cultivation of N-fixing plants, combustion of fossil fuels and biomass burning (Galloway et al. 2003, Galloway et al. 2008, Phoenix et al. 2006). Fine root production and turnover are among the response modes of plants that enable them to adjust to a changing environment and are highly sensitive to changes in environmental conditions (Vogt et al. 1993, Vogt et al. 1998). So far most studies on fine root responses to differences in nutrient supply in tropical forests have focused on differences in fine root mass along nutrient availability gradients (Nadelhoffer 2000) but have rarely investigated changes in fine root production and turnover due to enhanced N input. In addition, although effects of altered nutrient availability on biomass allocation between below- and above-ground parts of plants (root:shoot ratio or root:weight ratio) have received a lot of attention in past studies (Leuschner et al. 2007, Reich 2002, Reynolds & D´Antonio 1996 and references therein), changes in fine root allocation with soil depth in response to altered nutrient supply have so far received little attention. Large uncertainty exists about the possible effects of elevated N input on fine root production, turnover and allocation into different soil depths, which may affect soil carbon balance in forests (Gower & Vitousek 1989, Nadelhoffer 2000, Norby & Jackson 2000).

The frequently observed decrease in fine root biomass with increase in N availability had been discussed by Nadelhoffer (2000) to be due to the following possible scenarios of changes in fine root production and turnover: (1) fine root turnover is constant while fine root production decreases, (2) a decrease in fine root turnover might lead to a dramatic decrease in fine root production, (3) fine root production and turnover both increase sufficiently, while the proportion of net primary production (NPP) allocated below ground is constant, and (4) a slow increase in fine root production and turnover would lead to a decreasing proportion of below-ground NPP. Nadelhoffer (2000) stated that the most likely relationship between fine root biomass, production and turnover and N availability is that increasing N deposition results in an increasing fine root production and turnover, although fine root biomass declines. This hypothesis is contradicted by a number of studies suggesting that fine root production decreases as above-ground production increases due to increased N availability (Gower et al. 1992, Grier et al. 1981, Vogt et al. 1986).

We hypothesized that fine root production and turnover might decrease with increasing N availability, because the need for biomass investment into fine roots foraging for N is alleviated under N addition. We set up an N fertilization experiment in a lower montane rain forest in western Panama and measured fine root responses in three soil depths (organic layer, 0-10 and 10-20 cm mineral soil) that represent 71% of the total fine root biomass within 1 m depth (Hölscher et al. 2009). Our objectives were (1) to analyze whether fine root productivity and turnover change under elevated N availability, and (2) to assess in which soil depths these changes occur.

3.3 Methods

3.3.1 Site description and experimental design

The study area is situated in the western Panamanian province of Chiriquí. It lies within the Fortuna Forest Reserve which forms part of the La Amistad Biosphere Reserve. The Fortuna watershed forms a high valley in the Talamanca range. The study site is located at 1200-1300 m asl in the Quebrada Honda area (8°45’ N, 82°15’ W) at the northwestern site of the Fortuna lake. The vegetation is a mature lower montane rain forest (sensu Grubb 1977, Holdridge et al. 1971). The estimated number of tree species from two 1-ha plots in the Quebrada Honda valley is approximately 90 (J. Dalling, unpublished data). The most abundant species are Oreomunnea mexicana (Standl.) Leroy (Juglandaceae), Eschweilera panamensis Pittier (Lecythidaceae), Vochysia guatemalensis Donn. Sm. (Vochysiaceae), Cassipourea elliptica (Sw.) Poir. (Rhizophoraceae), Hedyosmum bonplandianum Mart. (Chloranthaceae) and Guarea glabra Vahl (Meliaceae). Also common is the palm Colpothrinax aphanopetala R. Evans. Mean annual precipitation is 5545 mm (1997-2007) without a clear dry season (no month < 100 mm precipitation); mean annual temperature is 20°C (1999-2007). Soil in the study site has developed on volcanic ash deposits and is classified as Aluandic Andosol (FAO) or Alic Hapludand (USDA) (Table 3-1). The mineral soil has a sandy loam texture and is covered by an organic layer with a mean thickness of 4.2 (± 0.4) cm.

Table 3-1. Soil characteristics (mean ± SE, n = 8 plots) of the study site (Quebrada Honda, Chiriqui, Panama) determined in January 2006 prior to N manipulation.

Soil characteristics	Organic layer	Mineral soil	
		0-10 cm	10-25 cm
Total C (g C/kg)	443.0 (18.7)	99.2 (25.1)	37.2 (5.4)
Total N (g N/kg)	22.4 (1.1)	6.0 (1.3)	2.2 (0.2)
C:N ratio	19.9 (0.4)	16.0 (0.7)	16.8 (0.8)
Total P (g P/kg)	0.7 (0.07)	0.5 (0.05)	0.3 (0.04)
Effective cation exchange capacity (mmol[+]/kg)		110.3 (20.4)	69.6 (17.7)
Base saturation (%)		16.2 (2.5)	8.0 (1.4)
pH (H_2O)	4.1 (0.1)	4.1 (0.1)	4.4 (0.1)

The experiment was set up in a paired-plots design with four replicates. Control and N-fertilized treatments were randomly assigned to each pair of plots. Each plot is 40 × 40 m, and plots are separated by at least 40 m. Plots lack streams or swampy areas, gaps, and slopes steeper than 15°. Measurements of side lengths were corrected for inclination (Condit 1998). The N-fertilized plots received 125 kg urea-N ha^{-1} year^{-1} divided into four applications per year (February 2006, May 2006, July 2006, October 2006, February 2007, June 2007, August 2007).

3.3.2 Below-ground biomass production estimation

Although several approaches for estimation of fine root production are debated (e.g. Hertel & Leuschner 2002, Vogt et al. 1998), a single valid method has so far not been established. Fine root sampling by sequential coring is probably the most commonly used approach to estimate fine root production in forest ecosystems (Hertel & Leuschner 2002, Vogt et al. 1998). Without the need for any installation prior to sampling, this method assesses fine root growth in an undisturbed soil. An important shortcoming of this method is that missing seasonal minima and maxima of fine root biomass results in an underestimation of fine root production. The ingrowth core method can be used to compare relative growth rates of fine roots between experimental manipulations (Vogt et al. 1998). However, recolonization of a root-free soil core can not be equated with fine root production in undisturbed soil as fine root growth may be delayed by recovery from the previous injury, may proceed at artificially low root density, and fine root biomass which may have died during the incubation period is not accounted for (Hertel & Leuschner 2002). As no consensus on how to estimate fine root production exists, we applied both independent methods to assess the response of fine root growth to N fertilization.

For each method, six sampling points were located in the inner 20 x 20 m area of each plot. Roots were sampled with a root borer and soil cores were divided into three depths: organic layer, 0-10 cm and 10-20 cm mineral soil. Roots were washed by hand and categorized into live (hereafter ‘fine root biomass’) and dead roots (hereafter ‘fine root necromass’) by examination under the stereomicroscope based on color, elasticity, and degree of cohesion of cortex, periderm and stele (Leuschner et al. 2001, Persson 1978). Roots were dried at 65°C for 24 hours. For the determination of fine root biomass and necromass in the organic layer, mean organic layer thickness was calculated for each plot based on measured organic layer thickness of the six sampling points.

For the sequential coring method (root borer Ø = 4 cm, length = 28 cm), fine roots were sampled in five sampling series with three to six months interval (April 2006, July 2006, November 2006, February 2007, August 2007). Soil cores were stored in a freezer until they were processed (between one and four months). Annual fine root production was calculated from changes in fine root mass for each sampling point and for the three depths separately with the minimum-maximum method (McClaugherty et al. 1982) across the five sampling series. Fine root turnover, the rate at which fine roots are being replaced, was calculated by dividing annual fine root production by mean standing fine root biomass (Hertel & Leuschner

2002) of the five sampling series. Fine root production and turnover from the six sampling points were averaged to represent each replicate plot.

For the ingrowth core method, three ingrowth cores were installed at six sampling points per plot to represent three sampling series (at 0.5, 1 and 1.5 years of incubation from February 2006 to September 2007). For the installation of the ingrowth cores, all visible roots were sorted out from the soil taken with a root borer (Ø = 8 cm, length = 28 cm). The root-free soil was filled back into the hole in the same sequence of soil layers as was found in the undisturbed soil. In the case that there was not enough soil left after sorting out the roots, root-free soil from a neighboring location within the same plot was used to fill up. Ingrowth cores were marked with flags made of aluminum wire and red duct tape. Roots that have grown into the root-free soil area were harvested with a root borer (Ø = 7 cm, length = 28 cm), and soil cores were stored in a refrigerator until they were processed (between one and three months). Diameter of ingrown roots did not exceed 3 mm so that not only dry mass of fine roots but of all roots was determined. Fine root biomasses from the six sampling points were averaged to represent each replicate plot.

