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Photosynthetic capacity and nitrogen nutrition of Ecuadorian montane forest trees

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CHAPTER 1

General introduction

Tropical montane forests and nitrogen limitation along elevational gradients

The majority of ecological studies about tropical forests have been conducted in the lowlands even though 12 % (204 million ha) of the tropical forests worldwide are situated in mountainous areas (FAO, 1993). Along mountain slopes, the lowland evergreen rain forest gives way to montane rain forest or montane cloud forest. Different types of tropical montane forests can be distinguished with increasing elevation, from premontane forest at lower elevations to elfin forest at high elevations (Grubb et al., 1963; Homeier et al., 2008; Scatena et al., 2011). The change from lowland to montane forest is related to the range of cloud formation and the average minimum temperature dropping below 18° C and is accompanied by the appearance of montane tree species and the disappearance of lowland species (Kitayama, 1992; Scatena et al., 2011). Compared to lowland forests, tropical montane forests have a reduced canopy height, a simpler canopy structure and a higher abundance of epiphytes and mosses (Frahm and Gradstein, 1991; Scatena et al., 2011).

Along elevational gradients, tropical montane forest trees experience modifications in form and function, these are adaptations in leaf morphology and physiology, tree stature, carbon allocation patterns, and productivity (e.g. Cordell et al., 1999; Moser, 2008; Moser et al., 2011, 2007). The most remarkable changes are the decrease in tree size (Aiba and Kitayama, 1999; Raich et al., 1997) and the reduction in aboveground net primary production with increasing altitude (Kitayama and Aiba, 2002; Moser et al., 2011; Raich et al., 1997). A decrease in stature, aboveground biomass, aboveground productivity and number of life forms with elevation has been attributed primarily to the increase in cloudiness and the decrease in temperature (Bruijnzeel and Veneklaas, 1998; Grubb, 1977). Other environmental factors that change with altitude are air pressure and the atmospheric concentrations of CO_2 and O_2 and UV-B radiation. In tropical montane forests, soil moisture tends to increase with elevation in many cases while the plant availability of nutrients, in particular of nitrogen, tends to decrease with altitude (Benner et al., 2010; Moser, 2008; Soethe et

al., 2008). Along some mountain slopes, a decrease of foliar nitrogen concentration and an increase in leaf longevity with elevation was found (e.g. Letts and Mulligan, 2005; Moser et al., 2010; Tanner et al., 1998) pointing to a nitrogen limitation of tree growth at high elevations. Leaves that are smaller, thicker and with lower nitrogen content were found to be characteristic for tropical montane forests at high elevation (Grubb, 1977). The structural changes are a consequence of rapid changes in the floristic composition with increasing elevation (Homeier et al., 2008).

Most tropical lowland forests are supposed to be phosphorus limited with nitrogen not being limiting for productivity (Paoli et al., 2005; Tanner et al., 1998). However, in tropical montane forests nitrogen limitation should become more crucial with elevation, mainly, due to reduced decomposition and mineralization rates with decreasing temperatures, increasing soil humidity and decreasing litter quality and, therefore, lower availability of nitrogen for plants at higher altitudes (Jones et al., 2009; Joshi et al., 2003; Marrs et al., 1988; Vitousek and Sanford, 1986). Moreover, reduced decomposition rates at higher altitudes lead to thicker organic soil layers and a change in available nitrogen forms from mostly inorganic at low elevations to mostly organic at high elevations (Iost, 2008; Wolf et al., 2011). Aside from phosphorus, nitrogen has been shown to limit productivity in tropical montane forests (Tanner et al., 1998; Vitousek, 1984). The nitrogen limitation of tropical montane forests seems to be linked to soil development (Walker and Syers, 1976). Due to erosion, the soils found in mountainous areas are often relatively young and shallow (Foster, 2001) compared to the highly weathered soils of the tropical lowland forests (McGroddy et al., 2008). With proceeding soil development, soils become poorer in phosphorus, which is almost exclusively provided by the soil parent material and is washed out over time, and richer in nitrogen, which is provided by deposition from the atmosphere and nitrogen fixation and accumulates over time (Hedin et al., 2009; Walker and Syers, 1976).

The dependence of photosynthetic capacity in tropical forests on altitude and nitrogen availability

Changes in nitrogen availability along elevational gradients in tropical montane forests should affect the photosynthetic capacity of trees. Maximum rate of photosynthesis A_{max} is related to foliar nitrogen content mainly because of the high nitrogen demand for the proteins of the Calvin cycle and thylakoids which together represent the majority of foliar nitrogen (Evans, 1989). Therefore, photosynthetic capacity has been found to be closely related to foliar nitrogen (Wright et al., 2004). Other possible factors controlling photosynthetic capacity which change along the altitudinal gradient are the availability of phosphorus, temperature, partial pressure of CO_2, VPD and radiation. In contrast to tropical lowland forests, the knowledge about photosynthetic capacity in tropical montane forests is rather scarce. In the rough terrain with often steep slopes, it is difficult to get access to the sun leaves of the canopies. The few studies accomplished in tropical high elevation forests, show slightly reduced leaf level photosynthetic rates when compared to lowland forests (Letts and Mulligan, 2005; Rada et al., 2009; van de Weg et al., 2012).

Knowledge about the photosynthetic capacity of the species composing tropical mountain forest is crucial to understand the functioning of this ecosystem. Information on productivity of tropical forests is needed for the development of realistic global carbon budgets and for projecting how these ecosystems will be affected by climatic and atmospheric changes (Clark et al., 2001).

Preference for different nitrogen forms and tropical montane forest trees

In tropical montane forests, reduced decomposition rates at higher altitudes lead to thicker organic soil layers and together with reduced mineralization and nitrification rates to a change in available nitrogen forms from mostly inorganic at low elevations

to mostly organic at high elevations (Iost, 2008; Wolf et al., 2011). However, the knowledge about tropical montane tree species' preferences for different nitrogen forms is very limited. Trees of different ecosystems have been shown to take up organic nitrogen (Kahmen et al., 2009; McFarland et al., 2010; Näsholm et al., 2009) but the mycorrhizal status of tropical tree species raises doubts about their possible ability to take up organic nitrogen. While ectomycorrhiza (ECM) are able to mobilize nitrogen from organic matter**,** AM are more effective in capturing inorganic nitrogen (Chalot and Brun, 1998 but note Hodge et al., 2001; Whiteside et al., 2012). Findings from Kottke et al (2004) at 2000 m elevation in the study region, indicate that arbuscular mycorrhiza (AM)-forming trees dominate the southern Ecuadorian montane forest.

Even the knowledge about preferences for different nitrogen forms of tropical lowland tree species is rather scarce. In two studies on hemi-epiphytic *Clusia* species, seedlings were able to take up nitrate, ammonium and glycine and preferred ammonium over the other two nitrogen forms (Arndt et al., 2002; Wanek et al., 2002).

So, to date, it remains an unanswered question, if tropical montane trees are able to take up significant amounts of organic nitrogen and how they adapt to different nitrogen forms being available at different altitudes.

Objectives and approach

This thesis is part of the DFG funded Research Unit 816 “Biodiversity and sustainable management of a megadiverse mountain ecosystem in South Ecuador”. The study aimed at investigating the photosynthetic capacity and nitrogen nutrition of forest trees in a South Ecuadorian mountain rainforest at three sites at 1000, 2000 and 3000 m asl.

Major aims of the study were

(1) to asses the photosynthetic capacity of adult tropical trees along the elevational transect and to analyse the possible controlling effects of temperature, partial pressure of CO_2 and nutrient availability on photosynthesis (chapter 2 and 3).

(2) to investigate altitudinal changes in the use of nitrate, ammonium or organic nitrogen sources by tropical forest trees by means of a stable isotope tracer study on seedlings in mesocosms in montane forest stands at 1000, 2000 and 3000 m elevation (chapter 4).

With these objectives, the following hypotheses were tested:

(1) Stand-level averages of mass-based A_{sat} are decreasing with elevation due to decreases in foliar N and P.

(2) Area-based A_{sat} remains unchanged because of the LMA increase with altitude.

(3) Area-based R_D does not change with elevation.

(4) Tropical trees at high elevations have higher N and P contents per leaf area.

(5) Tropical trees at high elevations possess a higher carboxylation efficiency than

trees at lower elevation.

(6) Due to these adaptations, the A_{max} of trees at high elevations responds in a homeostatic manner to the lowered CO_2 concentration and reduced temperature, thereby compensating for the less favourable environmental conditions.

(7) The saplings of tropical trees are capable of using organic N even though they are forming arbuscular mycorrhizas.

(8) With increasing elevation, tree saplings increasingly prefer ammonium and glycine over nitrate due to a lowered nitrification rate and increased humus accumulation.

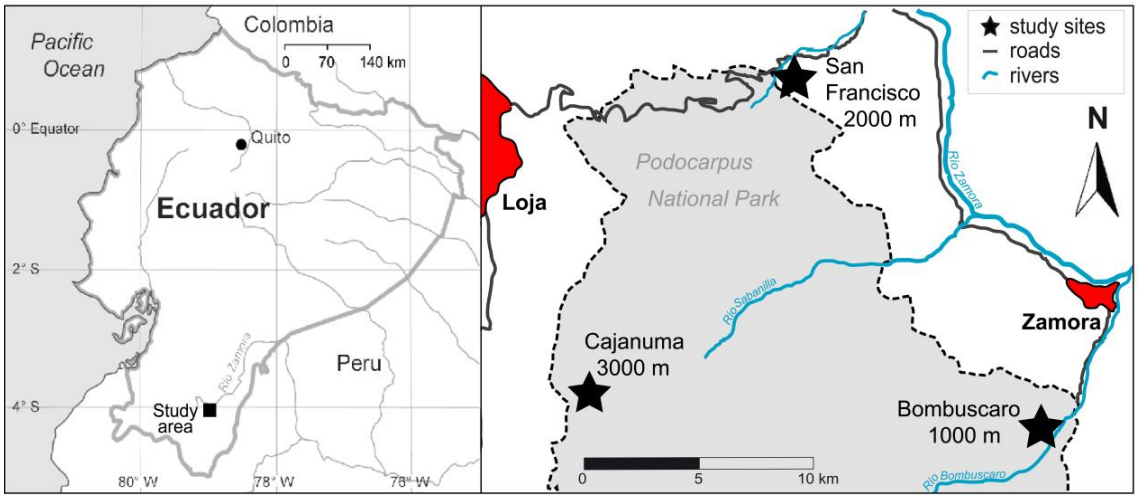

Figure 1. *Location of the study area in southern Ecuador with the three stands at 1000, 2000 and 3000 m a.s.l. Figure after Homeier et al. (2012).*

Study area

Measurements for this thesis were made along an altitudinal gradient in the tropical mountain forest on the eastern slope of the southern Ecuadorian Andes in the provinces of Loja and Zamora-Chinchipe. The study sites were located at 1000, 2000 and 3000 m a.s.l. inside the Podocarpus National Park and in the Reserva Biológica San Francisco. Maximum distance between the sites was 30 km (Fig. 1).

The study region has a tropical humid climate with an extremely wet season from April to July and a less humid period from September to December. The area is further characterized by high cloudiness and increasing cloud frequency with altitude (Bendix et al., 2006). The MAT along the elevational gradient, studied in this thesis, ranges from 19 °C at 1000 m to 9 °C at 3000 m a.s.l. (Table 1). The mountain ridge in the study region consists of a variety of acidic bedrocks with granites dominating at 1000 m and phyllites and sandstones being present at elevations >1500 m. The soil types change along the slope from Alumic Acrisols at 1000 m to Gleyic Cambisols (2000 m) and Podzols (3000 m) at higher elevation (Iost, 2008). The soils are generally acidic and nutrient-poor with increasing humus accumulation with increasing elevation; the organic layer thickness ranges from 48 mm at 1000 m to 435 mm at 3000 m a.s.l. (Moser et al., 2011; Wolf et al., 2011). While the organic layer thickness increases, the availability of nitrogen in this layer decreases: nitrogen mineralization rate and the amount of KCL-extractable inorganic nitrogen in the organic layers of the study sites are decreasing with altitude, indicating a reduced decomposition at high elevations (Wolf et al., 2011).

Table 1. Characteristics of the stands (climate data from (Moser et al., 2007; Wolf et al., 2011). Mean annual air temperature and relative air humidity were measured at 1.5 m height inside the stands at 1050, 1890 and 3060 m. Rainfall data are extrapolated from measurements in a forest gap at approximately 1050 m (measuring period May 2003 – May 2004), and from measurements in gaps at 1950, and 3170 m (Emck, 2007). C/N ratio, pH, net nitrification and net N mineralization rate (*in situ* buried bag method) refer to the topsoil (0-10 cm, after (Wolf et al., 2011). For the edaphic parameters, means ± SE of 4 soil profiles, each dug at lower slope, midslope and ridge position in the stands, are given.

Elevation	*m asl*	**1000**	**2000**	**3000**
Rainfall	*mm yr^{-1}*	c. 2230	c. 1950	c. 4500
Air temperature	*°C*			
Mean		19	16	9
Max		30	29	19
Min		12	8	3
Air humidity	*%*			
Mean		86	91	94
Max		100	100	100
Min		16	29	29
pH (H_2O)		4.9 ± 0.2	4.4 ± 0.2	3.9 ± 0.1
C/N		17.6 ± 0.8	14.8 ± 0.7	18.2 ± 0.9
Net Nitrification	*kg N ha^{-1} 10d^{-1}*	1.97 ± 0.73	0.89 ± 0.30	0.01 ± 0.01
Net N mineralization	*kg N ha^{-1} 10d^{-1}*	2.5 ± 0.6	1.5 ± 0.3	0.1 ± 0.2
KCl-extractable NO_3^-	*kg N ha^{-1}*	0.43 ± 0.10	0.24 ± 0.05	0.02 ± 0.01
KCl-extractable NH_4^+	*kg N ha^{-1}*	1.8 ± 0.3	0.9 ± 0.1	0.7 ± 0.1

The floristic composition of the tropical montane moist forests in the study region was described in detail by Homeier et al. (2008). The three elevations along the gradient correspond to three different forest types:

(1) at 1,000 m (S 4° 7´ W 78° 58´), in the transition zone between tropical lowland and lower montane forest, evergreen premontane forest with tree heights of up to 40 m is present. Common tree families of this forest type are Fabaceae, Melastomataceae, Moraceae, Myristicaceae, Rubiaceae and Sapotaceae.

(2) The evergreen lower montane forest at 2000 m (S 3° 58' W 79° 04') achieves a canopy height of 18 to 22 m. Characteristic tree families are Euphorbiaceae, Lauraceae, Melastomataceae and Rubiaceae.

(3) At 3000 m (S 4° 7´ W 79° 11´), evergreen upper montane forests and elfin-forests are found that extend up to the tree line; canopy height does rarely exceed 8-10 m. Dominant tree families are Aquifoliaceae, Clusiaceae, Cunionaceae, Lauraceae and Melastomataceae.

Stand structural characteristics of the three stands are summarized in Table 2. All three stands are located in protected forest sections. Natural disturbances in the past may have included landslides in the steeply sloped terrain.

Table 2. Stand structural characteristics of the three stands at 1000, 2000 and 3000 m (Homeier et al., unpublished; Leaf life span and BGB: Moser et al., 2011, 2007). Abbreviations: AGB, aboveground biomass; BGB, belowground biomass (coarse and fine roots); DBH, diameter in breast height; LAI, leaf area index. Given are means ± SE for each elevation. Means of tree DBH, stem density, basal area and AGB were calculated for 9-18 permanent plots (400 m² each) covering the whole range of topographic positions at the respective elevations (trees > 10 cm DBH). Estimates for nutrient pools in canopy leaf biomass were calculated from leaf biomass data (Moser et al., 2007) and mean foliar N and P concentrations according to Homeier et al. (unpublished). Different small letters indicate significant differences between elevations.

Elevation	*m asl*	**1000**		**2000**		**3000**	
Canopy height	*m*	25-30		16-20		8-10	
DBH	*cm*	19 ± 1	**a**	20 ± 1	**a**	18 ± 1	**a**
Stem density	*n ha^{-1}*	822 ± 50	**a**	900 ± 62	**a**	1061 ± 84	**a**
Basal area	*m^2 ha^{-1}*	29 ± 4	**a**	34 ± 3	**a**	30 ± 3	**a**
AGB	*Mg ha^{-1}*	177 ± 28	**a**	158 ± 22	**a**	89 ± 10	**b**
BGB	*Mg ha^{-1}*	32.1		26.1		62.8	
LAI	*m^2 m^{-2}*	6 ± 0.4	**a**	5.7 ± 0.5	**a**	2.2 ± 0.2	**b**
Leaf life span	*months*	16 ± 2.6	**a**	24 ± 2.3	**b**	25 ± 2.3	**b**
Leaf biomass	*Mg ha^{-1}*	6.8	**a**	9.7	**b**	3.6	**c**
Leaf biomass N pool	*kg ha^{-1}*	123		202		46	
Leaf biomass P pool	*kg ha^{-1}*	3.8		7.9		1.6	

Methods

Leaf gas exchange was measured between February and May 2009 on each three replicate leaves of the 41 trees (40 species; 123 leaves in total) using a portable IRGA system (LI-6400, LI-COR Biosciences, Lincoln, NE, USA) equipped with a LED red/blue light source (type 6400-02B). All measurements were carried out on sunny or over-cast days between 10:00 a.m. and 4:00 p.m. on intact fully expanded leaves of most distal insertion. The branches were part of the lateral canopy with exposure to full sunlight. For every leaf, a light and a CO_2 response curve was recorded. The temperature simulated in the cuvette was set to the air temperature found to be typical for the measurement time at the respective study site. The water vapour

saturation deficit in the cuvette was held constant at the respective ambient conditions of the three sites. The photosynthetic light response was determined at photon flux densities of 1500, 1000, 500, 200, 100, 50, 20 and 0 µmol m^{-2} s^{-1} starting at highest irradiance. In this measuring task, the CO_2/air mixing ratio was held constant at 370 ppm. The photosynthetic CO_2 response was measured under light saturation (1500 µmol photons m^{-2} s^{-1}) at CO_2 mixing ratios of 2000, 1300, 700, 360, 200, 100, 50 and 0 ppm starting at the highest ratio. The CO_2 release recorded in the dark at the end of the CO_2 response curve was assumed to give an estimate of leaf dark respiration (R_D). Prior to respiration measurement, the leaves were allowed to acclimate for 2-5 min to the dark in the cuvette.

A tracer experiment using the stable isotopes ^{15}N and ^{13}C was conducted with six tree species (each two per site) that were considered to be representative for the sites according to their relatively high local abundance: *Pouteria torta* (Mart.) Radlk. (Sapotaceae) and *Hedyosmum sprucei* Solms (Chloranthaceae, 1000 m asl.), *Myrcia* sp. nov (undescribed species, Myrtaceae) and *Hedyosmum translucidum* Cuatrec. (Chloranthaceae, 2000 m), *Graffenrieda harlingii* Wurdack (Melastomataceae), and *Hedyosmum purpurascens* Todzia (Chloranthaceae, 3000 m). Four to six months before the start of the experiment, saplings of all species were collected from the three stands and planted into plastic pots.

The cultivation pots of 25 cm diameter and 25 cm height were filled with local forest soil of the sites where the saplings had been collected. We used soil from 10-30 cm mineral soil depth in patches of undisturbed primary forest. The pots with each one sapling growing in it were placed on wooden tables at the three study sites in the interior of the local stands under a closed forest canopy.

For every tree species, four treatments with three- to fivefold replication were established: (1) control, (2) addition of labelled nitrate ($NH_4{}^{15}NO_3$, 98 atom-%), (3) addition of labelled ammonium ($^{15}NH_4NO_3$; 98 atom-%), and (4) addition of ^{15}N ^{13}C double-labelled glycine ($H_2{}^{15}N{}^{13}CH_2{}^{13}CO_2H$; 98 atom-%).

All investigated plants were harvested either five days after nutrient application (four species), or two, five and eight days after application (two species) to document the temporal course of ^{15}N acquisition in plant biomass.

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CHAPTER 2

Altitudinal change in the photosynthetic capacity of tropical trees: A case study from Ecuador and a pantropical literature analysis

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Abstract

In tropical mountains, trees are the dominant life form from sea level to more than 4000 m altitude under highly variable thermal conditions (range of mean annual temperatures: <8 to >28 °C). How light-saturated net photosynthesis of tropical trees adapts to variation in temperature, atmospheric CO_2 concentration and further environmental factors, that change along elevation gradients, is not precisely known. With gas exchange measurements in mature trees, we determined light-saturated net photosynthesis at ambient temperature (T) and [CO_2] (A_{sat}) of 40 tree species from 21 families in tropical mountain forests at 1000, 2000 and 3000 m elevation in southern Ecuador. We tested the hypothesis that stand-level averages of A_{sat} and leaf dark respiration (R_D) per leaf area remain constant with elevation. Stand-level means of A_{sat} were 8.8, 11.3 and 7.2 µmol CO_2 m^{-2} s^{-1}; those of R_D 0.8, 0.6 and 0.7 µmol CO_2 m^{-2} s^{-1} at 1000, 2000 and 3000 m elevation, respectively, with no significant altitudinal trend. We obtained coefficients of among-species variation in A_{sat} and R_D of 20 - 53% (*n* = 10 - 16 tree species per stand). Examining our data in the context of a pan-tropical A_{sat} data base for mature tropical trees (c. 170 species from 18 sites at variable elevation) revealed that area-based A_{sat} decreases in tropical mountains by, on average, 1.3 µmol CO_2 m^{-2} s^{-1} per km altitude increase (or by 0.2 µmol CO_2 m^{-2} s^{-1} per K temperature decrease). The A_{sat} decrease occurred despite an increase in leaf mass per area with altitude. Local geological and soil fertility conditions and related foliar N and P concentrations considerably influenced the altitudinal A_{sat} patterns. We conclude that elevation is an important influencing factor of the photosynthetic activity of tropical trees. Lowered A_{sat} together with a reduced stand leaf area decrease canopy C gain with elevation in tropical mountains.

Key words: altitudinal gradient, foliar N, foliar P, leaf dark respiration, light-saturated net photosynthesis, tropical lowland forests, mature trees, C source limitation, tropical montane forests

Introduction

With an estimated total of 37,000 woody plants (Odegaard 2000), tropical forests possess not only by far more tree species, but also exist under a broader spectrum of environmental conditions, than any other biome on earth. Moist forests once stretched from sea level to the alpine tree line at 3500 to 4800 m a.s.l., forming closed stands under a very broad range of mean annual temperatures (MAT; >28 to <8 °C), rainfall totals (from c. 2000 to >8000 mm yr^{-1}) and soil fertility conditions (very low to high fertility; Whitmore 1998, Ghazoul and Sheil 2010). As a consequence, tropical forests exhibit large changes in structure, physiognomy and species composition as one ascends from the lowlands to high elevation. Tropical mountain forests (TMFs) replace lowland forests at ~1000 m elevation, where the climate becomes cooler and often moister, and radiation is frequently reduced due to cloudiness (Hamilton and others 1995, Bruijnzeel and others 2010). Other influential environmental factors that change with altitude are air pressure and the atmospheric concentrations of CO_2 and O_2, and UV-B radiation. In many altitudinal transects in tropical mountains, soil moisture tends to increase and the plant availability of nutrients, in particular of nitrogen and phosphorus, to decrease with altitude (Soethe and others 2008, Benner and others 2010, Bruijnzeel and others 2010, Moser and others 2008).

Tropical trees seem to respond to altitudinal gradients by modifications in plant form and function, notably adaptation in leaf morphology and physiology, tree stature, carbon allocation patterns and productivity (e.g. Cordell and others 1999; Moser and others 2007, 2008, 2011) but the within-species variation in morphological and functional traits in response to increasing elevation is not well studied. More information exists about community level changes in tree stature, leaf form and function and forest productivity along elevation transects in tropical mountains which result from species turnover along the slope. One of the most obvious changes is the reduction in tree size (Liebermann and others 1996; Raich and others 1997; Aiba and Kitayama 1999; Pollmann and Hildebrand 2005; Shi and others 2008), which is accompanied by a reduction in aboveground NPP from tropical lowland to upper

montane forests (Hawaii: Raich and others 1997; Sabah, Malaysia: Kitayama and Aiba 2002; Puerto Rico: Weaver and Murphy 1990, Wang and others 2003; Peru: Girardin and others 2010; Ecuador: Moser and others 2011, Leuschner et al., in press). One of the possible underlying causes is the temperature decrease, but N limitation of tree growth may also be involved in certain mountains where a decrease of foliar N concentration and an increase in leaf longevity with elevation was found (e.g. Tanner and others 1998; Letts and Mulligan 2005, Moser and others 2010). Grubb and Tanner (1976) and Grubb (1977) identified smaller and thicker leaves with lower N concentrations as being characteristic for the trees at high elevations in tropical mountains.

Leaf level photosynthesis, the process that defines canopy carbon gain, has only rarely been investigated in TMFs (e.g. Hikosaka et al. 2002, Rada and others 2009, van de Weg and others 2012). In general, we know more about the photosynthetic activity of trees at the alpine tree line and their carbon relations than about altitudinal change in tree photosynthetic capacity in mountain forests, both in tropical and temperate regions (e.g. Rada and others. 1996, Hoch and others 2002; Körner 2003; Smith and others 2009). However, examining how light-saturated net photosynthesis (A_{sat}) and canopy C gain change along mountain slopes from lowland to upper montane elevation is crucial for a better understanding of the long-term tree adaptation of trees to changes in temperature, atmospheric CO_2 concentration and other abiotic factors, and it may also help to answer to the question about the causes of tropical alpine tree lines.

Altitudinal change in A_{sat} has been investigated in a few temperate and subtropical mountains (e.g. Benecke and others 1981; Zhang and others 2005; Premoli and Brewer 2007; Wieser and Tausz 2007; Bresson and others 2009) showing either no change (Benecke and others 1981; Wieser and Tausz 2007; Bresson and others 2009; Wieser and others 2010), an increase (Premoli and Brewer 2007), or a decrease with increasing altitude (Slayter and Morrow 1977; Zhang and others 2005). Thus, no consistent pattern has yet been detected. Even less is known about altitudinal change in the A_{sat} of tropical trees. A notable exception is the altitudinal

transect study in *Metrosideros polymorpha* in the tropical island forests of Hawaii between 100 and 2500 m a.s.l. (Cordell and others 1998, 1999).

In this transect study in southern Ecuadorian TMFs, we examined patterns of altitudinal variation in light-saturated net photosynthesis (A_{sat}) of mature (or pre-mature) trees along a transect from 1000 to 3000 m a.s.l. covering a large number of tree species (40). We further explored the possible dependence of A_{sat} on foliar nutrient concentration and leaf morphology along the elevation gradient. Complementary to this field study, we conducted a pan-tropical literature survey of published photosynthesis data from mature tropical trees at contrasting elevation (lowland to upper montane; 12 studies from 18 sites covering almost 170 tree species, excluding seedling and sapling studies). The main objectives of the study were (i) to clarify the position of tropical montane forest trees relative to tropical lowland, subtropical and temperate trees in terms of leaf form and function, and (ii) to search for a significant temperature and altitude dependence of A_{sat} and R_D in tropical trees. We tested the hypotheses that (1) stand-level averages of mass-based A_{sat} are decreasing with elevation due to decreases in foliar N and P, while (2) area-based A_{sat} remains unchanged because of the LMA increase with altitude. We also hypothesized that (3) area-based R_D does not change with elevation.