3.3.3 Statistical analyses

Tests for normality using Kolmogorov-Smirnov D statistics and equality of variance using Levene statistic (Sokal & Rohlf 1981) were conducted for each parameter. For fine root biomass and necromass by sequential coring, treatment effects were assessed using linear mixed effects models (Crawley 2002) in which treatment is considered as fixed effect and spatial replication (experimental plots) nested in time (five sampling series) as random effect. Details are described in a related study conducted in our site (Koehler et al. 2009); in short, the model includes (1) a variance function which allows different variances of the response variable per level of the fixed effect, and/or (2) a first-order temporal autoregressive process which assumes that the correlation between measurements decreases with increasing time difference. For fine root production and turnover by sequential coring and fine root biomass by the ingrowth core method, treatment differences for each depth were assessed using Mann-Whitney U test, while differences among depths for each treatment were assessed using Kruskal-Wallis H test followed by multiple comparison extension test. Differences among three sampling times in ingrowth fine root biomass were also tested using Kruskal-Wallis H test with multiple comparison extension. Means and standard errors, determined from 4 replicate plots per treatment, are reported as measures of central tendency and dispersion. Treatment effects were considered significant at $P \leq 0.05$.

3.4 Results

From the sequential coring approach, fine root biomass and necromass across the five sampling periods at each depth did not differ between control and N-fertilized plots. Fine root production was higher in the 0-10 cm mineral soil than in the 10-20 cm mineral soil in the control ($P = 0.03$) and N-fertilized plots ($P = 0.03$) but did not differ from the organic layer. Fine root turnover was not different among depths. During 1.5 years of N addition, fine root production and turnover in all depths were not affected by N fertilization (Table 3-2).

Table 3-2. Mean (SE; n = 4 plots) fine root biomass, necromass, production and turnover in control and N-fertilized plots (starting February 2006) measured by sequential coring in five sampling series between April 2006 – August 2007. There were no statistically significant differences between treatments (Mann-Whitney U test at P ≤ 0.05).

Depth	Fine root biomass ($g\ m^{-2}$)		Fine root necromass ($g\ m^{-2}$)		Fine root production ($g\ m^{-2}\ y^{-1}$)		Fine root turnover ($\%\ y^{-1}$)	
	Control	N-fertilized	Control	N-fertilized	Control	N-fertilized	Control	N-fertilized
overall	446 (17)	454 (49)	111 (7)	133 (10)	320 (21)	396 (52)	72 (6)	93 (20)
organic layer	162 (9)	183 (40)	34 (1)	51 (13)	195 (6)	180 (21)	113 (3)	102 (12)
0-10 cm	224 (16)	217 (41)	59 (4)	62 (9)	210 (23)	247 (42)	96 (12)	118 (15)
10-20 cm	84 (7)	90 (13)	24 (3)	28 (5)	100 (8)	122 (9)	129 (15)	147 (23)

From the ingrowth core approach, overall fine root biomass (sum of three depths) did not increase beyond approximately 200 g m^{-2} after one year in both the control and N-fertilized plots (Table 3-3). In the control plots, fine root biomass that has grown into the organic layer was nine times higher than into the 10-20 cm mineral soil depth after 1.5 years ($P = 0.01$), while there were no difference among soil depths in the N-fertilized plots (Table 3-3). Fine root allocation to the mineral soil, calculated as the ratio of fine root biomass that has grown into the different mineral soil depths to the fine root biomass that has grown into the organic layer, was higher in the 10-20 cm mineral soil in the N-fertilized plots at 1.5 years compared to the control ($P = 0.06$; Figure 3-1).

Table 3-3. Fine root biomass in ingrowth cores in the control and N-fertilized plots (starting February 2006) harvested at three incubation times between August 2006 – October 2007.

Depth	Fine root biomass (ingrowth) (g m^{-2})					
	0.5 y		1 y		1.5 y	
	Control	N-fertilized	Control	N-fertilized	Control	N-fertilized
overall	70^a (16)	85^a (28)	198^a (46)	197^a (55)	201^a (37)	177^a (30)
organic layer	32^a (14)	29^a (9)	94^a (25)	59^a (24)	126^a (21)	84^a (32)
0-10 cm	29^a (6)	31^a (7)	104^a (27)	120^a (42)	78^a (13)	85^a (17)
10-20 cm	16^a (5)	31^a (15)	27^a (7)	38^a (15)	13^a (2)	28^b (4)

Within each row, means (SE; n = 4 plots) followed by a different letter indicate significant differences between treatments (Mann-Whitney U test at $P \leq 0.05$).

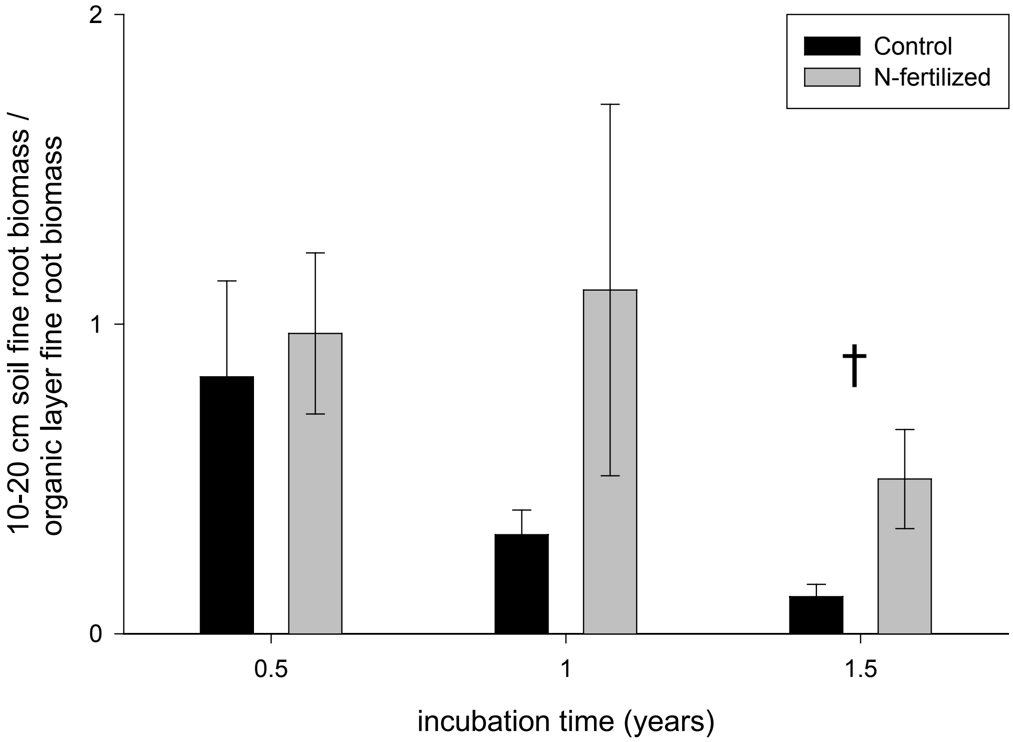

Figure 3-1. Ratio of ingrown fine root biomass in the 10-20 cm mineral soil to ingrown fine root biomass in the organic layer of control and N-fertilized plots (starting February 2006) harvested at three incubation times between August 2006 – October 2007. † The ratio tended to be higher in N-fertilized than control plots (Mann-Whitney U test at $P = 0.06$).

3.5 Discussion

3.5.1 Fine root mass, production and turnover in the control plots

The mean fine root biomass of our site (Table 3-2) was comparable to the mean (357 ± 51 g m^{-2}) for tropical lower montane rain forests (Hertel & Leuschner, in press). Total fine root mass was lower than the mean (804 ± 75 g m^{-2}; Hertel & Leuschner, in press) for tropical lower montane rain forests but was comparable to two other fine root mass measurements in the Fortuna area: Cavelier (1992) reported a total fine root mass of 400 g m^{-2} in the upper 25 cm of soil (organic layer and mineral soil) and Hölscher et al. (2009) found a total fine root mass of 484 g m^{-2} in the organic layer and the 10 cm mineral soil. In our study site, the decrease in fine root biomass with depth is paralleled by a 10-fold decline in total N concentration over the same depth (Table 3-1) and a 7-fold decrease in net N mineralization and nitrification rates from the organic layer to the 0-5 cm mineral soil (Koehler et al. 2009). Fine root production in our site lies below the mean for tropical lower montane rain forests (512 ± 91 g m^{-2} $year^{-1}$ from 12 studies reviewed by Hertel & Leuschner, in press); this mean, however, was influenced by an extraordinarily high value from a Venezuelan forest growing on an extremely acidic Oxisol with low Ca availability (Priess et al. 1999), and excluding this value rendered a mean (457 ± 76 g m^{-2} $year^{-1}$) comparable to our estimates. Total net primary production was 15.9 ± 0.3 Mg ha^{-1} $year^{-1}$ of which 80 ± 1 % was above-ground NPP (unpublished results from our separate study) and 20 ± 1 % was fine root production. The ratio of fine root production to leaf litterfall in our study site (0.63 ± 0.04) was similar to the 1890-m Ecuadorian forest on Humic Cambisol soil (Röderstein et al. 2005). Overall fine root turnover rate (equivalent to a mean residence time of 1.4 ± 0.1 years) in our study site was comparable to the mean for tropical tree fine roots (80% $year^{-1}$) (Gill & Jackson 2000).