Methods

Study sites and selection of trees

The measurements were conducted along a 2000-m elevation transect in tropical mountain forests on the eastern slope of the southern Ecuadorian Andes between February and May 2009. The study sites were located at ca. 1000, 2000 and 3000 m a.s.l. in the Podocarpus National Park and the Reserva Biológica San Francisco in the Provinces of Loja and Zamora-Chinchipe. The maximum distance between the sites was 30 km (Figure 1).

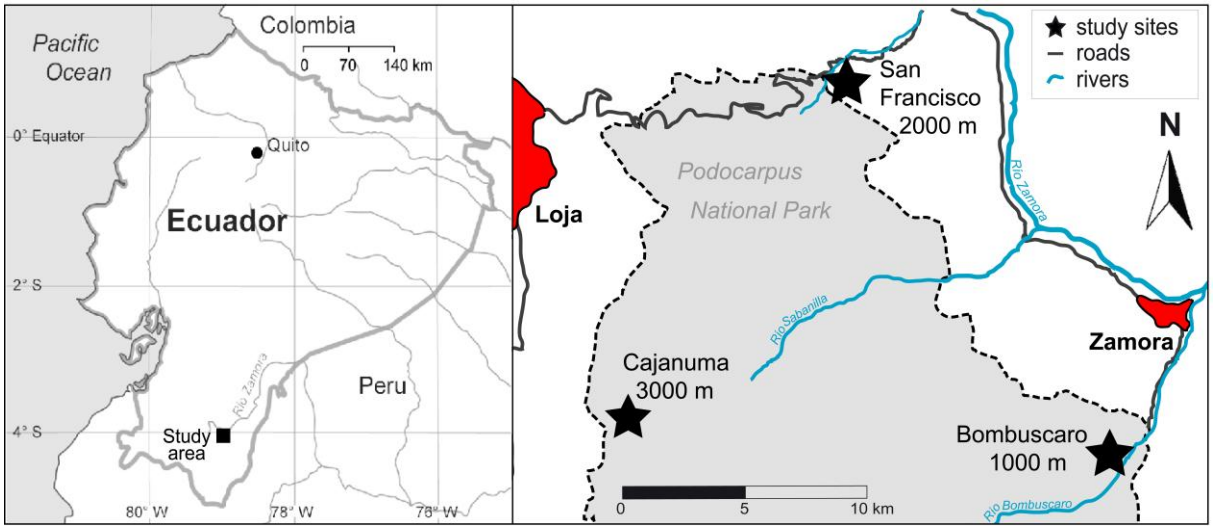

***Figure 1**. Location of the study area in southern Ecuador with the three stands at 1000, 2000 and 3000 m a.s.l.*

The research area has a tropical humid climate with an extremely wet season from April to July and a less humid period from September to December (Bendix and others 2006). Regularly occurring longer dry periods do not exist. Further details on the climatic conditions at the study sites are given in Table 1.

All three stands are located in protected forest sections. Natural disturbances in the past may have included landslides in the steeply sloped terrain. The three elevations along the gradient correspond to three different forest types (Homeier and others 2008): (1) At 1000 m (S 4° 7´ W 78° 58´), in the transition zone between tropical lowland and lower montane forest, we find evergreen premontane forest whose trees attain heights of up to 40 m. Common tree families of this forest type are Fabaceae, Melastomataceae, Moraceae, Myristicaceae, Rubiaceae and Sapotaceae. In this forest, we selected one tree individual per species, totaling to 15 species, at elevations between 950 and 1050 m a.s.l. Ten species were identified to the species level (see Table 3), the remaining to the genus level. (2) The evergreen lower montane forest at 2000 m (S 3° 58' W 79° 04') achieves a canopy height of 18 to 22 m. Characteristic tree families are Euphorbiaceae, Lauraceae, Melastomataceae and

Rubiaceae. Sixteen tree species were investigated at elevations between 1800 and 1900 m a.s.l. Fourteen of the sixteen species could be identified to the species level. (3) The evergreen elfin-forest at 3000 m (S 4° 7´ W 79° 11´) extends up to the tree line, and the canopy height is rarely higher than 8 to 10 m. Dominant tree families are Aquifoliaceae, Clusiaceae, Cunionaceae, Lauraceae and Melastomataceae. Ten tree species were investigated at elevations between 2850 and 3000 m a.s.l. situated about 100 - 200 m below the tree line; nine were identified to the species level. Stand structural characteristics of the three stands are summarized in Table 2.

In the three stands, photosynthesis measurements were conducted on a total of 41 trees representing 40 different species (in one species, *Clethra revoluta*, we selected one individual at 2000 m and at 3000 m). Only medium to tall trees with a minimum breast height diameter (dbh) of 10 cm were investigated (the mean dbh of the sampled trees was 16 ± 2 cm at 1000 m, 19 ± 2 cm at 2000 m, and 12 ± 1 cm at 3000 m). Tree size of the measured trees was 10 - 20 m at 1000 m, 8 - 15 m at 2000 m and 4 - 12 m at 3000 m. To gain access to the sun-lit parts of the tree canopies, we selected tree individuals in the forest that grew on the steep slope beneath walking paths or below ribs on the slope, so that part of the canopy exposed to the sun could be approached from the ground.

We compiled data on the geographical distribution of the sampled families and the known altitudinal distribution of the investigated species using the online databases established by Stevens (2008) and the Missouri Botanical Gardens (2010). We used APG III (2009) for family classification.

Table 1. Climatic and edaphic characteristics of the sampled stands (data from Moser and others (2007) and Wolf and others, unpubl.). Mean annual air temperature and relative air humidity were measured at 1.5 m height inside the stands at 1050, 1890 and 3060 m. Rainfall data are extrapolated from measurements in a forest gap at approximately 1050 m (measuring period May 2003 – May 2004), and from measurements in gaps at 1950, and 3170 m performed by P. Emck (3-year means, unpublished). [CO_2] is the CO_2 concentration of the air above the boundary layer as estimated from air pressure and by assuming a constant mixing ratio of 370 µmol CO_2 mol air^{-1} along the slope. C/N ratio, available phosphorus (P_{av}) and net N mineralization rate (*in situ* buried bag method) refer to the topsoil (0-10 cm, after K. Wolf, unpubl.). Available P was determined by the modified Hedley fractionation (extraction with anion exchange resins combined with $NaHCO_3$ percolation). For the edaphic parameters, means ± SE of 4 soil profiles dug at midslope position in the stands are given.

Elevation	*m asl*	**1000**		**2000**		**3000**	
Rainfall	*mm* yr^{-1}	c. 2230		c. 1950		c. 4500	
Air temperature	*°C*						
Mean		19		16		9	
Max		30		29		19	
Min		12		8		3	
Air humidity	*%*						
Mean		86		91		94	
Max		100		100		100	
Min		16		29		29	
[CO_2]	*Pa*	33		30		27	
pH (H_2O)		4.3 ± 0.6	**a**	4.8 ± 0.5	**a**	3.7 ± 0.1	**a**
C/N	*g* g^{-1}	19.0 ± 2.5	**ab**	15.6 ± 0.6	**a**	23.9 ± 1.4	**b**
P_{av}	*kg* ha^{-1}	11.8 ± 5.3	**a**	12.5 ± 4.9	**a**	5.1 ± 0.6	**a**
Net N mineralization	*kg N* ha^{-1} $10d^{-1}$	4.0 ± 1.6	**a**	1.4 ± 0.7	**ab**	0.6 ± 0.4	**b**

Table 2. Structural characteristics of the stands at 1000, 2000 and 3000 m (Homeier and others, unpublished; LAI, leaf lifespan, BGB and leaf biomass: Moser and others, 2007; 2011). Presented are means ± 1 SE for the three elevations (except for BGB). Abbreviations: AGB, aboveground biomass; BGB, belowground biomass (coarse and fine roots); DBH, diameter at breast height; LAI, leaf area index. Means of DBH, stem density, basal area and AGB were calculated for 9-18 permanent plots (400 m^2 each) covering the whole range of topographic positions at the respective elevations (trees > 10cm DBH).

Elevation	Canopy height	DBH	Stem density	Basal area	LAI	Leaf lifespan	AGB	BGB
m asl	*m*	*cm*	*n ha^{-1}*	*m^2 ha^{-1}*	*m^2 m^{-2}*	*months*	*Mg ha^{-1}*	*Mg ha^{-1}*
1000	25-30	19 ± 1 **a**	822 ± 50 **a**	29 ± 4 **a**	6.0 ± 0.4 **a**	16 ± 3 **a**	177 ± 28 **a**	32.1
2000	16-20	20 ± 1 **a**	900 ± 62 **a**	34 ± 3 **a**	5.7 ± 0.5 **a**	24 ± 2 **b**	158 ± 22 **a**	26.1
3000	8-10	18 ± 1 **a**	1061 ± 84 **a**	30 ± 3 **a**	2.2 ± 0.2 **b**	25 ± 2 **b**	89 ± 10 **b**	62.8

Photosynthesis measurements

Light-saturated net photosynthesis A_{sat} was measured on about 50 days between February and May 2009 with a portable IRGA system (LI-6400, LI-COR Biosciences, Lincoln, NE, USA) with a standard leaf chamber equipped with a LED red/blue light source (type 6400-02B). All measurements were performed between 10:00 a.m. and 4:00 p.m. Three fully expanded leaves of most distal insertion on intact twigs exposed to full sunlight were investigated per tree. Thus, every species was represented by one tree individual and three leaves. Light-saturated net photosynthesis was determined at a photon flux density of 1500 µmol m^{-2} s^{-1} under ambient CO_2 concentration and temperature. Before starting the measurement cycle of a light response curve, the leaves were exposed to high irradiance (1500 µmol m^{-2} s^{-1}) until apparent photosynthesis was stable (CV ≤ 10%); this was achieved after 5-20 min. In

the subsequently measured light levels, the gas exchange system was programmed to 5 min for stable values before taking a reading. The measurements were conducted at mixing ratios of 370 ppm CO_2 at all sites and at cuvette temperatures of 22 - 24 °C at 1000 m, 19 – 21 °C at 2000 m and 15 - 17 °C at 3000 m to simulate the typical local sun canopy temperatures on a sunny day at noon (based on temperature measurements by Rollenbeck and Peters, unpublished data). The vapor pressure deficit was held constant at ambient conditions at the three sites. The CO_2 release in the dark was used as an estimate of leaf dark respiration (R_D). Prior to R_D measurement, the intact leaves on the branches were allowed to acclimate to the dark in the chamber for 2 - 5 min. At the beginning of the measuring campaign, the CO_2 analyzer was calibrated against a gas standard of 400 ppm CO_2 in N_2. The IRGA channels were matched before each measurement. We did not check the respiration data for the possible occurrence of post-illumination burst effects; however, our R_D data compare well with leaf dark respiration rates reported from other neo- and paleotropical tree species (e.g. Eschenbach and others 1998, Carswell and others 2000, Kenzo and others 2004, Meir and others 2007).

Morphological and chemical leaf traits

All investigated leaves were harvested for analysis of foliar N and P concentrations. Total concentrations of foliar N were determined with a C/N elemental analyzer (Vario EL III, Elementar, Hanau, Germany). Total P concentrations were analyzed using an Inductively Coupled Plasma Analyzer (Optima 5300DV ICP-OES, Perkin Elmer, Waltham, Massachusetts, USA) after digesting the samples with concentrated HNO_3.

Data analysis

The relationship between net photosynthesis rate and PPFD was described with a non-rectangular hyperbolic function; 90% of the CO_2 assimilation rate at 1500 µmol

photons m^{-2} s^{-1} was taken as A_{sat}. The initial slope of the light response curve was used to calculate the apparent quantum yield of CO_2 assimilation (α). The data were analyzed at the stand level by pooling the 10 to 16 species of a study site. Analysis of variance (Scheffé`s test) was used to conduct multiple comparisons among the means of the three stands. If the data was not normally distributed according to a Shapiro-Wilk test, the Mann–Whitney two-sample test (Wilcoxon U test) was used instead of Scheffé`s test. All calculations were conducted with SAS software (version 9.1; SAS Institute, Cary, NC, USA). A significance level of 5% was used throughout the analyses.

Pan-tropical literature survey

A literature survey was conducted to compile a data base of A_{sat} values of trees from all over the tropics covering sites at variable altitudes from lowland to upper montane elevation (see Table 5 in the Appendix). This data base covers 9 studies (including the present one) with 157 tree species in 16 stands located at elevations between 100 and 3050 m. Only measurements referring to mature or pre-mature tree individuals of non-pioneer stands were considered. Studies referring to seedlings or saplings were excluded. All study sites are located in moist tropical forests with >1800 mm rainfall yr^{-1}. A_{sat} values obtained at irradiances <1200 µmol photons m^{-2} s^{-1} were only included if that flux density was identified as being saturating. If information was available, only data referring to sun-lit, fully expanded leaves of the upper canopy were included. Wet season data were given preference over dry season data if both were available.

Results

Among the 40 tree species from 21 families investigated, light-saturated net photosynthesis at ambient temperature and [CO_2] conditions (A_{sat}) varied in a broad

range from 2.1 to 12.9 µmol CO_2 m^{-2} s^{-1} (Table 3, lowest value in *Ilex teratopis* (3000 m), highest value in *Tibouchina ochipetala*, 1000 m). A_{sat} varied from 3.4 to 16.0 µmol m^{-2} s^{-1} in the stand at 1000 m (15 species), from 7.7 to 15.4 µmol m^{-2} s^{-1} at 2000 m (16 species), and from 2.6 to 10.3 µmol m^{-2} s^{-1} in the uppermost stand at 3000 m (10 species). We obtained coefficients of variation (standard deviation expressed as percent of mean) for the species collectives investigated of 36% (1000 m), 20% (2000 m) and 38% (3000 m) for the three stands.

We found no evidence in support of the assumption that trees from families restricted to the Tropics generally had higher A_{sat} values than members from families with tropical and extratropical distribution range. The 21 families investigated (each represented with 1 to 6 species in the study) are arranged in Fig. 2 according to their mean photosynthetic capacity. Relatively high A_{sat} values were determined in members from the Clethraceae, Rubiaceae, Siparunaceae and Anonaceae; low rates, in trees from the Clusiaceae, Nyctaginaceae, Sapotaceae and Aquifoliceae.

In our species sample from southern Ecuador, we found no clear elevational trend in A_{sat} with stand means of 8.8 (1000 m), 11.3 (2000 m) and 7.2 µmol CO_2 m^{-2} s^{-1} (3000 m). However, the 16 species of the montane stand at 2000 m had on average significantly higher photosynthetic capacities than the trees at 1000 or 3000 m (Table 3). This is also visible in Fig. 2 when comparing the species of a family at 2000 m (triangles) with the members from 1000 or 3000 m (squares and circles).

We found apparent quantum yields (α, the initial slope of the light response curve under ambient T and [CO_2]) in the range of 0.037 – 0.080 mol CO_2 mol quanta $^{-1}$ for the 40 species (Table 3). The tree samples from the stands at 1000, 2000 and 3000 m were not significantly different with respect to their α means.

Leaf dark respiration (R_D) at ambient temperature varied between 0.25 and 1.52 µmol CO_2 m^{-2} s^{-1} with most species means ranging between 0.4 and 0.9 µmol m^{-2} s^{-1} (Table 3). The stand means of R_D did not differ significantly at 1000, 2000 and 3000 m a.s.l.

Table 3. Light-saturated net photosynthesis of sun leaves measured at ambient temperature and [CO_2] (A_{sat}), apparent quantum yield of photosynthesis (α), leaf dark respiration (R_D), foliar nitrogen (N_m) and phosphorus concentrations (P_m) (mass-based), leaf mass per area (LMA) and leaf conductance at light saturation (mean and maximum values, g_{mean} and g_{max}) of 40 tree species at three elevations. c = Canopy species, s = subcanopy species. Presented are means ± SE for three leaves each per species (species level) and means ± SE over all species of a stand. Significantly different stand level means (three elevations) are indicated by different letters ($p < 0.05$).

		A_{sat}	α	R_D	N_m	P_m	LMA	g_{mean}	g_{max}
		µmol m^{-2} s^{-1}	*mol mol^{-1}*	*µmol m^{-2} s^{-1}*	*mg g^{-1}*	*mg g^{-1}*	*g m^{-2}*	*mol m^{-2} s^{-1}*	*mol m^{-2} s^{-1}*
1000 m asl (n = 15)									
Saurauia spec.1	s	11.0 ± 0.8	0.054 ± 0.001	0.47 ± 0.15	18	0.8	102 ± 5	0.16 ± 0.01	0.22
Guatteria pastazae	c	11.3 ± 0.3	0.066 ± 0.001	0.77 ± 0.04	18	0.5	137 ± 3	0.13 ± 0.01	0.33
Hedyosmum sprucei	s	12.0 ± 0.5	0.080 ± 0.007	1.33 ± 0.16	18	0.8	79 ± 2	0.08 ± 0.01	0.12
Inga spec.	c	9.4 ± 2.5	0.060 ± 0.001	0.95 ± 0.12	26	0.7	119 ± 3	0.08 ± 0.02	0.20
Lozania klugii	s	9.4 ± 0.3	0.070 ± 0.002	0.66 ± 0.05	19	0.5	86 ± 2	0.17 ± 0.01	0.19
Licaria cf *terminalis*	c	6.1 ± 1.0	0.054 ± 0.002	0.46 ± 0.06	26	0.5	94 ± 1	0.08 ± 0.02	0.13
Mollia cf gracilis	c	5.4 ± 0.4	0.054 ± 0.003	0.56 ± 0.01	21	0.6	58 ± 1	0.14 ± 0.01	0.18
Centronia laurifolia	s	7.9 ± 0.7	0.070 ± 0.001	0.58 ± 0.06	10	0.2	138 ± 4	0.08 ± 0.01	0.11
Miconia spec.	s	6.9 ± 0.5	0.058 ± 0.006	0.51 ± 0.15	14	0.4	166 ± 10	0.11 ± 0.01	0.14
Tibouchina ochipetala	s	16.0 ± 1.0	0.063 ± 0.002	1.05 ± 0.05	16	0.5		0.20 ± 0.02	0.29
Ficus cervantesiana	c	7.3 ± 0.6	0.060 ± 0.006	0.82 ± 0.08	13	0.6	155 ± 18	0.11 ± 0.02	0.15
Ficus spec.	c	8.5 ± 0.6	0.059 ± 0.001	1.06 ± 0.13	11	0.5		0.19 ± 0.01	0.21
Neea cf *divaricata*	s	6.0 ± 0.6	0.072 ± 0.003	0.67 ± 0.08	42	0.7	73 ± 1	0.06 ± 0.01	0.09
Cinchona spec.	c	11.1 ± 0.5	0.062 ± 0.002	1.03 ± 0.07	14	0.4	120 ± 4	0.11 ± 0.02	0.21
Pouteria torta	c	3.4 ± 1.7	0.054 ± 0.009	0.35 ± 0.03	14	0.4	103 ± 9	0.05 ± 0.02	0.07
Mean		**8.8 ± 0.8** [a]	**0.062 ± 0.002** [a]	**0.75 ± 0.07** [a]	**19 ± 2** [a]	**0.5 ± 0.04** [a]	**110 ± 6** [a]	**0.12 ± 0.01**	

		A_{sat}	α	R_D	N_m	P_m	LMA	g_{mean}	g_{max}
		$\mu mol\ m^{-2}\ s^{-1}$	$mol\ mol^{-1}$	$\mu mol\ m^{-2}\ s^{-1}$	$mg\ g^{-1}$	$mg\ g^{-1}$	$g\ m^{-2}$	$mol\ m^{-2}\ s^{-1}$	$mol\ m^{-2}\ s^{-1}$
2000 m asl (n = 16)									
Saurauia spec.2	s	8.6 ± 1.3	0.051 ± 0.003	0.41 ± 0.13	20	1.2	121 ± 6	0.12 ± 0.01	0.15
Piptocoma discolor	c	12.8 ± 1.0	0.063 ± 0.003	1.52 ± 0.47	26	1.9	78 ± 9	0.12 ± 0.00	0.17
Critoniopsis zamorensis	c	8.4 ± 0.8	0.071 ± 0.003	0.73 ± 0.18	23	1.9	74 ± 9	0.06 ± 0.01	0.11
Clethra revoluta	c	13.0 ± 1.6	0.052 ± 0.004	0.37 ± 0.08	13	0.5	196 ± 2	0.16 ± 0.00	0.37
Vismia cf *tomentosa*	c	12.2 ± 0.4	0.059 ± 0.003	0.41 ± 0.05	15	0.9	168 ± 2	0.14 ± 0.01	0.19
Aniba spec.	c	12.1 ± 1.3	0.059 ± 0.005	0.32 ± 0.04	17	1.2	135 ± 3	0.22 ± 0.00	0.30
Rhodostemonodaphne kunthiana	c	11.4 ± 1.0	0.071 ± 0.002	0.28 ± 0.10	26	0.9	137 ± 4	0.13 ± 0.08	0.44
Heliocarpus americanus	c	14.7 ± 1.0	0.064 ± 0.001	1.10 ± 0.20	33	2.5	59 ± 5	0.17 ± 0.02	0.27
Meriania hexamera	c	8.5 ± 1.3	0.058 ± 0.008	0.32 ± 0.07	18	0.8	121 ± 6	0.14 ± 0.02	0.23
Tibouchina lepidota	s	15.4 ± 1.2	0.054 ± 0.002	0.48 ± 0.06	17	0.6	144 ± 2	0.18 ± 0.03	0.25
Ficus pertusa	c	11.3 ± 1.6	0.058 ± 0.001	0.44 ± 0.05	19	1.1	119 ± 6	0.11 ± 0.01	0.14
Ficus citrifolia	c	10.1 ± 1.8	0.064 ± 0.007	0.78 ± 0.15	21	0.9	146 ± 2	0.05 ± 0.01	0.12
Isertia laevis	c	13.4 ± 2.1	0.048 ± 0.004	0.62 ± 0.22	16	0.8	215 ± 5	0.13 ± 0.02	0.20
Ladenbergia acutifolia	c	9.8 ± 2.0	0.071 ± 0.007	0.68 ± 0.33	18	0.8	98 ± 9	0.18 ± 0.07	0.45
Siparuna aspera	s	11.4 ± 1.7	0.054 ± 0.002	0.60 ± 0.05	29	1.5	96 ± 7	0.17 ± 0.04	0.51
Cecropia andina	c	7.7 ± 1.6	0.057 ± 0.002	0.57 ± 0.02	18	0.7	126 ± 1	0.08 ± 0.01	0.10
Mean		**11.3 ± 0.6** ^{b}	**0.060 ± 0.002** ^{a}	**0.60 ± 0.08** ^{a}	**21 ± 2** ^{a}	**1.1 ± 0.15** ^{b}	**127 ± 6** ^{a}	**0.13 ± 0.01**	
3000 m asl (n = 10)									
Ilex teratopis	c	2.6 ± 0.2	0.054 ± 0.008	0.25 ± 0.15	9	0.2	326 ± 1	0.03 ± 0.00	0.04
Hedyosmum purpurascens	c	7.5 ± 1.0	0.064 ± 0.005	0.45 ± 0.08	16	0.5	140 ± 6	0.07 ± 0.01	0.11
Clethra revoluta	c	10.0 ± 0.4	0.050 ± 0.002	0.54 ± 0.04	16	0.7	171 ± 6	0.41 ± 0.09	1.11
Clusia alata	c	4.4 ± 1.2	0.059 ± 0.020	1.09 ± 0.06	10	0.5	286 ± 5	0.03 ± 0.02	0.06

		A_{sat}	α	R_D	N_m	P_m	LMA	g_{mean}	g_{max}
		µmol m^{-2} s^{-1}	*mol mol^{-1}*	*µmol m^{-2} s^{-1}*	*mg g^{-1}*	*mg g^{-1}*	*g m^{-2}*	*mol m^{-2} s^{-1}*	*mol m^{-2} s^{-1}*
Clusia elliptica	c	5.8 ± 1.5	0.068 ± 0.002	0.77 ± 0.19	13	0.6	164 ± 7	0.05 ± 0.01	0.08
Weinmannia pubescens	c	8.8 ± 1.1	0.058 ± 0.004	0.76 ± 0.11	13	0.5	113 ± 4	0.10 ± 0.01	0.17
Persea ferruginea	c	8.0 ± 1.2	0.038 ± 0.005	0.67 ± 0.17	10	0.4	487 ± 9	0.08 ± 0.00	0.09
Miconia spec.	c	4.6 ± 1.3	0.071 ± 0.001	0.56 ± 0.22	20	0.7	98 ± 2	0.06 ± 0.01	0.09
Myrica pubescens	c	10.3 ± 0.6	0.064 ± 0.005	0.74 ± 0.29	22	0.5	156 ± 5	0.11 ± 0.01	0.33
Styrax foveolaria	s	10.2 ± 0.9	0.044 ± 0.002	0.78 ± 0.04	10	0.4	304 ± 2	0.11 ± 0.03	0.27
Mean		**7.2 ± 0.9** [a]	**0.057 ± 0.003** [a]	**0.66 ± 0.07** [a]	**14 ± 1** [b]	**0.5 ± 0.05** [a]	**216 ± 19** [b]	**0.11 ± 0.04**	

Discussion

Among-species variation in A_{sat} in species-rich tropical forests

This study focuses on the photosynthetic capacity of mature trees with a size of at least 4 m (uppermost stand), typically 8 to 20 m, in order to examine the maximum assimilation rate of sun-canopy leaves in their natural position in the canopy. We excluded seedling and sapling studies because leaf physiology may be subject to a considerable ontogenetic shift with tree ageing (e.g. Thomas and Winner 2002, Mediavilla and Escudero 2003). Measurements conducted in nearly 170 species in about 18 stands in the Americas, South-east Asia and Africa met the criteria defined in this study (see Table 5 in the Appendix).

In terms of species numbers, our study is the most comprehensive investigation conducted so far on the leaf-level photosynthesis of tropical trees; nevertheless, 170 species examined from a pool of ~37.000 taxa is still a tiny number. Similarly, our in-depth study on the 40 species of the Ecuadorian TMFs represents only c. 5% of the 800 or so tree species in this study area (J. Homeier, unpubl. data). Thus, the two species samples investigated may well be not representative neither for the local nor the pantropical tree flora. The among-species variation in A_{sat} in the three Ecuadorian stands was indeed considerable (coefficients of variation of 20 to 38%). Further bias may have been added to our data sets by the fact that we were not able to conduct a random selection of species or to choose them according to dominance in the three stands. Rather, species selection in Ecuador was confined to tree individuals growing on steep slopes because this allowed us to access part of their sun canopies with gas exchange equipment. This procedure guaranteed that the measured leaves were exposed to full sunlight at least part of the day and thus must have possessed partial or full sun leaf adaptation. The selection of tree species based on extensive knowledge of the ecology and distribution of the taxa in the Loja region of South Ecuador ; we chose only typical members of the closed natural forest and excluded shade-intolerant pioneer tree species. Furthermore, the phylogenetic diversity

covered by our species sample was relatively large, including taxa from 21 families in the whole study, or 10 to 16 species from 9-12 families in each of the three stands. However, we cannot rule out the possibility that a larger survey covering additional species and families would lead to different A_{sat} stand averages than the figures reported here for the Ecuadorian and pantropical analysis.