3.5.2 Effects of N fertilization on fine root production, turnover and fine root biomass allocation

We did not observe N fertilization effects on fine root production and turnover during 1.5 years. In a forest in the Fortuna watershed, fine root production was also not affected by nine months of combined N and P fertilization (Cavelier 1989; Appendix 3-1). In a Hawaiian montane forest, despite N limitation to different components of above-ground productivity (e.g. stem diameter growth, leaf litter production and foliar N content) (Vitousek et al. 1993, Vitousek & Farrington 1997), ten years of N fertilization to an N-limited 300-y-old site (Hydric Lithic Dystrandept) had no significant effect on fine root production, fine root turnover rates and standing stock of fine root necromass, with the only exception being a small increase in standing fine root biomass (Ostertag 2001; Appendix 3-1). At the same site, Gower and Vitousek (1989; Appendix 3-1) found reduced fine root biomass after 1.5 years of N fertilization. Thus, short- and long-term effects of N fertilization on fine roots can be even opposite in their direction. Comparison with the Hawaiian sites is difficult because the ecosystem is dominated by mainly one tree species (*Metrosideros polymorpha)*; hence, all trees at such sites can be expected to react in the same way to N fertilization. On the other hand, species in a mixed-species ecosystem may respond differently to the same exogenous stimulus, because the overall effect is influenced by each species´ response to the change in N availability of the ecosystem.

Changes of fine root distribution after experimental manipulation were observed by Sayer et al. (2006) who doubled the monthly litter input in an old-growth lowland forest in Panama over a period of 1.75 years and measured fine root biomass that had grown into the litter layer, 0-5 cm and 5-10 cm mineral soils. Higher fine root biomass in the litter layer was found to be related to a decreased fine root biomass in the 5-10 cm mineral soil and was suggested to be promoted by the more easily obtainable nutrients in the doubled litter layer (Sayer et al. 2006). In our study, the increased fine root biomass that has grown into the 10-20 cm mineral soil in the N-fertilized plots suggests that fine roots allocate to the deeper mineral soil at the expense of the organic layer. N fertilization in our site has changed the magnitude of available N and vertical distribution of mineral N, as indicated by a 3-fold increase in net rates of N mineralization and nitrification in the organic layer and 0-5 cm mineral soil combined (Koehler et al. 2009) and by a 5-fold increase in nitrate concentrations in soil solution at 1.5 m depth during 2006-2007 measurements (M.D. Corre, unpublished data). The increased available N in N-fertilized plots might have allowed a change in fine root allocation to permit a more extensive exploration of the mineral soil for other nutrients without being constrained by the otherwise low N availability of the unamended soil. A stronger fine root allocation to the mineral soil might be caused by fine roots foraging for other nutrients, e.g. P, which may cause additional limitation to above-ground production once N limitation is alleviated. In a recent review, Benner et al. (in press) stated that tropical mountain forests are usually not only N-limited but also often P-limited. This was also shown by the increased production of phosphatase enzymes by roots in N-fertilized Hawaiian montane forests (Olander & Vitousek 2000, Treseder & Vitousek 2001) despite no change in fine root production (Ostertag 2001). Their results show that there might be different paths (allocation vs. exudation) for fine roots to invest in the acquisition of a possibly limiting nutrient. In a comparison of three Amazonian forest types, fine roots were more concentrated in the organic layer of a relatively N-rich tierra firme forest on an Oxisol than in two relatively P-rich caatinga and bana forests growing on Spodosols (Sanford 1989). Wood et al. (2006) reported that trees in the tropical lowland rain forest of La Selva, Costa Rica, exploit deeper nutrient pools for P than for other nutrients. If this also holds true for montane forests, it seems reasonable to infer that in our N-fertilized plots fine roots were allocated to deeper soil depths to forage for P after limitation by N is alleviated.

In conclusion, N fertilization for 1.5 years did not affect fine root production and turnover. Instead, fine root biomass allocation into the 10-20 cm mineral soil increased at 1.5

years of N fertilization. The increased available N in the N-fertilized plots may have favoured the change in fine root allocation to explore the deeper mineral soil for other nutrients which may cause additional limitation to above-ground production once N limitation is alleviated.

Acknowledgements

The Smithsonian Tropical Research Institute provided administrative and technical support. We highly appreciate the help of the research assistants Arturo Morris, Rodolfo Rojas, Evidelio Garcia and Ignacio Delcid. We are grateful to Catharina Meinen and Marieke Harteveld for advice on root sampling, processing and production calculation, and to François Jost for help with the translation of the abstract into Spanish. Dr. James Dalling provided background information and assisted in the site selection. This study is under the NITROF project funded by the Robert Bosch Foundation (Germany) and awarded to M. D. Corre as independent research group leader.

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Appendix 3-1: Effects of N fertilization on fine roots (diameter ≤ 2 mm) in tropical montane forests.

Location	Altitude (m asl)	Precipitation; mean annual temperature	Forest type	Soil description	Effect of N fertilization on fine roots	N fertilizer rate (source) and N fertilization period	Method
Hawaii (Gower & Vitousek 1989)	1150	2400 mm y^{-1}; 14°C (January), 17°C (July)	Tropical montane forest dominated by *Metrosideros polymorpha*	Hydric Lithic Dystrandept	FRB decreased	100 kg N ha^{-1} y^{-1} (n.a.) in 1985, thereafter 50 kg N ha^{-1} y^{-1}, split in 2 applications per y	Soil coring in March 1987
Fortuna watershed, Panama (Cavelier 1989)	1200	3900 mm y^{-1}; 19°C	Lower montane rain forest	Inceptisol	No effect on FRP. No effect on root ingrowth.	Mixture of 300 kg N ha^{-1} y^{-1} (ammonium nitrate) and 100 kg P ha^{-1} y^{-1} (superphosphate) split in three quarterly applications from April – December 1988	Sequential coring with 9 sampling series from February to October 1988; summing positive differences and decision matrix. Ingrowth core with 8 sampling series from March to October 1988
Hawaii; 300-y-old, N-limited site (Ostertag 2001)	1176	2500 mm y^{-1}; 16°C	Dominated by *Metrosideros polymorpha*	Hydric Lithic Dystrandept	FRB increased; no effect on FRP and FRT	100 kg N ha^{-1} y^{-1} (50% as urea, and 50% as ammonium nitrate) at 6 month interval since 1985 and 1991 in Hawaii and Kauai, respectively	Sequential coring with 12 sampling series from October 1995 to October 1996; compartment flow model
Kauai; 4.1 x 10^6-y-old, P-limited site (Ostertag 2001)	1134	2500 mm y^{-1}; 16°C	Dominated by *Metrosideros polymorpha*	Plinthic Acrudox	FRT increased; no effect on FRP		

Mount Kinabalu, Sabah, East Malaysia (Nomura & Kikuzawa 2003)	1708	2714 mm y^{-1}; 18.1°C	Lower montane forest	n.a.	Root growth length shorter	180 kg N ha^{-1} (50% as urea, and 50% as ammonium nitrate) split in three biweekly applications from November – December 2000	Biweekly measurement of root growth length from November to December 2000 using an optical document scanner
	2425	2083 mm y^{-1}; 12.8°C	Upper montane forest	n.a.	Root growth length larger		
	3080	3285 mm y^{-1}; 10.2°C	Subalpine forest	n.a.	Root growth length shorter		
Fortuna watershed, Panama (this study)	1200-1300	5545 mm y^{-1}; 20°C	Tropical lower montane rain forest	Aluandic Andosol (FAO) or Alic Hapludand (USDA)	No effect on FRP and turnover. Increased FRB allocation to 10-20 cm mineral soil depth after 1.5 y	125 kg N ha^{-1} y^{-1} (urea) split in four applications per y since 2006 to present	Sequential coring with 5 sampling series from April 2006 to August 2007; minimum-maximum calculation. Ingrowth core with 3 sampling times from August 2006 to October 2007

FRB = fine root biomass, FRP = fine root production, FRT = fine root turnover, n.a. = not available

Chapter

4

Carbon and nitrogen sequestration by a lower montane rain forest in Panama

Markus Adamek, Marife D. Corre, Dirk Hölscher

Manuscript in preparation

4.1 Abstract

Anthropogenic nitrogen (N) deposition to tropical ecosystems is projected to double by 2020 but the potential effects on carbon (C) and N sequestration by the vegetation of different forest ecosystems is widely understudied. In a lower montane rain forest, an N fertilization experiment (125 kg urea-N ha^{-1} $year^{-1}$) with four replicate plots per treatment (control and N-fertilized) was set up to study 1) how much of the added N is sequestered by trees, and 2) to which plant tissues a change in C and N sequestration is attributable. Fresh leaves, wood and bark, leaf litter, and fine roots were sampled and analyzed for C and N content. Foliar, wood, bark and fine root concentrations of C and N were not affected by N fertilization. N concentration of litter leaves of the tree species *Oreomunnea mexicana* was higher in the first and second year and C:N ratio was lower in the second year under N addition as was total leaf litter C:N ratio. Due to an increase in net primary production, total C sequestration in N-fertilized plots exceeded the one in controls by 1.48 Mg C ha^{-1} $year^{-1}$ in the first year. Leaf litter N sequestration increased under N fertilization for both years combined and was 16.8% of the added N. Hence, the additionally sequestered N was primarily used for leaf litter production. The increased N return to the forest floor and the improved leaf litter quality may accelerate leaf litter decomposition and nutrient cycling.