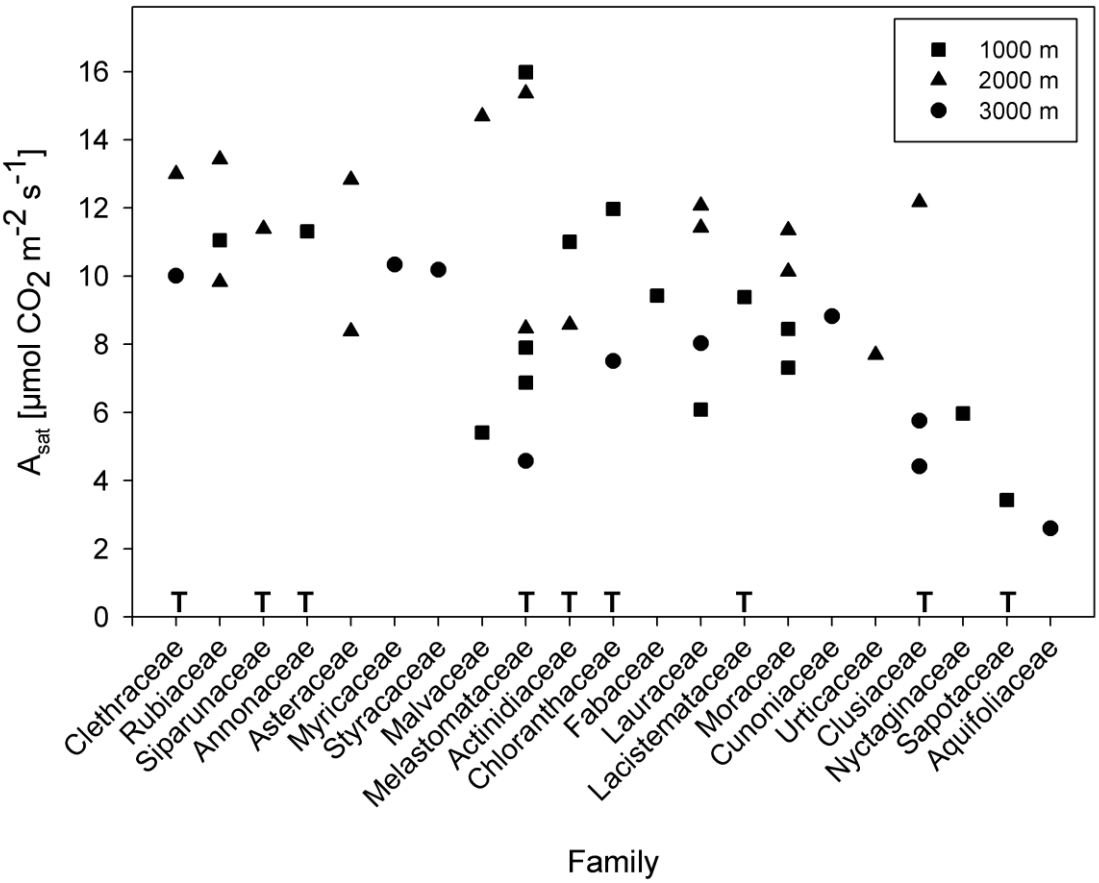

***Figure 2**. Net photosynthesis rate of sun leaves at ambient temperature and [CO_2] (A_{sat}) averaged over all species (morphotypes) of a family at a given elevation. The 21 families are arranged from left to right according to their mean A_{sat} rate. T: families with restriction to the Tropics.*

Constant or decreasing photosynthetic capacity with elevation?

From our 40-species sample in Ecuador, a significant altitudinal trend in photosynthetic capacity at ambient [CO_2] and temperature did not manifest. However, we found a significantly higher A_{sat} stand mean for the mid-elevation stand at 2000 m than at lower and higher elevation, corresponding to higher foliar P and N concentrations at this elevation. Correspondingly, species with relatively high A_{sat} values (>10 µmol CO_2 m^{-2} s^{-1}) occurred mostly in this mid-elevation stand. To our knowledge, our investigation is the first that examined altitudinal changes in A_{sat} in species-rich tropical moist forests by measuring a large number of species in the adult stage and thus accounting for the characteristic species turnover along the slope. A few studies on altitudinal change in photosynthetic performance measured daily means of actual photosynthesis but not A_{sat} (e.g. Cavieres and others 2000) or derived estimates of photosynthetic activity from productivity data (e.g. Kitayama and Aiba 2002). The only other transect study on altitudinal patterns in A_{sat} in mature tropical trees focused on a single, polymorphic tree species (*Metrosideros polymorpha*, Myrtaceae) in the species-poor Hawaiian rainforests (Cordell and others 1999). Similar to our study, these authors found no change in A_{sat} under ambient temperature and [CO_2] conditions between 100 and 2500 m a.s.l. However, A_{sat} varied at unusually low levels (2.3 - 3.8 µmol CO_2 m^{-2} s^{-1}) across the Hawaiian transect. It may well be that this rather unproductive species reflects the specific conditions of isolated island forests, and that the results cannot simply be extrapolated to tropical forests on the continents. Moreover, altitudinal patterns in A_{sat} derived from a single species with wide altitudinal distribution should differ from trends obtained from transect studies that cover a large number of species of different phylogenies, each adapted to the specific site conditions at the respective elevation level.

Table 4. List of the 40 species from 21 families investigated in the three stands at 1000, 2000 and 3000 m a.s.l. in South Ecuador and their altitudinal distribution according to Missouri Botanical Garden (1981). Geographical distribution of the families according to Stevens (2010).

Family	**Geographical distribution**	**1000 m stand**		**2000 m stand**		**3000 m stand**	
		Sampled species	**Altitudinal distribution** *m a.s.l.*	**Sampled species**	**Altitudinal distribution** *m a.s.l.*	**Sampled species**	**Altitudinal distribution** *m a.s.l.*
Actinidiaceae	Largely tropical, not Africa	*Saurauia* spec.1		*Saurauia* spec.2			
Annonaceae	Largely tropical, pantropical	*Guatteria pastazae*	390-2120				
Aquifoliaceae	Cosmopolitan					*Ilex teratopis*	1350-3316
Asteraceae	Cosmopolitan			*Critoniopsis zamorensis*			
				Piptocoma discolor	100-3890		
Chloranthaceae	Tropical and subtropical, not Africa	*Hedyosmum sprucei*	40-3250			*Hedyosmum purpurascens*	2400-3300
Clethraceae	Largely tropical montane or subtropical			*Clethra revoluta*	456-3450	*Clethra revoluta*	456-3450
Clusiaceae	Pantropical			*Vismia cf tomentosa*	100-2615	*Clusia alata*	100-3200
						Clusia elliptica	1900-3200
Cunoniaceae	Largely temperate and tropical, S. hemisphere, few African species					*Weinmannia pubescens*	1350-2800
Fabaceae	Cosmopolitan	*Inga sp.*					
Lacistemataceae	Neotropical	*Lozania klugii*	100-2140				
Lauraceae	Pantropical (temperate)	*Licaria terminalis*	1050-1440	*Aniba* spec.		*Persea ferruginea*	1400-3950

Family	**Geographical distribution**	**1000 m stand** **Sampled species**	**Altitudinal distribution** *m a.s.l.*	**2000 m stand** **Sampled species**	**Altitudinal distribution** *m a.s.l.*	**3000 m stand** **Sampled species**	**Altitudinal distribution** *m a.s.l.*
				Rhodoste monodaphne kunthiana	1-2150		
Malvaceae	Pantropical, also temperate	*Mollia gracilis*	100-910	*Heliocarpus americanus*	0-2615		
Melastomataceae	Largely tropical, also subtropical	*Centronia laurifolia* *Miconia* spec. *Tibouchina ochypetala*	600-2050 140-2900	*Meriania hexamera* *Tibouchina lepidota*	1100-2900 400-3230	*Miconia* spec.	
Moraceae	Mostly tropical to warm temperate	*Ficus cervantesiana* *Ficus* spec.	100-2400	*Ficus citrifolia* *Ficus pertusa*	0-3000 0-2100		
Myricaceae	Cosmopolitan					*Myrica pubescens*	1500-3605
Nyctaginaceae	Pantropical to warm temperate	*Neea divaricata*	45-2500				
Rubiaceae	Cosmopolitan, but especially tropical	*Cinchona* spec.		*Isertia laevis* *Ladenbergia acutifolia*	0-2100 220-1630		
Sapotaceae	Pantropical	*Pouteria torta*	0-2440				
Siparunaceae	Neotropical and W. African			*Siparuna aspera*	30-3300		
Styracaceae	Warm N. temperate to tropical					*Styrax foveolaria*	2400-3430
Urticaceae	Cosmopolitan, but mainly tropical			*Cecropia andina*	1540-2800		

In the absence of other transect studies on gas exchange in tropical mountains, additional information on altitudinal change in A_{sat} is provided by our pan-tropical literature survey (Table 5). The data incorporated in this analysis were selected according to strict criteria with respect to tree size (mature or pre-mature trees only), successional status (no pioneer species), canopy position (sun-exposed branches only), measuring conditions (saturating light, typically >1200 µmol m^{-2} s^{-1} PPFD; ambient temperature and [CO_2]) and climate (tropical moist); this protocol excluded a number of studies on maximum photosynthesis of tropical trees that either worked with saplings, understorey or pioneer trees or apparently used non-saturating irradiances. According to this data compilation, sun leaf-A_{sat} significantly decreases from tropical lowland to upper montane elevation which contradicts our first hypothesis. The linear regression equation indicates a decrease in A_{sat} by about 1.3 µmol CO_2 m^{-2} s^{-1} per km altitude (Fig. 3: left column) or by about 0.20 µmol CO_2 m^{-2} s^{-1} per K air temperature reduction (Fig. 3: centre column). The trees from lowland forests reached average photosynthetic capacities of about 10-11 µmol CO_2 m^{-2} s^{-1}, those of the upper montane forests of about 7 µmol CO_2 m^{-2} s^{-1}. The decrease in area-based A_{sat} coincides not only with the temperature decrease but also with a significant decrease in foliar P while no significant N_m decrease with elevation was detected in the pantropical survey. Not only area-based, but mass-based A_{sat} decreased also from lowland to upper montane elevation ($P = 0.004$; data of Table 5); this indicates that the altitudinal increase in LMA, which occurred between lowland and montane elevation, was on the average not large enough to compensate the mass-based A_{sat} reduction and thus to prevent a decrease in area-based A_{sat}.

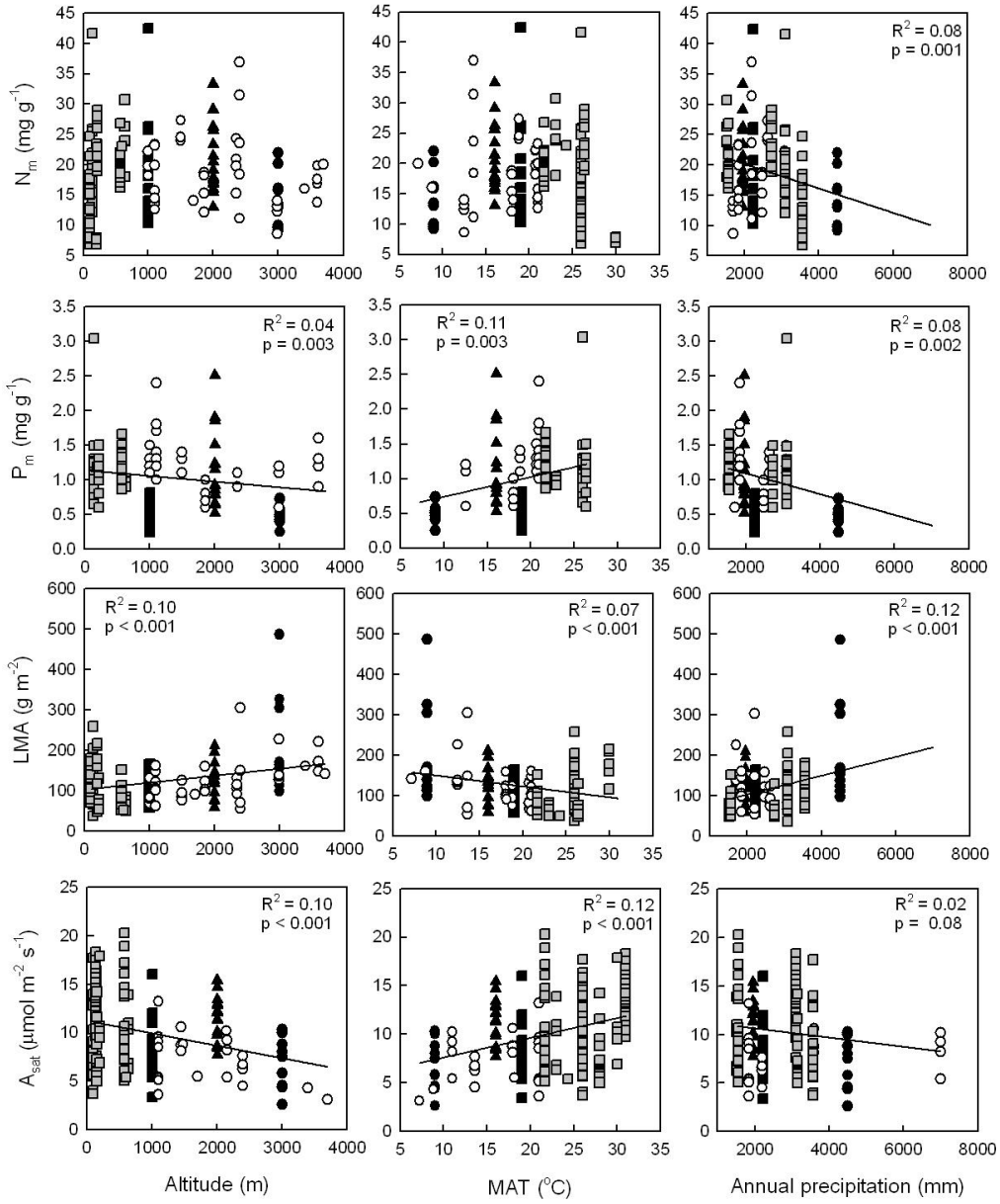

Figure 3. *Dependence of light-saturated net photosynthesis at ambient temperature and [CO_2] (A_{sat}), foliar nitrogen (N_m) and phosporous (P_m) concentrations (mass-based), and leaf mass per area (LMA) on elevation, temperature and precipitation in a pan-tropical data set of c. 170 tree species and 18 forest stands. Only data referring to mature or pre-mature tree individuals (no seedlings or saplings) of non-pioneer stands were included. For further explanations and references see Table 5 in the Appendix. Filled grey symbols mark the data from lowland forests, black filling indicates the data from the present study in southern Ecuador (squares – 1000 m, triangles – 2000 m, circles – 3000 m stand).*

Local edaphic and climatic conditions seem to modify this more general picture of altitudinal change in area- and mass-based A_{sat} considerably. While the Ecuadorian data are generally fitting quite well into to the patterns extracted from the pantropical analysis, A_{sat} deviates with relatively high rates at mid-slope position (2000 m) from the overall trend which is explained by the relatively high soil P availability in this stand. With a mean foliar P concentration of 1.1 mg g^{-1}, the 2000 m-stand fits well to the 'moderately fertile' category of Vitousek and Sanford (1986) for lowland forests, while the 1000 m- and 3000 m-stands with 0.5 mg P g^{-1} must be classified as 'infertile'. The very high annual rainfall amount in the 3000 m-stand, in combination with high cloudiness, poor soil aeration and low decomposition rates, is another site-specific factor in the Ecuador transect that may have influenced A_{sat}. Upper montane forests studied in Peru at similar elevation received only half the rainfall amount (van de Weg and others, 2009) and thus grew under less extreme conditions.

The lowered A_{sat} of TMF trees was evident from our analysis, even though the among-species variation was large and local edaphic and climatic factors seem to modify the effect of elevation and temperature considerably. The relatively low A_{sat} differentiates tropical mountain forest trees functionally from tropical lowland trees. This difference matches with the MAT and MAP characteristics of the investigated TMF stands which place this tree group closer to the trees of temperate evergreen rainforests or temperate deciduous forests than to tropical lowland forests (Fig. 4); certain TMF stands with extremely high precipitation are even falling outside any biome range as defined by Wright and others (2004). A more detailed analysis of leaf trait relationships in the TMF tree sample shows that this group is partly deviating from the pattern of A_{sat} – foliar N-LMA inter-relationships as they appear from a global perspective in the leaf economics spectrum of Wright and others (2004) (Fig. 5). One striking difference is large scatter of the data in the plot of mass-based A_{sat} against SLA and N_m in TMF trees. The surprisingly low mass-based A_{sat} rates in several leaves of the TMF sample with high SLA might point at plant-internal N allocation patterns that are directed to an optimization of light capture at the expense of maximum carboxylation rate (Evans 1989), in a similar manner as it has been found

in tropical understorey plants (Santiago and Wright 2007). In contrast to tropical lowland trees, many montane forest trees are exposed to continuously high cloudiness and low-light conditions which could explain this apparent functional difference to lowland trees. High variation in SLA at a relatively narrow range of mass-based A_{sat} rates may offer a greater potential for optimizing CO_2 assimilation in this cool, often light-limited environment (Givnish 1988, Sims and others 1994, Santiago and Wright 2007).

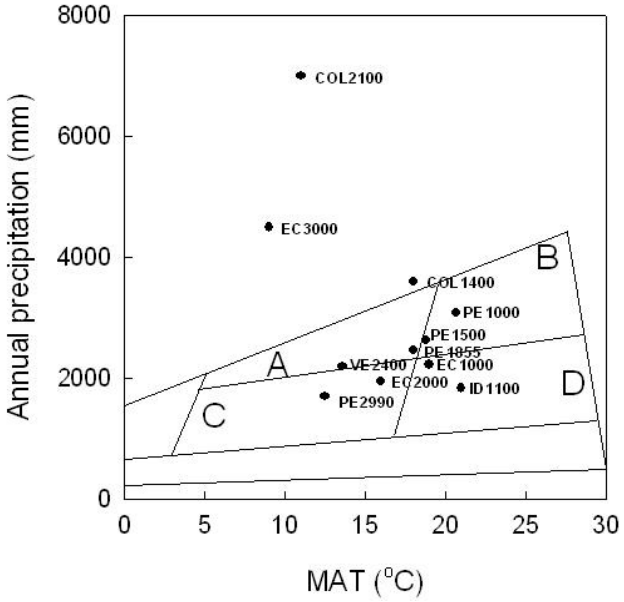

Figure 4. *Grouping of the tropical montane forests considered in this study in the major biomes of the world (after Wright et al. 2004) using annual precipitation and mean annual temperature as criteria. Accordingly, these tropical montane forests have affililations either to temperate rain forests (A), tropical rainforests (B), temperate forests (C) or tropical seasonal forests (D). Only sites located between 1000 and 3600 m asl are included. Locations: Ecuador (EC1000, EC2000, and EC3000, this study), Peru (PE1000, PE1500, PE1855, and PE2990, Van der Weg and others 2012), Colombia (CO1445, CO2145, Letts and Mulligan, 2005), Venezuela (VE2400, Rada and others), and Indonesia (ID1100, Hölscher and others 2006).*

Our literature survey further suggests that the range of A_{sat} species means occurring in lowland forests (3.7 to 20.3 µmol CO_2 m^{-2} s^{-1}) is larger than that in montane and upper montane stands (2.1 to 13.2 µmol m^{-2} s^{-1}). The apparent decrease in A_{sat} variation with increasing elevation is partly caused by the uneven distribution of data over the elevation range with fewer trees being studied at altitudes >1500 m than at lower elevation. However, the upslope decrease in the number of investigated species mirrors the general decrease in tree species richness from tropical lowland to montane elevation (Gentry 1988, 1995; Aiba and Kitayama 1999; Slik and others 2009) and thus seems to be justified.

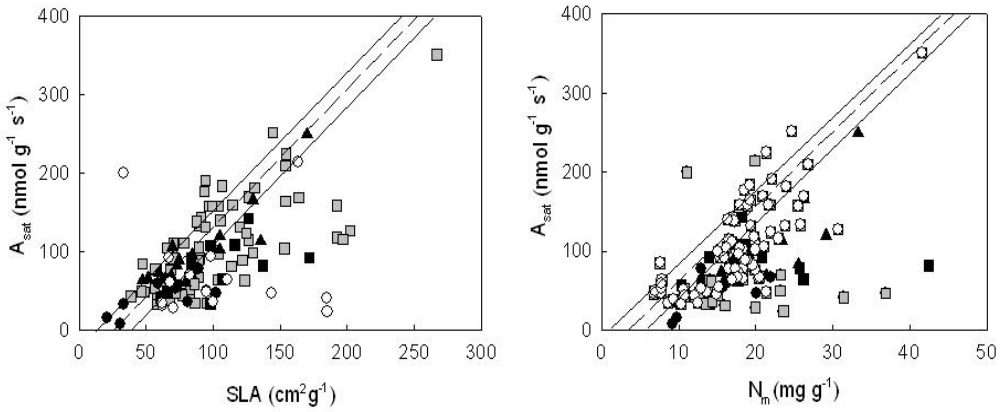

Figure 5. *Relationships between photosynthetic rate per mass (A_{sat}) and (left) specific leaf area (SLA) or (right) leaf nitrogen per mass (N_m) for the tree species in the survey. Filled symbols are for species from montane locations with black filling indicating the Ecuadorian species of this study (squares – 1000 m, triangles – 2000 m, circles – 3000 m stand) and grey filling species from other montane sites, open symbols stand for all other species in the survey. Dashed lines represent model II regressions with 95% confidence intervals for the global data set of Wright and Santiago (2007) (GLOPNET).*

A functional explanation of the upslope reduction in A_{sat} variation is provided by stand differences in canopy structure. We expect that taller multi-layered lowland forests should generate a spatially more heterogeneous light climate than the structurally simpler high-elevation forests. This would allow at lower altitude the coexistence of

more tree species with different strategies of light use and carbon assimilation. We assume that the large A_{sat} variation in the lowland data of our literature survey (maxima up to 20 µmol CO_2 m^{-2} s^{-1}) is mirroring the co-existence of species from the full spectrum of tree functional types that are present in old-growth lowland forests, notably shade-tolerant late-successional and light-demanding gap species (Turner 2001). High-elevation forests of low stature, in contrast, are typically dense with only a single main canopy layer in which the among-species variation in photosynthetic capacity (A_{sat} typically <8 µmol CO_2 m^{-2} s^{-1}) is indeed reduced. In the neotropical high-elevation tree genus *Polylepis*, which forms the highest forests of the world, the largest observed A_{sat} values do not exceed 9 µmol CO_2 m^{-2} s^{-1} in Argentina (2100 m), 7 µmol CO_2 m^{-2} s^{-1} in Venezuela (4200 m) and 3 µmol CO_2 m^{-2} s^{-1} in Bolivia (4300 m, Rada and others 1996; Azocar and others 2007). Accordingly, it is not very likely that a future more extensive set of photosynthesis data from tropical high-elevation tree species would result in higher mean A_{sat} values than those reported in this study.

What are the underlying causes of the apparent A_{sat} decrease with elevation? The direct or indirect air pressure effects on A_{sat} are probably small, in particular at low temperatures, as indicated by modeling studies of Terashima and others (1995) and Smith and Hughes (2009). A more likely explanation would be the effect of lowered temperature on the rate of RuBP carboxylation, i.e. reduced light-saturated photosynthesis rates of montane and upper montane tree species under the lowered ambient temperatures of their growing sites. Low temperatures can reduce enzyme activity in the photosynthetitic apparatus or may cause feedback inhibition of photosynthesis because of impaired sucrose metabolism and phloem loading. However, alpine and arctic plants have been found to compensate effectively for low temperatures by increasing Rubisco concentration and carboxylation capacity which is reflected by the generally relatively high foliar N concentrations (Körner and Larcher 1988, Körner 1989). A prerequisite of such a compensatory response is a sufficiently high N and P supply which existed in the Ecuador TMF at 2000 m, but N and P were short in supply at 3000 m (Moser and others 2011). Suppression of photosynthetic capacity by shortage of N or P (or other elements) in montane and

upper montane forests is a possible scenario because the rate of nutrient cycling generally decreases at higher elevations with a reduction in temperature (Benner and others 2010). We found markedly smaller average foliar N concentrations in the 3000-m stand than in the other two forests (difference significant to the 2000 m-stand). Growing deterioration of N and/or P supply with increasing elevation could limit the photosynthetic capacity of high-altitude trees by two mechanisms, by restricting the amount of N and P available for allocation to the photosynthetic machinery or by increasing mean leaf longevity and thus mean leaf age; longer-living leaves are typically more sclerophyllous and physiologically less active (Reich and others 1991; Wright and others 2004). In fact, Moser and others (2007) found tree leaf longevity to increase from 16 to 25 months on average between 1000 and 3000 m elevation; Further studies are needed to identify the biotic and abiotic factors causing A_{sat} to decrease with elevation in tropical mountains.

As predicted, the stand means of leaf dark respiration measured at ambient temperature remained constant along the elevation transect indicating effective adaptation of mitochondrial respiration of the montane and upper montane trees to the 10 K-temperature gradient in Ecuador. Similar homeostatic responses of leaf dark respiration were reported from gradient studies with *Eucalyptus pauciflora* and herbaceous plants growing at different elevations .

Conclusions

Altitudinal gradient studies may provide valuable insights into the temperature dependence of tree growth and the forest carbon balance. Canopy C gain is a key element of the C balance, but how this flux is altered with altitude in response to decreasing temperature, atmospheric [CO_2] and other factors, that change with elevation, is not well understood. The detection of more general patterns of altitudinal change in A_{sat} in tropical forests has long been hindered by the often high species diversity and difficult canopy access. Our combined approach of gas exchange

screening in a large sample of pre-montane to upper montane neotropical tree species and a pan-tropical literature survey of carefully selected A_{sat} data from low to high elevation revealed (1) a linear decrease from sea level to the alpine tree in the stand-level average of (mass- and area-related) A_{sat} measured at ambient conditions, and (2) a decreasing range of among-species A_{sat} variation with altitude. However, the data also suggest that (3) mountain transects with different geology and thus soil N and P availabilities might differ in their altitudinal A_{sat} patterns. Our findings have implications for the search for the causes of alpine tree lines in tropical mountains. It appears that stands close to the tropical alpine tree line are built by tree species with reduced A_{sat} rates, but, in addition, a reduced stand leaf area also contributes to a decease in canopy C gain with increasing altitude in tropical mountains (Moser et al. 2007). We interpret this result as a strong hint to carbon source limitation in tropical high-elevation forests, not supporting the hypothesis of C sink limitation as the principal cause of alpine tree lines (Körner 2003). Our results are in accordance with several recent studies that questioned the idea of carbon saturation in trees at high elevations (e.g. Millard and others 2007; Susiluoto and others 2007; Li and others 2008).