4.2 Introduction

Litter and fine root production play an important role in biogeochemical nutrient cycling in forest ecosystems. Among above-ground litter categories, leaf litter represents the most important pathway of nutrients to the forest floor. Leaf litter is the most easily decomposable litter category that is produced constantly in large quantities (compared to woody litter which is hard to decompose and reproductive litter which is produced spatially and temporally heterogenous); hence, leaf litter quality (e.g. carbon-to-nitrogen stoichiometry, C:N ratio) strongly affects decomposition rates and soil nutrient availability in the longer term (Aerts & Chapin 2000). In tropical moist forests carbon (C) and nutrient inputs to soil via fine root decomposition are estimated to equal or surpass the nutrient return from leaf litter production (Joslin & Henderson 1987, Raich & Nadelhoffer 1989) and fine root turnover even gains increasing importance as source of soil organic matter with increasing elevation (Röderstein et al. 2005). Consequently, roots may constitute the main pathway of C and nutrients to the soil on tropical mountains. However, C and nutrient stocks in below-ground biomass have rarely been investigated in tropical montane forests (Soethe et al. 2007). Uncertainties in nutrient contents of different tree tissues set limitations to our understanding of biogeochemical cycles in tropical rain forests.

Tropical rain forests play an important role in the global C budget with one-third of terrestrial net primary production (NPP) generated by this biome alone (Field et al. 1998). Estimation of C sequestration by tropical rain forest NPP is dependent on measurements of the C content of different tissues and tree species (Elias & Potvin 2003, Kraenzel et al. 2003). Nitrogen (N) limitation to production is a strong constraint on C sequestration in many terrestrial ecosystems (Vitousek & Howarth 1991). But while NPP in most tropical lowland forests which grow on highly weathered soils is considered to be limited rather by phosphorus than by N, productivity in most tropical montane forests on younger soils should be N-limited

(Tanner et al. 1998). The projected increase of anthropogenic N deposition to natural ecosystems in the tropics from agricultural and industrial use, legume cultivation, combustion of fossil fuels, and biomass burning (Galloway et al. 2008) rises the question of how biogeochemical cycles in tropical montane rain forests will be affected by this elevated N input, into which part of the vegetation the extra N will be taken up, and if the extra N may cause an increase in C sequestration by either changing C:N stoichiometry of plant tissues or by increasing NPP.

To assess how sequestration of C and N change under increased N input, we set up an N fertilization experiment in a lower montane rain forest in western Panama. Our objectives were (1) to determine how much of the added N is sequestered by trees and (2) to identify which plant tissues were affected by a possible change in C and N sequestration under N fertilization.

4.3 Material and methods

4.3.1 Study area

The study area is situated in the western Panamanian province of Chiriquí. It lies within the Fortuna Forest Reserve which forms part of the La Amistad Biosphere Reserve. The Fortuna watershed forms a high valley in the Talamanca range. The study site is located at 1,200-1,300 m a.s.l in the Quebrada Honda area (8°45’ N, 82°15’ W) at the northwestern site of the Fortuna lake.

The vegetation is a mature lower montane rain forest (*sensu* Grubb 1977, Holdridge et al. 1971). The estimated number of tree species from two 1-ha plots in the Quebrada Honda valley is approximately 90 (J. Dalling, unpublished data). Mean annual precipitation is 5545 mm (1997-2007) without a clear dry season (no month < 100 mm precipitation); mean annual temperature is 20°C (1999-2007). Soil characteristics were determined in January 2006 prior to treatment application (for methodology see Koehler et al., in press). These characteristics (Table 4-1) did not differ between plots which were later randomly assigned as control and N-fertilized. The soil is derived from volcanic ash deposits, has a sandy loam texture, and is classified as Aluandic Andosol (FAO classification) or Alic Hapludand (USDA).

Table 4-1. Soil characteristics of the lower montane rain forest in Quebrada Honda (Fortuna Forest Reserve), Chiriquí, Panama (means ± SE; n = 8 plots). Soil characteristics were determined in January 2006 prior to treatment application and did not differ between plots.

Characteristics	Organic layer	Mineral soil	
		0-5 cm	5-50 cm
Total C (g C kg^{-1})	443.0 (18.7)	73.0 (8.3)	30.7 (5.1)
Total N (g N kg^{-1})	22.4 (1.1)	5.0 (0.6)	1.9 (0.2)
C:N ratio	19.9 (0.4)	14.5 (0.5)	16.5 (0.6)
Total P (g P kg^{-1})	0.7 (0.1)	0.6 (0.1)	0.3 (0.1)
Effective cation exchange capacity ($mmol_c$ kg^{-1})	-	132 (25.0)	71 (18.0)
Base saturation (%)	-	21 (4.0)	11 (4.0)
Al saturation (%)	-	72 (6.0)	86 (4.0)
pH (H_2O)	4.1 (0.1)	4.1 (0.1)	4.6 (0.1)

4.3.2 Experimental design

In December 2005 eight plots of 40 x 40 m were established in the Quebrada Honda area. Plots are paired and separated by at least 40 m interval to avoid transfer of fertilizer from a fertilized to an unfertilized plot. While choosing the plot areas care was taken that the plots had no streams or swampy areas, no gaps and no slopes steeper than 15° again to avoid transfer of fertilizer by water flow. Measurements of side lengths were corrected for inclination (Condit 1998). Four plots served as a the control while the other four – chosen randomly out of each pair – were fertilized with 125 kg urea-N ha^{-1} $year^{-1}$ spread in four applications per year (February 2006, May 2006, July 2006, October 2006, February 2007, June 2007, August 2007, November 2007, March 2008).

4.3.3 Sampling and chemical analyses

From March 2006 on, every three month leaf litter (separated into leaves of *Oreomunnea mexicana* and other leaves) from the biweekly litter sampling (four 0.5 m^2-traps per plot) were retained for analyses of C and N concentrations. Samples from March 2007 and 2008 and from September 2006 and 2007 were additionally analyzed for base cation concentrations (P, S, Na, K, Ca, Mg, Mn, Fe, Al).

In September 2006 and September 2007 fresh tissue samples (sun leaves, bark, wood) were taken from one individual of each of the five most abundant species per plot. Leaves were sampled with a slingshot. Bark and wood samples were taken with a knife; thus, only the growing outer part of the wood, where changes in element concentrations are most likely to occur, was obtained. Leaf, bark and wood samples were analyzed for C and N concentrations, and leaf samples were additionally analyzed for total element concentrations. Sampled trees were marked, to ensure that the same individuals could be sampled again.

Fine roots (diameter $\leq$ 2 mm) were sampled with a root borer (Ø = 4 cm) in five sampling series (April 2006, July 2006, November 2006, February 2007, August 2007) from six soil cores per plot. Soil cores were divided into three depths (organic layer, 0-10 cm and 10-20 cm mineral soil), and fine roots were washed and separated into live and dead roots. Per sample date, a mixed sample of the live fine roots per depth and plot was retained for analyses of C and N concentrations, and the samples from April 2006 and August 2007 were also analyzed for total element concentrations.

All samples were dried (65°C, 72h; 24h for fine roots) and brought to the laboratory of the Department of Soil Science of Tropical and Subtropical Ecosystems of the University of

Göttingen, Germany, for chemical analyses. C and N concentrations were measured by a CNS Elemental Analyzer (Elementar Vario EL, Hanau, Germany) and P and base cation concentrations by pressure digestion in concentrated HNO_3 (König & Fortmann 1996) followed by analysis of the digests using inductively coupled plasma-atomic emission spectrometer (ICP-AES; Spectro Analytical Instruments, Kleve, Germany).

The quarterly measurements of C and N concentrations and C:N ratios in leaf litterfall were averaged to represent the annual mean of these concentrations. Annual C and N return with leaf litter is the product of the quarterly measured C and N concentration and the amount of leaf litter produced in the respective quarter. For the annual C and N return with wood and bark, measured C and N concentrations of fresh wood and bark were multiplied with wood and bark litter production of the respective year. The annual C and N return with all other litter categories (flowers, fruits, epiphytes, rest), which were not analyzed for C and N content, was calculated using leaf litter C and N concentrations. Measured C and N concentrations in stem wood and bark were weighted with the number of trees per sampled species in each plot. Stem wood C and N sequestration is the product of above-ground woody biomass production and the weighted wood C and N concentrations. Above-ground C and N sequestration was estimated by summing the return with total litter and stem wood sequestration. Below-ground C and N sequestration is the product of fine root production and the mean C and N concentration in live fine roots of the five sample dates.

Base cation concentrations of leaf litter and fine roots were averaged over the sampling dates.