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CHAPTER 3

Environmental and biotic controls of photosynthetic capacity in tropical trees (southern Ecuador): the role of elevation, [CO2], temperature and nutrient availability

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Abstract

How the photosynthetic capacity (A_{max}) of tropical trees is modified in response to the temperature and atmospheric [CO_2] reduction with increasing elevation in mountains, is not precisely known but is relevant for understanding climate warming effects on tropical forests. We measured photosynthesis in 40 tropical tree species in an elevation transect (1000 - 3000 m a.s.l.) in Ecuador and analysed the dependence of A_{max} and leaf dark respiration (R_D) on important abiotic (temperature; [CO_2]) and biotic factors (SLA; foliar N and P contents; wood specific gravity). Neither A_{max} nor R_D showed significant elevational trends but A_{max} tended to be lowest at highest elevation. Thus, A_{max} was subject to partial, and R_D to full homeostatic adjustment to the reductions in temperature and [CO_2] at higher elevations, mainly through a large reduction in SLA and the resulting increase in foliar N and P per leaf area. No elevational increase in carboxylation efficiency was detected. P content per leaf mass was the factor with tightest correlation to A_{max} across the elevation gradient. We conclude that the elevational decrease in both SLA and canopy leaf area are more important determinants of carbon gain in tropical high-elevation forests than changes in the performance of the photosynthetic apparatus.

Keywords: Elevation, A_{max}, Foliar nitrogen, Foliar phosphorus, Homeostatic response, Leaf dark respiration, Photosynthesis, Tropical montane forests

Abbreviations:

A_{max} – light-saturated net photosynthesis rate

$A_{maxTACA}$ – A_{max} at ambient temperature and [CO_2]

$A_{maxTAC33}$ – A_{max} at ambient temperature and 33 Pa CO_2 pressure

$A_{maxT25CA}$ – A_{max} at 25 °C and ambient CO_2 pressure

$A_{maxT25C33}$ – A_{max} at 25 °C and 33 Pa CO_2 pressure

CE – carboxylation efficiency

[CO_2] – CO_2 partial pressure

DBH – diameter at breast height

J_{maxTA} – maximum electron transport rate at ambient T

J_{maxT25} - maximum electron transport rate at simulated conditions of 25 °C

LAI – leaf area index

LCP – light compensation point

LSP –irradiance needed to saturated photosynthesis

N_a – N content per leaf area

N_m – N concentration per leaf mass

P_a – P content per leaf area

P_m – P concentration per leaf mass

PNUE – photosynthetic nitrogen use efficiency

PPUE – photosynthetic phosphorus use efficiency

R_D – leaf dark respiration rate

R_{DTA} – leaf dark respiration at ambient temperature

R_{DT25} – leaf dark respiration at 25 °C

SLA – specific leaf area (leaf area per mass)

Suffix '$_{area}$' – A_{max} or R_D related to leaf area

Suffix '$_{mass}$' – A_{max} or R_D related to leaf mass

T – temperature

V_{cmaxTA} – maximum carboxylation rate at ambient T

$V_{cmaxT25}$ – maximum carboxylation rate at simulated conditions of 25 °C

WSG – wood specific gravity

α – quantum efficiency

Introduction

The transition from lowland to upper montane forest in tropical mountains is typically characterised by a gradual decrease in tree height and a sequence of different tree species assemblages reflecting a high species turnover across the elevational belts (Gentry 1988; Kitayama 1995; Pendry and Proctor 1996; Ashton 2003; Moser et al. 2008; Bruijnzeel et al. 2010). While the changes in forest structure and species composition with increasing elevation have 'puzzled and irritated' tropical ecologists over many decades (Whitmore 1989), the underlying causes are not well understood yet because many relevant environmental factors are closely interrelated and thus are changing simultaneously with elevation. Not only does temperature decrease more or less continuously with elevation but the partial pressures of CO_2 and O_2 as well, while the changes in irradiance, precipitation and nutrient availability are more site-specific and less predictable (Hastenrath 1991).

Trees growing at high elevations typically exhibit characteristic morphological, chemical and physiological features such as reduced tree size, smaller but thicker leaves, a relatively high foliar N content per leaf area, less negative foliar $\delta^{13}C$ values, high root:shoot ratios, and often relatively high wood specific gravity (Bruijnzeel and Hamilton 2000; Moser et al. 2007; Bruijnzeel et al. 2010; Milla and Reich 2011). Some of these modifications may allow the trees to maintain growth at relatively high rates despite less favourable environmental conditions at high elevations (Cordell et al. 1999). While a considerable number of studies in tropical mountains have investigated the tree size decrease and reductions in aboveground biomass and productivity with increasing elevation (e.g. Raich et al. 1997; Moser et al. 2011; Van de Weg et al. 2012; Leuschner et al. 2013), much less is known about elevational change in the photosynthetic capacity (A_{max}) of tropical trees from lowland to montane elevation.

Whether canopy carbon gain changes with elevation or not and how A_{max} adapts to the less favourable growing conditions at high elevations, is of relevance for understanding the climate warming response of tropical trees and when explanations

for the conspicuous tree size reduction along mountain slopes are sought. Elevational change in photosynthetic capacity may be investigated from two perspectives, either by focussing on gas exchange data obtained at different elevations under ambient [CO_2] and temperature conditions, or by analysing assimilation data standardised to low-elevation (higher than ambient) CO_2 concentrations and temperatures. The first type of data can be used for characterizing elevational change in canopy carbon gain and thus C source strength along mountain slopes when photosynthesis is up-scaled to the stand level, while the second information source can help to assess the adaptive potential of the photosynthetic apparatus to respond to the decreased temperature and [CO_2] at high elevation. For tropical mountain forests, both aspects are poorly studied. The few available studies on the photosynthetic performance of trees in tropical mountains fall into two categories, (1) transect studies focussing on a single tree species with wide elevational range (e.g. Cordell et al. 1998, 1999), and (2) transect studies covering the characteristic tree species turnover along tropical mountain slopes (e.g. Cavieres *et al.* 2000; Kitayama and Aiba 2002). In the Hawaiian tree species *Metrosideros polymorpha* (Myrtaceae), photosynthetic capacity was found to respond in a homeostatic manner to the less favourable [CO_2] and temperature conditions at higher elevations probably due to an increase in carboxylation efficiency (Cordell et al. 1999). However, a generally valid picture as to whether the A_{max} of tropical trees changes with elevation and how the photosynthetic apparatus of high-elevation trees has adapted to this specific environment, has not yet emerged.

Recently, Wittich et al. (2012) compiled a comprehensive A_{max} data set for tropical trees spanning an elevation distance of 2000 m in the southern Ecuadorian Andes that included 40 tree species from pre-montane to upper montane forests. They detected no statistically significant elevational change in the stand mean of light-saturated net photosynthesis rate measured at ambient conditions ($A_{maxTACA}$). However, when the data were placed in the broader context of a pan-tropical literature survey of published A_{max} values from mature tropical trees, a significant decrease in $A_{maxTACA}$ by *c.* 1.7 µmol CO_2 m^{-2} s^{-1} per km of elevation increase emerged

as a global average (Wittich et al. 2012.). This result raises the question on the causes of elevational change or constancy in A_{max}.

In this paper, we analyse possible controlling factors of the photosynthetic capacity of tropical trees along elevation transects using the 40-species A_{max}-data set compiled by Wittich et al. (2012). We paid special attention to effects of nitrogen and phosphorus availability on the photosynthetic capacity at high elevations because earlier investigations had produced evidence for N and P limitation of tree growth in these Ecuadorian mountain forests (Graefe et al. 2010; Moser et al. 2011). The specific objectives of the study were (1) to analyse the dependence of A_{max} (measured at ambient conditions) on important abiotic factors (temperature, $[CO_2]$) and plant traits (leaf morphology, foliar nutrient content, wood specific gravity, productivity), and (2) to examine the degree of up-regulation of the photosynthetic apparatus of tropical high-ele**v**ation trees in response to reduced $[CO_2]$ and lowered temperature, assessed by comparing A_{max} rates measured at ambient conditions and rates standardised to higher (low-elevation) $[CO_2]$ and temperature conditions. With reference to earlier studies on the photosynthetic capacity of tropical mountain forest trees (e.g. Cordell et al. 1998, 1999; Letts et al. 2010), we tested the hypotheses that (i) trees in tropical high elevation forests have higher N and P contents per leaf area, but lower contents per leaf mass, than trees at lower elevation, and (ii) they possess a higher carboxylation efficiency than trees at lower elevation. (iii) The homeostatic response in A_{max} and leaf dark respiration to lowered temperature and $[CO_2]$ is sufficient to compensate for the less favourable environmental conditions at high elevations.

Materials and methods

Study sites and tree species

The study was conducted between February and May 2009 in tropical pre-montane to upper montane forests along a 2000-m elevation gradient on the eastern slope of the southern Ecuadorian Andes. Three study sites were selected at c. 1000, 2000 and 3000 m a.s.l. within the borders of Podocarpus National Park and Reserva Biológica San Francisco in the provinces of Loja and Zamora-Chinchipe (see Fig. A1 in the Appendix). The maximum distance between the three sites was 30 km.

The research area has a tropical humid climate with an extremely wet season from April to July (precipitation maximum in June/July) and a less humid season from September to December without regularly occurring longer dry periods (Bendix et al. 2006; Homeier et al. 2010). The microclimate close to the investigated stands was monitored from January 2008 to December 2009 (24 months) with three microclimate stations erected in gaps in the forest (ca. 20 m in diameter). Table A1 in the Appendix gives 2-yr averages of rainfall, air temperature, air humidity and atmospheric [CO_2] for the three elevations. While air temperature decreased more or less continuously from 1000 to 3000 m a.s.l. with a lapse rate of c. 6 K km^{-1}, rainfall decreased slightly from 1000 to 2000 m (from about 2200 to 1800 mm yr^{-1}) but increased again from 2000 to 3000 m, where clouds are frequent (to c. 2600 mm yr^{-1}). Against the climate variability recorded at the mid-elevation site (Breuer et al. 2013), the year 2009 received average precipitation amounts and had a slightly higher mean air temperature compared to the previous years. We estimated the CO_2 partial pressure in the turbulent air above the forest canopy from local air pressure by assuming a constant mixing ratio of 370 µmol CO_2 mol air^{-1}. Accordingly, [CO_2] decreased by c. 3 Pa km^{-1} from 33 Pa at 1000 m to 27 Pa at 3000 m (see Table A1 in the Appendix).

The mountain ridge in the study region consists of a variety of acidic bedrocks with granites dominating at 1000 m and phyllites and sandstones being present at elevations >1500 m. The soil types change along the slope from Alumic Acrisols at

1000 m to Gleyic Cambisols (2000 m) and Podzols (3000 m) at higher elevation. The availability of nitrogen (N) and phosphorus (P) generally decreased with elevation, but the trend was not uniform along the slope due to a geological discontinuity in the transect between 1000 and 2000 m elevation (Table A2 in the Appendix). The pools of total and available P in the organic layer and in the mineral topsoil (0-10 cm) and soil pH were highest in the montane forest at 2000 m, which stocks on moderately fertile phyllites and sandstones; the soils at 3000 m and also at 1000 m on less fertile granite had lower values. In contrast, the extractable pools of NH_4^+ and NO_3^- and net N mineralisation and nitrification rates in the topsoil decreased four- to 30-fold from 1000 to 3000 m despite the geological substrate change in the lower part of the transect.

The three investigated stands are located in protected forest sections on relatively steep slopes (10-40°) and refer to natural forest with no signs of major human disturbance. Important stand structural characteristics of the three stands are summarized in Table A3 in the Appendix. The forest inventory data reveal large decreases in mean canopy height (from 25 - 30 to 8 - 10 m), leaf area index (from 6.0 to 2.2) and aboveground biomass (from 177 to 89 Mg DM ha^{-1}) between 1000 and 3000 m elevation but only minor change in stem density, stand basal area and mean DBH. In contrast, belowground biomass increased greatly with elevation from 32 to 63 Mg ha^{-1}. The pool of N stored in stand leaf biomass increased from 123 to 202 kg ha^{-1} between 1000 and 2000 m, probably as a consequence of the geological discontinuity, and dropped to only 46 kg ha^{-1} toward the uppermost stand. Similarly, the foliar P pool doubled between 1000 and 2000 m and reached its minimum at 3000 m. Mean leaf lifespan as determined by leaf tagging and repeated censuses increased from 16 (1000 m) to 25 months (3000 m).

With ≥ 800 tree species the forests in the study area are extremely species rich (J. Homeier, unpubl. data). At every site, we selected 10-16 tree species that belonged to the more abundant taxa at this elevation and thus were considered as being more or less representative of the local tree assemblage. Another selection criterion was that at least a few trees were accessible from the ground for gas exchange

measurements.

The three elevations correspond to different forest types (Homeier et al. 2008): The 1000 m stand (S 4° 7´ W 78° 58´) is situated at pre-montane elevation in the transition zone between tropical lowland and lower montane forest with a canopy up to 40 m high. Common tree families are Fabaceae, Melastomataceae, Moraceae, Myristicaceae, Rubiaceae and Sapotaceae. In this stand, one tree individual of each of the 15 species was selected for photosynthesis measurement at elevations between 950 to 1050 m a.s.l. Ten species could be identified to the species level, the remaining to the genus level (Table A4 in the Appendix).

The 2000 m stand (S 3° 58' W 79° 04') is an evergreen lower montane forest of 18 to 22 m canopy height. Characteristic tree families are Euphorbiaceae, Lauraceae, Melastomataceae and Rubiaceae. Sixteen tree species were investigated at elevations between 1800 and 1900 m a.s.l. with 14 of the species being identified to the species level.

The stand at 3000 m (S 4° 7´ W 79° 11´) is an evergreen elfin forest located 100-200 m below the alpine tree line with a canopy height rarely exceeding 8 to 10 m. Dominant tree families are Aquifoliaceae, Clusiaceae, Cunionaceae, Lauraceae and Melastomataceae. Here, ten tree species were investigated at elevations between 2850 and 3000 m a.s.l.; nine could be identified to the species level.