4.3.4 Statistical analyses

For C and N concentrations and C:N ratios in leaf litter and fine roots and concentrations of base cations in leaf litter, treatment effects were assessed using linear mixed effects models (Crawley 2002) in which treatment is considered as fixed effect and spatial replication (experimental plots) nested in time (five sampling series) as random effect. Details are described in a related study conducted in our site (Koehler *et al.* 2009); in short, the model includes (1) a variance function which allows different variances of the response variable per level of the fixed effect, and/or (2) a first-order temporal autoregressive process which assumes that the correlation between measurements decreases with increasing time difference. For C and N concentrations in fresh material, concentrations of base cations in fresh leaves and fine roots, and for C and N sequestration, treatment differences were assessed using Mann-Whitney U test. Differences in C and N concentrations between fresh material of different species and between fine roots from different soil depths for each treatment were assessed using One-way ANOVA with Tukey´s HSD test for uneven sample numbers (Spjotvoll/Stoline). Means and standard errors, determined from 4 replicate plots per treatment, are reported as measures of central tendency and dispersion. $P \leq 0.05$ indicates significant difference.

4.4 Results

In control plots foliar C concentration did not differ between species in September 2006 while it was lower in *V. guatemalensis* in September 2007. Wood C concentrations of *V. guatemalensis* were higher than in the two other species. *E. panamensis* had higher foliar N concentrations and lower foliar C:N ratios than *O. mexicana* and higher wood N concentrations and lower wood C:N ratios than the two other species (Table 4-2). Element concentrations of fresh material (leaves, wood, bark) were generally not affected by N fertilization, although wood C in September 2006 was higher under N fertilization, but the change was not persistent.

Table 4-2. C and N concentrations and C:N ratios in fresh leaves and wood of different species and in bark. Within each column, means (SE; n = 4 replicate plots) followed by a different letter indicate significant differences among species (One-way ANOVA with Tukey´s HSD test at $P \leq 0.05$). There was no difference between treatments, the only exception being a higher total wood C concentration in the N-fertilized plots in the first year.

		First year (Sep 06)		Second year (Sep 07)	
tissue	species	Control	N-fertilized	Control	N-fertilized
C (mg g^{-1})					
total leaves[1]		478.9 (6.0)	485.2 (4.3)	490.9 (3.1)	490.6 (3.0)
	O. mexicana	491.0^{a} (5.0)	501.9^{ab} (1.8)	504.2^{a} (2.2)	495.3^{a} (1.4)
	E. panamensis	490.1^{a} (7.4)	507.1^{a} (2.9)	509.1^{a} (1.6)	509.2^{a} (6.5)
	V. guatemalensis	470.3^{a} (13.0)	477.1^{b} (6.9)	468.9^{b} (10.9)	497.4^{a} (5.4)
total wood[1]		459.2 (3.3)	473.1 (3.3)	477.2 (1.8)	482.6 (5.3)
	O. mexicana	467.9^{a} (3.4)	474.1^{ab} (15.2)	485.5^{a} (4.9)	476.2^{a} (20.6)
	E. panamensis	461.5^{a} (3.7)	468.6^{a} (5.2)	482.3^{a} (2.7)	474.2^{a} (8.7)
	V. guatemalensis	496.2^{b} (1.3)	503.4^{b} (2.7)	510.3^{b} (0.2)	515.7^{a} (3.0)
total bark[1]		487.3 (4.5)	498.7 (8.7)	494.8 (5.9)	506.3 (7.0)
N (mg g^{-1})					
total leaves[1]		18.4 (1.3)	18.2 (1.0)	17.8 (0.7)	17.5 (0.9)
	O. mexicana	14.8^{a} (0.9)	14.7^{a} (0.7)	15.6^{a} (0.5)	14.3^{a} (0.5)
	E. panamensis	20.3^{b} (1.0)	20.0^{b} (0.3)	19.7^{b} (1.3)	17.4^{a} (1.1)
	V. guatemalensis	17.6^{ab} (1.3)	15.8^{a} (0.5)	16.9^{ab} (0.8)	16.3^{a} (0.2)
total wood[1]		5.8 (0.8)	4.5 (0.4)	5.4 (0.7)	4.6 (0.5)
	O. mexicana	3.0^{a} (0.2)	2.7^{a} (0.2)	3.1^{a} (0.2)	2.8^{a} (0.3)
	E. panamensis	7.3^{b} (0.6)	7.6^{b} (0.3)	7.6^{b} (0.7)	7.2^{b} (0.2)
	V. guatemalensis	2.7^{a} (0.2)	2.3^{a} (0.1)	2.8^{a} (0.3)	2.5^{a} (0.3)
total bark[1]		11.7 (1.1)	9.1 (0.8)	10.7 (1.1)	8.5 (0.6)
C:N ratio					
total leaves		27.4 (1.6)	28.1 (1.7)	28.3 (0.9)	29.4 (1.5)
	O. mexicana	33.5^{a} (2.5)	34.1^{a} (1.4)	32.4^{a} (1.0)	34.6^{a} (1.3)
	E. panamensis	24.3^{b} (1.0)	25.4^{b} (0.4)	26.1^{b} (1.6)	29.4^{a} (1.6)
	V. guatemalensis	26.8^{ab} (1.2)	30.3^{a} (1.3)	27.9^{ab} (2.0)	30.5^{a} (0.6)
total wood		106.0 (5.5)	130.4 (12.3)	116.1 (10.8)	128.8 (16.3)
	O. mexicana	159.6^{a} (8.5)	176.4^{a} (18.5)	158.7^{a} (10.6)	175.5^{a} (26.6)
	E. panamensis	64.1^{b} (5.2)	61.6^{b} (2.9)	64.7^{b} (5.4)	66.0^{b} (0.9)
	V. guatemalensis	187.5^{a} (15.0)	219.8^{b} (6.8)	184.4^{a} (16.4)	210.7^{a} (20.7)
total bark		50.5 (3.4)	61.2 (5.5)	53.7 (2.7)	66.7 (6.7)

[1] Total leaf, wood, and bark C and N concentrations were calculated by weighing C and N concentrations with the number of trees per species from a sample of 40 trees per plot used for above-ground woody biomass calculation.

C and N concentrations and C:N ratios of total leaf litter were not affected by N fertilization. N concentrations of *O. mexicana* leaves from N-fertilized plots were higher in both years and for both years combined. C:N ratios of *O. mexicana* leaves were lower in the second year and for both years combined under N fertilization (Table 4-3).

Table 4-3. C and N concentrations, and C:N ratios in leaf litterfall. Within each row, means (SE; n = 4 plots) followed by a different letter indicate significant differences between treatments (Linear mixed effects model at $P \leq 0.05$).

	First year		Second year		Combined 2 years	
Leaf litter category	Control	N-fertilized	Control	N-fertilized	Control	N-fertilized
C (mg g^{-1})						
total leaf litter	497.3^a (10.0)	511.4^a (6.1)	529.0^a (9.7)	536.2^a (10.6)	503.9^a (2.1)	509.2^a (2.0)
O. mexicana	486.2^a (10.2)	507.2^a (11.2)	522.1^a (1.6)	522.4^a (0.5)	504.2^a (4.5)	514.8^a (5.8)
N (mg g^{-1})						
total leaf litter	12.1^a (0.2)	13.8^a (0.8)	12.7^a (0.5)	14.7^a (1.0)	12.3^a (0.1)	14.0^a (0.7)
O. mexicana	11.7^a (0.3)	12.9^b (0.3)	12.1^a (0.2)	13.4^b (0.1)	11.9^a (0.2)	13.2^b (0.1)
C:N ratio						
total leaf litter	41.3^a (0.5)	37.8^a (1.8)	41.9^a (0.8)	37.0^a (1.8)	41.3^a (0.2)	37.1^a (2.0)
O. mexicana	41.6^a (1.3)	39.4^a (1.4)	43.5^a (0.7)	39.3^b (0.4)	42.6^a (0.9)	39.3^b (0.7)

In control plots C concentration of fine roots decreased with depth while N concentration decreased only from the 0-10 cm to the 10-20 cm depth (Table 4-4). N fertilization did not affect C and N concentrations, C:N ratios, and C and N stocks in live fine roots from any depth.

Table 4-4. Mean C and N concentrations, C:N ratios, and C and N stocks in fine root biomass at different soil depths. Within each column, means (SE; n = 4 plots) followed by a different letter indicate significant differences among depths (One-way ANOVA with Tukey´s HSD test at $P \leq 0.05$). There was no difference between treatments during 1.5 years of N addition (Linear mixed effects model at $P \leq 0.05$).

Depth	C (mg g^{-1})		N (mg g^{-1})		C:N		C stock (g m^{-2})		N stock (g m^{-2})	
	Control	N-fert.	Control	N-fert.	Control	N-fert.	Control	N-fert.	Control	N-fert.
overall	507.0	511.1	14.1	14.1	36.1	36.6	225.1	231.5	6.3	6.3
	(8.0)	(9.8)	(0.2)	(0.7)	(0.3)	(2.3)	(7.4)	(25.3)	(0.2)	(0.6)
organic layer	488.1^{a}	477.7^{a}	14.4^{a}	15.1^{a}	34.2^{a}	32.9^{a}	79.2^{a}	87.3^{a}	2.4^{a}	2.6^{ab}
	(3.0)	(8.4)	(0.4)	(1.0)	(1.0)	(2.5)	(4.1)	(21.1)	(0.2)	(0.5)
0-10 cm	475.2^{b}	475.0^{a}	13.3^{a}	12.9^{ab}	36.0^{ab}	37.7^{a}	107.2^{b}	103.8^{a}	3.0^{a}	2.8^{a}
	(3.1)	(2.6)	(0.3)	(0.8)	(0.7)	(2.4)	(8.2)	(19.6)	(0.2)	(0.7)
10-20 cm	453.1^{c}	444.8^{b}	10.6^{b}	10.3^{b}	43.3^{b}	45.0^{a}	38.7^{c}	40.4^{a}	0.9^{b}	0.9^{b}
	(3.0)	(6.0)	(0.5)	(1.0)	(1.7)	(5.3)	(2.9)	(6.5)	(0.1)	(0.1)

Total and above-ground carbon sequestration was higher under N fertilization in the first year. Total and above-ground nitrogen sequestration did not differ between treatments. C and N return with total leaf litter were increased by N fertilization in the second year and in both years combined, while C and N sequestration into stem wood were not affected. Below-ground carbon and nitrogen sequestration did not change under N fertilization (Table 4-5).

Table 4-5. Above- and below-ground C and N sequestration, and C and N return with litter. Below-ground sequestration, and hence total sequestration, are available only for the first year. Within each row, means (SE; n = 4 plots) followed by a different letter indicate significant differences between treatments (Mann-Whitney U test at $P \leq 0.05$).

	C (g m^{-2})		N (g m^{-2})	
category	Control	N-fertilized	Control	N-fertilized
First year				
total	751.7^{a} (24.5)	900.1^{b} (35.5)	16.1^{a} (0.8)	19.4^{a} (1.1)
below-ground[1]	162.3^{a} (10.8)	203.0^{a} (28.9)	4.5^{a} (0.3)	5.7^{a} (1.0)
above-ground[2]	589.4^{a} (27.5)	697.2^{b} (8.2)	11.6 (0.8)	13.7 (0.7)
stem wood[3]	195.6^{a} (17.2)	241.9^{a} (18.3)	2.5^{a} (0.5)	2.3^{a} (0.4)
total litter[4]	393.8^{a} (12.3)	455.3^{a} (14.4)	9.1^{a} (0.4)	11.4^{a} (0.7)
leaf litter[5]	250.3^{a} (10.7)	303.5^{a} (15.1)	6.2^{a} (0.2)	8.2^{a} (0.6)
Second year				
above-ground[2]	647.2^{a} (44.9)	718.0^{a} (30.4)	12.4^{a} (1.0)	14.7^{a} (1.0)
stem wood[3]	186.8^{a} (24.3)	209.7^{a} (19.2)	2.1^{a} (0.5)	2.0^{a} (0.3)
total litter[4]	460.4^{a} (23.2)	510.3^{a} (12.2)	10.3^{a} (0.6)	12.6^{a} (0.9)
leaf litter[5]	282.4^{a} (8.4)	323.9^{b} (9.6)	6.8^{a} (0.2)	8.9^{b} (0.7)
Combined 2 years				
above-ground[2]	1237.5^{a} (71.9)	1410.1^{a} (39.1)	24.0^{a} (1.8)	28.3^{a} (1.6)
stem wood[3]	383.3^{a} (41.6)	444.6^{a} (33.6)	4.6^{a} (0.9)	4.3^{a} (0.6)
total litter[4]	854.2^{a} (34.1)	965.5^{a} (16.8)	19.4^{a} (0.9)	24.0^{a} (1.3)
leaf litter[5]	532.7^{a} (14.1)	627.5^{b} (19.0)	13.0^{a} (0.3)	17.2^{b} (1.2)

[1] Below-ground C and N sequestration (for the organic layer and 0-20 cm mineral soil combined) is the product of fine root production times the mean C and N concentration of the five sample dates.

[2] Above-ground C and N sequestration is the sum of C and N sequestration in total litter and new stem wood.

[3] Stem wood C and N sequestration is the product of above-ground woody biomass production times the mean wood C and N concentrations weighted with the number of trees per sampled species.

[4] C and N return with total litter is the sum of the product of C and N concentrations of fresh wood and bark times wood and bark litter production, respectively, and the product of the mean leaf litter C and N concentrations times production in all other litter categories.

[5] Annual C and N return with leaf litter is the sum of the products of the C and N concentration of the four quarterly collections per year and the amount of leaf litter in the respective quarter.

The concentrations of base cations (P, S, Na, K, Ca, Mg, Mn, Fe, Al) did generally not differ between treatments with the only exceptions being a lower S concentration in fresh leaves in the second year under N fertilization (Appendix 4-1).

4.5 Discussion

Foliar N concentrations at our site are at the upper end of the range reported for tropical montane forests (Veneklaas 1991). Neither foliar N concentrations of all species pooled together nor of a single species responded to N fertilization. Similarly, N addition did not increase foliar N concentration of four species in a mixed-species forest in Jamaica (Tanner et al. 1990). N fertilization increased foliar N in the Hawaiian sites when stem growth and/or leaf litter production were N-limited (Harrington et al. 2001, Raich et al. 1996, Vitousek et al. 1993, Vitousek & Farrington 1997). In a P-limited site in Kauai, N treatment increased foliar N after 6 years, while there was no difference after 1.5 years of N fertilization (Harrington et al. 2001, Herbert & Fownes 1995). As the latter example shows, foliar N concentrations may respond to N addition only after a longer time than our fertilization experiment lasts, especially in a site that may not be limited by N alone, but that may also be co-limited by P. Foliar N at our site is already high compared to other montane forests and a further increase might not be possible. A response of N concentrations in bark and wood may take even longer than in leaves as production of those tissues is slower and a separation of tissue produced before (having the same N content like in the control) and after N addition (which may have an increased N content) during sampling is difficult. Furthermore, extra N might not change tissue N concentration despite an increase in production (Tanner et al. 1998). In contrast to the findings of Tanner et al. (1990), canopy species with low foliar N concentrations in control plots (in our site *O. mexicana* and *V. guatemalensis*) did not increase their stem growth under N fertilization (Adamek et al., in preparation). *E. panamensis*, a species mostly from the lower canopy, which has higher foliar and wood N concentrations in controls than the two other species, responded to N addition with an increased stem growth.

Concentrations of leaf litter N are within the range for tropical montane forests (Heaney & Proctor 1989, Veneklaas 1991). Although foliar N concentrations of *O. mexicana* were not affected by N fertilization, N concentrations in litter leaves of *O. mexicana* were increased under N fertilization. Comparison with a Venezuelan montane forest where N addition did not increase N concentration in litter (Tanner et al. 1992) is difficult, because in this study a composite sample of woody and non-woody material was analyzed and litter was collected only monthly leading to increasing leaching losses with increasing residence time of litter in the traps which may level out differences between treatments. C and N return with total leaf litter increased under N addition as a result of increased leaf litter production (Adamek et al., in preparation).

Concentrations of C and N in live fine roots of the organic layer are comparable to the values reported by Soethe et al. (2007) from a forest at 1900 m asl in the Ecuadorian Andes. Consistent with our findings, N fertilization did not affect fine root N concentrations in two sites in the Hawaiian montane forest (Ostertag 2001). As fine roots are short-lived plant organs which's primary function is the uptake and transmission of nutrients (and water) and not its storage, an increase in their N content due to N fertilization is unlikely.

In the first year, N fertilization increased total and above-ground C sequestration by 148.5 g C m^{-2} $year^{-1}$ (or 1.49 Mg C ha^{-1} $year^{-1}$) and 107.8 g C m^{-2} $year^{-1}$ (or 1.08 Mg C ha^{-1} $year^{-1}$), respectively. For the two years combined N fertilization increased C and N return with leaf litter by 94.7 g C m^{-2} $year^{-1}$ (0.95 Mg C ha^{-1} $year^{-1}$) and 4.2 g N m^{-2} $year^{-1}$ (0.04 Mg N ha^{-1} $year^{-1}$), respectively. Hence, 16.8% of the added N were returned with leaf litter. As tissue concentrations were not affected by N fertilization these changes are due to the increases in ANPP in the first year and in leaf litter production for the two years combined (M. Adamek et al., in preparation). In control plots, litter made up for 52% and 57% of the total C and N sequestration in the first year, thereby constituting the main sink of C and N taken up by trees. If no retranslocation of nutrients from fine roots occurred (Nambiar 1987), below-ground carbon and nitrogen sequestration can be equated with C and N inputs from fine roots to soil and C and N inputs from fine roots constituted 65% and 74%, respectively, of that from leaf litter. Hence, in our study site fine roots were nearly as important as leaf litter for returning N to the soil.

4.6 Conclusions

N addition increased total C sequestration by 1.48 Mg C ha^{-1} $year^{-1}$ in the first year mostly due to an increased net primary production. The added N taken up by plants was primarily channelled into total leaf litter production than into production of other tissues or an increase of tissue N concentrations. As C:N ratio of total leaf litter decreased under N fertilization, the greater N availability of this high quality leaf litter may accelerate microbial decomposition on the forest floor, thereby enhancing plants´ access also to other nutrients than the added N.

Although foliar N concentrations of *O. mexicana* did not respond to N fertilization, the higher N concentrations in its litter leaves may indicate that retranslocation of N from senescing leaves decreased under N addition.

Acknowledgements

The Smithsonian Tropical Research Institute provided administrative and technical support. We highly appreciate the help of the research assistants Arturo Morris, Rodolfo Rojas, Evidelio Garcia and Ignacio Delcid. We are grateful to the laboratory staff of the Soil Science of Tropical and Subtropical Ecosystems department, especially Heike Strutz and Kerstin Langs. Dr. James Dalling provided background information and assisted in the site selection. This study is under the NITROF project funded by the Robert Bosch Foundation (Germany) and awarded to M. D. Corre as independent research group leader.

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Appendix 4-1. Base cation concentrations in fresh leaves, leaf litter and fine roots from different soil depths. Within each row, means (SE; n = 4 plots) followed by a different letter indicate significant differences between treatments (Linear mixed effects model at *P* ≤ 0.05).

	Treatment	P	S	Na	K	Ca	Mg	Mn	Fe	Al
Fresh leaves	Control 1.y	0.8^{a} (0.0)	3.0^{a} (0.1)	1.7^{a} (0.4)	8.3^{a} (0.4)	9.6^{a} (0.9)	2.7^{a} (0.3)	0.6^{a} (0.2)	0.1^{a} (0.0)	1.7^{a} (0.6)
	N-fertilized 1.y	0.9^{a} (0.0)	2.4^{a} (0.3)	1.6^{a} (0.6)	8.3^{a} (0.8)	8.8^{a} (0.5)	2.1^{a} (0.2)	0.3^{a} (0.0)	0.1^{a} (0.0)	2.4^{a} (0.9)
	Control 2.y	0.8^{a} (0.0)	3.0^{a} (0.3)	1.6^{a} (0.4)	7.9^{a} (0.4)	10.1^{a} (1.0)	2.6^{a} (0.3)	0.5^{a} (0.2)	0.1^{a} (0.0)	2.0^{a} (0.8)
	N-fertilized 2.y	0.9^{a} (0.1)	2.0^{b} (0.1)	1.2^{a} (0.4)	8.3^{a} (1.5)	8.4^{a} (0.8)	2.1^{a} (0.2)	0.3^{a} (0.0)	0.1^{a} (0.0)	2.0^{a} (0.7)
Leaf litter[1]	Control	0.4^{a} (0.0)	1.5^{a} (0.0)	1.0^{a} (0.1)	3.2^{a} (0.3)	11.3^{a} (0.6)	2.5^{a} (0.1)	0.7^{a} (0.1)	0.1^{a} (0.0)	0.7^{a} (0.2)
	N-fertilized	0.5^{a} (0.1)	1.5^{a} (0.2)	0.9^{a} (0.1)	3.7^{a} (0.2)	9.9^{a} (0.8)	2.0^{a} (0.2)	0.4^{a} (0.1)	0.1^{a} (0.0)	1.4^{a} (0.5)
Fine roots[1]										
Organic layer	Control	0.5^{a} (0.0)	1.5^{a} (0.1)	0.3^{a} (0.1)	0.8^{a} (0.1)	5.2^{a} (0.7)	0.9^{a} (0.1)	0.1^{a} (0.0)	1.0^{a} (0.4)	2.0^{a} (1.1)
	N-fertilized	0.6^{a} (0.1)	1.4^{a} (0.3)	0.3^{a} (0.1)	1.0^{a} (0.1)	6.2^{a} (0.7)	1.0^{a} (0.2)	0.2^{a} (0.1)	0.6^{a} (0.3)	1.5^{a} (0.6)
0-10 cm	Control	0.4^{a} (0.0)	1.7^{a} (0.1)	0.5^{a} (0.2)	0.7^{a} (0.1)	3.9^{a} (0.5)	0.9^{a} (0.0)	0.1^{a} (0.0)	4.0^{a} (1.4)	6.5^{a} (1.0)
	N-fertilized	0.5^{a} (0.1)	1.6^{a} (0.1)	0.4^{a} (0.0)	0.9^{a} (0.1)	4.0^{a} (0.5)	1.0^{a} (0.1)	0.2^{a} (0.1)	3.1^{a} (0.3)	7.6^{a} (0.5)
10-20 cm	Control	0.4^{a} (0.0)	1.9^{a} (0.2)	0.6^{a} (0.1)	0.8^{a} (0.1)	3.3^{a} (0.9)	1.0^{a} (0.1)	0.1^{a} (0.0)	6.4^{a} (2.3)	12.6^{a} (2.4)
	N-fertilized	0.4^{a} (0.1)	1.7^{a} (0.2)	0.6^{a} (0.1)	1.1^{a} (0.2)	3.1^{a} (0.5)	1.0^{a} (0.1)	0.1^{a} (0.0)	5.1^{a} (1.1)	14.3^{a} (2.0)

[1] **Base cation concentrations of leaf litter and fine roots are averages of the sampling dates.**

Chapter

5

Synthesis

5.1 The response of different DBH classes, tree species, and components of ANPP to N fertilization

The response of overall stem growth rates to N fertilization in the two years of the measuring period was influenced by the disparity of the response of different DBH classes and species as well as by climatic factors. In the first year, overall stem growth rates in the control correlated very strongly with the difference between stem growth rates in control and N-fertilized plots but there was no correlation in the second year. Hence, N fertilization stimulated stem growth especially during the high-growth period in the first year but as there was no such high-growth-period in the second year, stem growth showed no response to N fertilization. The climatic factors driving the intra- and inter-annual variation in stem growth rates could not be detected.

Structure had a great influence on the overall stem growth response to N fertilization. Trees ≥ 50 cm DBH were unresponsive to N fertilization, while trees 10-30 cm DBH increased their stem growth quickly after N fertilization had begun; but the 70% of stems they made up for had – with 31% of basal area – a disproportionately low influence on overall stem growth.

The influence of different tree species on the overall stem growth response was also of interest for the first objective with the three most abundant tree species (*Eschweilera panamensis*, *Oreomunnea mexicana*, *Vochysia guatemalensis*) taken into focus. *O. mexicana* and *V. guatemalensis* did not respond to N addition, while *E. panamensis* 10-30 cm DBH increased its stem growth in the first year. It was concluded that stem growth of *E. panamensis* was mainly N-limited, while stem growth of *O. mexicana* and *V. guatemalensis* was – at least in the short term – not limited by N alone. However, their fast growth let assume that they are not strongly limited by other factors. As these two unresponsive species exert a disproportionate influence over overall stem growth (26% of stems but 49% of stem growth), the ecosystem is dominated by species that are not strongly constrained by the N availability of the study site.

As only a small group of trees (*E. panamensis* 10-30 cm DBH) responded to N addition during a short time span (high-growth period of the first year), overall stem growth, and hence, AWBP were not increased by N fertilization.

Which factors influence the different stem growth responses of the investigated species?

O. mexicana and *V. guatemalensis* had wood C:N ratios that were double or triple that of *E. panamensis*. Hence, they should have lower costs of stem growth (if C is not limiting) because they need less N to sustain their relatively high stem growth rates. These high stem growth rates indicate that those species are not strongly constrained by other limiting factors. In contrast, the low wood C:N ratio of *E. panamensis* makes stem growth very cost-intensive for this species, as it has to sequester relatively more N for a given amount of C used for stem growth and thus stem growth rates of *E. panamensis* in control plots are very low compared to the mean. These high N costs cause stem growth rates of *E. panamensis* to be mainly limited by N supply. Hence, this species is responsive to N fertilization, while the low N costs for stem growth of *O. mexicana* and *V. guatemalensis* cause no N limitation to their stem growth under the site's inherent N availability and makes them unresponsive to N fertilization. At least in the case of *V. guatemalensis* these findings correspond well with what is known about its ecology: *V. guatemalensis* is an early- to mid-successional species from the upper forest canopy (Redondo-Brenes & Montagnini 2006). For species which have to establish themselves in the upper canopy during ecosystem succession by fast growth stem growth costs have to be low to be able to outcompete their competitor species.

N fertilization increased fine litterfall in the first year and for the two study years combined. Although leaf litter production increased for the two years combined, PAI did not change, indicating that leaf turnover increased and leaf lifespan decreased.

ANPP increased in the first year under N fertilization but not all components of ANPP were uniformly affected by N fertilization. Since AWBP did not change under N addition, it was fine litterfall driving the response of ANPP to N fertilization. Fine litter production was 66% of ANPP and was therefore not only the most important ANPP component but also responded stronger to N fertilization than AWBP. Leaf production, with 66% the most important litter category, has a higher priority in the allocation of newly synthesized biomass than AWBP, as the photosynthesis of these primary assimilatory organs enables all other life processes (Malhi et al. 2004, Tanner et al. 1998). As maximum net photosynthesis and above-ground production efficiency (ANPP/foliar biomass) decrease with increasing leaf life-span (Reich et al. 1992), the production of leaves with shorter lifespan should be prioritized in order to increase ANPP.

5.2 Fine root responses to N fertilization

For the second objective, two different root sampling methods were applied. Fine root biomass and necromass were measured in three soil depths (organic layer, 0-10 cm and 10-20 cm mineral soil) by sequential coring and the obtained data were used to estimate fine root production and turnover with the minimum-maximum calculation (McClaugherty et al. 1982). Fine root biomass allocation to different soil depths was assessed with the ingrowth core approach.

Fine root production and turnover did not change under N addition with the only exception being a tendency towards an increase in fine root production in the 10-20 cm mineral soil. As results from investigations in Hawaii showed, short- and long-term responses of fine roots to N fertilization can differ strongly and can be even opposite in their direction. Gower & Vitousek (1989) found a reduced fine root biomass after 1.5 years of N fertilization, while Ostertag (2001) found no treatment effects on fine root biomass after ten years of N addition at the same site, despite N limitation to above-ground productivity (stem growth, leaf litter production, foliar N) (Vitousek et al. 1993, Vitousek & Farrington 1997).

In N-fertilized plots, fine roots biomass ingrowth into the 10-20 cm mineral soil increased two-fold, which – together with the higher fine root production in the same soil depth from the sequential coring – gave a second line of evidence for an increased fine root biomass allocation to this deeper soil depth at the expense of the organic layer. In unamended soil, plant-available N was concentrated in the organic layer (Koehler et al. 2009), which may be partly responsible for the superficial distribution of fine roots. In N-fertilized soil, the distribution of mineral N through the soil profile was changed, as indicated by an increase in nitrate concentration in the soil solution at 1.5 m depth (Corre, unpublished data). If NPP in the study forest is not only limited by N but also by other nutrients (e.g. phosphorus, like was recently stated for tropical montane rain forests; Benner et al., in press), the observed increase in fine root allocation to the mineral soil can be explained by fine roots foraging for these rock-derived nutrients, which might limit the increased ANPP, without being constrained by the low N availability of the mineral soil. Hence, the trade-off for fine roots to either forage for N in the organic layer or for rock-derived nutrients in the mineral soil is alleviated under N addition.

5.3 The potential of the vegetation to serve as a sink for N and C

In the first year, C sequestration in above-ground biomass increased by 1.07 Mg C ha^{-1} $year^{-1}$. As ANPP exerted a strong influence over total NPP (79%), also C sequestration in total biomass (above- and below-ground) increased. Trees in N-fertilized plots sequestered 1.48 Mg C ha^{-1} $year^{-1}$ more than trees in control plots. Leaf litter C and N sequestration for the two years combined increased by 0.92 Mg C ha^{-1} year^{-1} and 0.04 Mg N ha^{-1} year^{-1}. Hence, 16.5% of the added N were returned to the forest floor with leaf litter. Leaf litter C and N sequestration did not increase as a consequence of an increase in C and N concentrations (as they remained unchanged) but the added N was channelled into a higher leaf litter production. As pointed out before, leaf production should have a high priority in whole tree C and N allocation as photosynthetic processes sustain all other plant life processes. In control plots, leaf litter accounted for 33% of C sequestration and 38% of N sequestration. Fine roots made up for 22% of C and 28% of N sequestration and were nearly as important as leaf litter for returning C and N to the soil, given that no retranslocation from fine roots occurred (Nambiar 1987). AWBP equals fine root production in importance as C sink (26%) but is – due to the higher C:N ratios of wood – much less important for N sequestration (15%).

The amount of C and N sequestered in response to N addition, and hence, the potential of the vegetation to serve as a sink for C and N, does not only depend on whether an increase in NPP will occur, but also on the C:N stoichiometry of the responsive NPP component(s) and on whether the C:N ratio of the responsive component(s) will be subject to change. If tissue C:N ratios remain equal to pre-fertilization levels a higher C and N sequestration can only occur through an increase in NPP. A production increase in a low-C:N tissue (e.g. leaves) would increase N sink strength, while a production increase in a high-C:N tissue (e.g. wood) would favor C sink strength. If, however, mainly the stem growth of the slow-growing species with low wood C:N ratios is responsive to N, as the presented results suggest, then the C sequestering benefit from an increased AWBP would be minimal. This is because the amount of C sequestered by species with a low wood C:N ratio is lower than in species with a high wood C:N ratio but that are not responsive to additional N.

5.4 Effects of N on other forest processes

A higher NPP of tropical montane forests under elevated N deposition is likely to be not only beneficial for the ecosystem. The above discussed impacts of elevated N availability on NPP and C sequestration are likely to be short-term effects. In the long term, faster tree growth and turnover can lead to higher mortality and may favor disturbance-adapted species, which are likely to have less dense wood (Malhi & Phillips 2004). NPP in tropical montane forests may not only be limited by N alone but also by other nutrients such as P (Benner et al., in press) and an increase in NPP caused by higher N availability might cause an increased competition for these other limiting nutrients. Species which are stronger competitors for special nutrients might gain increasing importance over species which were dominating in a mainly N-limited ecosystem. The stronger fine root allocation to the deeper mineral soil at the expense of the organic layer, probably due to fine roots foraging for nutrients concentrated in the mineral soil, might be seen as a hint for a shift in resource limitation.

In N-fertilized temperate forest soils, higher nitrate mobility can change the availability of nutrient cations. Nitrate leaching is accompanied by leaching losses of cations (e.g. Ca and Mg) and increases soil acidity, which in turn enhances Al availability. The latter two processes might immobilize P and counteract the higher P demand for the increased NPP. The changing soil nutrient availability can cause nutrient imbalances in trees. Most frequently, nutrient imbalances have been expressed as foliar or root Ca:Al or Mg:N ratios. Such imbalances have been reported to reduce net photosynthesis, tree growth or even increase tree mortality (Aber et al. 1995). As tropical forest soils already have relatively low pH values and high Al contents (Matson et al. 1999, Tanner at al. 1998), the increase in NPP may be a short-term effect, while the long-term effects of changes in soil chemical characteristics on NPP may be deleterious.

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Acknowledgements

First of all, I want to thank Dr. Marife Corre and Prof. Dirk Hölscher for providing me an interesting and challenging research topic. Many thanks for always having an open ear, for the constructive discussions, critical advice and the great interest for my work.

I am grateful to the Robert Bosch Foundation for funding the NITROF project.

Great thanks to the Smithsonian Tropical Research Institute, especially to Orelis Arosemena, Marcela Paz and Celideth de Leon, for the friendly way in which they gave invaluable administrative support in Panama.

Special thanks go to Dr. James Dalling who assisted in the selection of the study site, provided structural and floristic data on the study area, and was always interested for my work.

Especially great thanks goes to the field assistants in Panama: Arturo Morris, Rodolfo Rojas, Evidelio Garcia and Ignacio Delcid. Without their countless help field work would have been simply impossible to accomplish. ¡Muchissimas Gracias!

Intrinsically tied to my time in Fortuna is Carlos Espinosa whom I will remember for his enthusiasm and friendliness with which he manages the "casa verde" and for the uncomplicated help he provided in many ways. For me, also belonging to the "casa verde" is Kelly Andersen whom I would like to thank for many good conversations which brought diversion into everyday life during field stays. For the same reason I am thankful to Bianca Dunker, Marco Harbusch and Stefanie Berghoff for being such a great company during the "Hornitos" time and to Jonas Loss for the fun we had during his stay in "casa B".

The laboratory team of the Büsgen Institute carried out extensive laboratory work; here I am especially thankful to Heike Strutz and Kerstin Langs.

I am grateful to Ute Krökel for the uncomplicated way she eased complicated financial issues.

Thanks to Ulrike Talkner, Inga Krämer and Johannes Dietz for help with statistics, the CAN_EYE program and instructions on the fisheye camera.

Many thanks to Julia Arnold, Nadine Eickenscheidt and Nadine Jäger for looking thoroughly into and giving constructive comments on the introduction and synthesis of this thesis – and for everything else!

My greatest thank goes to my parents for their everlasting support and to my girlfriend Anja for accompanying me through the ups and downs of the last years.

Declaration of originality and certificate of authorship

I hereby declare that this thesis entitled "Effects of increased nitrogen input on the net primary production of a tropical lower montane rain forest, Panama" is my own work and has been written by me. It has not previously been submitted in any form for another degree at any university or other institute of tertiary education. Information derived from the published and unpublished work of others has been acknowledged in the text and a list of references is given in the bibliography. I certify that the manuscripts presented in chapters 2, 3 and 4 have been written by me as first author.

Göttingen, May 2009

Markus Adamek

Curriculum vitae

Personal details

Date of birth: March 7, 1977

Place of birth: Lüdenscheid, Germany

Nationality: German

Education and Research Experience

10/2005 – 09/2008	PhD study in Biological Diversity and Ecology at the Georg-August-University, Göttingen
10/2000 – 09/2004	Study of biology at the University of Leipzig
10/1997 – 09/2000	Study of biology at the Ruhr-University, Bochum
08/1987 – 06/1996	Geschwister-Scholl-Gymnasium, Lüdenscheid High School graduation: Allgemeine Hochschulreife
09/1983 – 06/1987	Primary School, Lüdenscheid

Contributions to Conferences

Adamek, M., Corre, M.D., Hölscher, D. Elevated N input increased above-ground net primary production in a tropical lower montane rain forest, Panama. International Conference of the Society for Tropical Ecology. 18 – 22 February 2008, Hohenheim, Germany.

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