In total, we were able to conduct photosynthesis measurements in 41 trees representing 40 different species (for one species, *Clethra revoluta*, we selected each one individual at 2000 m and 3000 m). Maximum distance between the trees at a site was 1.5 km. Only medium to tall trees with a minimum breast height diameter (DBH) of 10 cm were investigated (the mean DBH of the sampled trees was 16 ± 2 cm at 1000 m, 19 ± 2 cm at 2000 m, and 12 ± 1 cm at 3000 m). The measured trees were 10-20 m tall at 1000 m, 8-15 m at 2000 m and 4-12 m at 3000 m. For accessing the sun-lit parts of the tree canopies with the gas exchange system, we selected tree individuals in the forest that grew on the steep slope below walking paths or trees growing below ribs on the slope, so that part of the sun-exposed canopy was

accessible from the ground.

Data on stand structure, aboveground biomass fractions, DBH increment (in percent of basal area) and wood specific gravity of the studied species were collected in the course of a comprehensive inventory on stand structure and productivity in 54 plots of 400 m^2 size in close vicinity of the trees used for photosynthesis measurement at 1000, 2000 and 3000 m elevation (J. Homeier, unpubl. data). Data on belowground biomass, leaf life span and leaf area index refer to three additional plots located at 1000, 2000 and 3000 m in close vicinity of the photosynthesis trees (data from Moser et al. 2007; consult this source also for description of methods).

Photosynthesis measurements

Between February and May 2009, we measured leaf gas exchange on each three replicate leaves of the 41 trees (40 species; 123 leaves in total) using a portable IRGA system (LI-6400, LI-COR Biosciences, Lincoln, NE, USA) equipped with a LED red/blue light source (type 6400-02B). At the beginning of the measuring campaign, the CO_2 analyser was calibrated against a gas standard of 400 ppm CO_2 in N_2. The IRGA channels were matched before every measurement. All measurements were carried out between 10:00 a.m. and 4:00 p.m. on intact fully expanded leaves of most distal insertion on branches that were not detached during measurement. The branches were part of the lateral canopy with exposure to full sunlight. For every leaf, a light and a CO_2 response curve was recorded. The temperature simulated in the cuvette was set to the air temperature found to be typical for the measurement time at the respective study site based on temperature measurements by Rollenbeck and Peters (unpublished data), i.e. 22-24 °C at 1000 m, 19-21 °C at 2000 m and 15-17 °C at 3000 m. The water vapour saturation deficit in the cuvette was held constant at vpd levels that were characteristic for the mean ambient conditions at the respective sites, i.e. 1.6 kPa at 1000 m, 1.2 kPa at 2000 m and 0.9 kPa at 3000 m. The photosynthetic light response was determined at photon flux densities of 1500, 1000,

500, 200, 100, 50, 20 and 0 µmol m^{-2} s^{-1} starting at highest irradiance. In this measuring task, the CO_2/air mixing ratio was held constant at 370 ppm. The photosynthetic CO_2 response was measured under light saturation (1500 µmol photons m^{-2} s^{-1}) at CO_2 mixing ratios of 2000, 1300, 700, 360, 200, 100, 50 and 0 ppm starting at the highest ratio. The CO_2 release recorded in the dark at the end of the CO_2 response curve was assumed to give an estimate of leaf dark respiration (R_D). Prior to respiration measurement, the leaves were allowed to acclimate for 2-5 min to the dark in the cuvette.

Besides the A_{max} measurements conducted at ambient temperature and [CO_2] conditions, we also calculated for the trees at 2000 and 3000 m the expected photosynthetic capacity under low-elevation (1000 m) conditions, i.e. at a temperature of 25 °C and/or CO_2 partial pressure of 33 Pa ($A_{maxT25CA}$, $A_{maxTAC33}$ and $A_{maxT25C33}$). This allowed comparing the higher elevation trees with those at 1000 m under standardised conditions. The $A_{maxTAC33}$ value was read from the respective A/c_a curve, while the A_{max} rate at 25 °C was calculated by correcting the $A_{maxTACA}$ value according to a temperature function of A_{max} derived for wheat plants by de Pury and Farquhar (1997) because of the lack of adequate data for tropical trees. A similar approach was chosen by earlier authors (e.g. Meir et al. 2007). The CO_2 partial pressure at 1000, 2000 and 3000 m was calculated from the equation

$$P_{CO2} = X \times P_{atm} \times 10^{-6}$$

where P_{CO2} is the partial pressure of CO_2 at a given elevation, X the molar fraction of CO_2 in ppm (which was assumed to be constant with elevation), and P_{atm} the local air pressure at this elevation. The mean air pressure was *c*. 90 kPa at 1000 m, 80 kPa at 2000 m and 72 kPa at 3000 m.

The photosynthetic response to a variable PPFD at ambient [CO_2] was described with a non-rectangular hyperbolic function (Norman et al. 1992); ninety percent of the measured assimilation rate at 1500 µmol photons m^{-2} s^{-1} was taken as A_{max} and the corresponding photon flux density as the saturating irradiance of photosynthesis (light saturation point, LSP). The initial slope α of the light response curve was used

to calculate the quantum yield of photosynthesis. For fitting the A/c_i curves, the program RACCIA (Fleck 2002) was used which is based on the equations of Farquhar et al. Berry (1980), Harley and Tenhunen (1991) and Ball et al. (1987). Carboxylation efficiency (CE) was derived from the initial slope of the A/c_i curve.

Morphological and chemical leaf trait analysis

For all 123 leaves measured, we determined dry weight, leaf size and foliar nutrient content. The harvested fresh leaves were scanned and their areas measured with an optical analysis system (WinFolia, Quebec, Régent, Canada). After drying at 70 °C for 48 h, the leaves were weighed and the specific leaf area calculated. The N concentration of the dry leaf mass was measured with an elemental analyser (Vario EL III, Elementar, Hanau, Germany), the P concentration with an Inductively Coupled Plasma Analyser (Optima 5300DV ICP-OES, Perkin Elmer, Waltham, Mass., USA) after HNO_3 pressure digestion. The foliar N and P contents were expressed either per leaf area (N_a, P_a) or per leaf mass (N_m, P_m). The $\delta^{13}C$ signature of the leaf mass was analysed by mass spectroscopy (Delta plus, ThermoFinnigan, USA) in the Stable Isotope Laboratory of Göttingen University.

Statistical analysis

The photosynthesis data were analysed for their elevation dependence by pooling the 10 to 16 species of a study site; the species averages consist of the each three leaves per species examined. Analysis of variance (Scheffé's test) was used to conduct multiple comparisons among the means of the three sites. If the data were not normally distributed according to a Shapiro-Wilk test, the Mann–Whitney two-sample test (Wilcoxon U test) was used instead of Scheffé's test. All calculations were done with SAS software (version 9.1; SAS Institute, Cary, NC, USA). A significance level of 5 % was used throughout the analyses.

The relationship between photosynthetic parameters and leaf traits or productivity variables was analysed by simple linear regressions (based on species averages; conducted with the software SigmaPlot, version 11.0, Systat Software, Inc, Washington, USA). Multiple linear regression analyses with backward variable elimination (software R 2.13.0, R Development Core Team 2011) were applied to test which model built with the possibly influencing factors CO_2, temperature, P_a, N_a, SLA and wood specific gravity predicted A_{max} best. We conducted two separate analyses, one with the complete set of variables but excluding CO_2, and a second one excluding temperature, since these two variables were highly correlated (Table 1). A correlation coefficient of R > 0.7 was used as a threshold to exclude predictor variables in the same model. Criterion for selecting the best model was the AIC (Akaike information criterion) score. We selected the model with the minimum AIC value.

Table 1. Results of Pearson correlation analyses between seven biophysical and biotic variables with a possible influence on photosynthesis. Given are the R values and the direction on the relationships.

		T	**P_a**	**N_a**	**P_m**	**N_m**	**SLA**	**WSG**
		°C	*mg m^{-2}*	*g m^{-2}*	*mg g^{-1}*	*mg g^{-1}*	*cm^2 g^{-1}*	
[CO_2]	*Pa*	0.96	-0.45	-0.41	-0.02	0.26	0.48	-0.23
T	*°C*		-0.49	-0.39	-0.09	0.23	0.45	-0.24
P_a	*mg m^{-2}*			0.56	0.54	0.00	-0.29	-0.03
N_a	*g m^{-2}*				-0.11	0.05	-0.58	0.14
P_m	*mg g^{-1}*					0.59	0.57	-0.27
N_m	*mg g^{-1}*						0.70	-0.23
SLA	*cm^2 g^{-1}*							-0.21

Table 2. Means over all investigated species (± SE) for about 30 parameters of photosynthetic activity, leaf dark respiration, and leaf morphology and chemistry in the three stands at 1000, 2000 and 3000 m a.s.l. (N = 10 – 16 species, three leaves per species investigated). N_m, P_m – N or P per leaf mass; N_a, P_a – N or P per leaf area; $A_{maxTACA}$ – light-saturated net photosynthesis rate per leaf area at ambient temperature and [CO_2]; $A_{maxTAC33}$, $A_{maxT25CA}$, $A_{maxT25C33}$ – A_{max} at simulated conditions of ambient T and 33 Pa CO_2, or 25 °C and ambient CO_2, or 25 °C and 33 Pa CO_2; V_{cmaxTA}, $V_{cmaxT25}$ – maximum carboxylation rate at simulated conditions of ambient T or at 25 °C; J_{maxTA}, J_{maxT25} – maximum electron transport rate at simulated conditions of ambient T or at 25 °C; α - quantum efficiency; CE – carboxylation efficiency; LCP and LSP - light compensation point and light saturation point of photosynthesis; CO_2CP – CO_2 compensation point; PNUE and PPUE – instantaneous photosynthetic N and P use efficiency; $A_{maxmassTACA}$ – mass-specific A_{max} at ambient T and [CO_2]; $A_{maxmassTAC33}$, $A_{maxmassT25CA}$ and $A_{maxmassT25C33}$ – mass-specific A_{max} at simulated conditions of ambient T and 33 Pa CO_2, or 25 °C and ambient CO_2, or 25 °C and 33 Pa CO_2; R_{DTA} and R_{DT25} – leaf dark respiration rate at ambient T or 25 °C; $\delta^{13}C$ – leaf $\delta^{13}C$ signature. Significantly different means between the three elevations are indicated by different letters ($P < 0.05$). SLA, N_m, P_m, N_a, P_a, $A_{maxTACA}$, α and R_{DTA} data after Wittich *et al.* (2012).

		1000 m		2000 m		3000 m	
No. of tree species		15		16		10	
No. of families		12		11		9	
Leaf and stand structural traits							
Leaf size	cm^2	127 ± 24	**a**	145 ± 32	**a**	40 ± 11	**b**
SLA	$cm^2\ g^{-1}$	100 ± 9	**a**	90 ± 8	**a**	57 ± 8	**b**
Rel. dbh increment	$\%\ yr^{-1}$	1.20 ± 0.21	**a**	0.88 ± 0.18	**a**	0.92 ± 0.16	**a**
Wood spec. gravity		0.52 ± 0.03	**a**	0.48 ± 0.02	**a**	0.56 ± 0.02	**a**
N_m	$mg\ g^{-1}$	19 ± 2	**ab**	21 ± 2	**a**	14 ± 1	**b**
P_m	$mg\ g^{-1}$	0.5 ± 0.04	**a**	1.1 ± 0.15	**b**	0.5 ± 0.05	**a**
N_a	$g\ m^{-2}$	2.0 ± 0.2	**a**	2.4 ± 0.1	**b**	2.7 ± 0.3	**b**
P_a	$mg\ m^{-2}$	57 ± 5	**a**	128 ± 7	**b**	104 ± 14	**b**
N per leaf	mg	24.5 ± 4.4	**a**	38.8 ± 10.3	**a**	11.1 ± 3.4	**b**
P per leaf	mg	0.71 ± 0.14	**ab**	2.06 ± 0.53	**a**	0.40 ± 0.09	**b**
N/P		35.2 ± 2.8	**a**	19.9 ± 1.3	**b**	27.8 ± 2.3	**ab**

		1000 m		2000 m		3000 m	
Gas exchange traits							
a) Area-related							
$A_{maxTACA}$	$\mu mol\ m^{-2}\ s^{-1}$	8.8 ± 0.8	**a**	11.3 ± 0.6	**b**	7.2 ± 0.9	**a**
$A_{maxTAC33}$	$\mu mol\ m^{-2}\ s^{-1}$	8.8 ± 0.8	**a**	11.5 ± 0.7	**b**	8.9 ± 0.99	**ab**
$A_{maxT25CA}$	$\mu mol\ m^{-2}\ s^{-1}$	9.6 ± 0.8	**a**	18.9 ± 1.0	**b**	16.3 ± 2.0	**b**
$A_{maxT25C33}$	$\mu mol\ m^{-2}\ s^{-1}$	9.6 ± 0.8	**a**	19.0 ± 1.1	**b**	18.8 ± 2.1	**b**
V_{cmaxTA}	$\mu mol\ m^{-2}\ s^{-1}$	49 ± 5	**a**	43 ± 2	**a**	24 ± 2	**b**
$V_{cmaxT25}$	$\mu mol\ m^{-2}\ s^{-1}$	49 ± 4	**a**	69 ± 4	**b**	52 ± 5	**a**
J_{maxTA}	$\mu mol\ electrons\ m^{-2}\ s^{-1}$	67 ± 4	**a**	84 ± 4	**b**	80 ± 3	**b**
J_{maxT25}	$\mu mol\ electrons\ m^{-2}\ s^{-1}$	68 ± 4	**a**	111 ± 5	**b**	123 ± 5	**c**
α	$mol\ mol^{-1}$	0.062 ± 0.002	**a**	0.060 ± 0.002	**a**	0.057 ± 0.003	**a**
CE		48 ± 1	**a**	33 ± 3	**b**	41 ± 2	**a**
LCP	$\mu mol\ m^{-2}\ s^{-1}$	12 ± 1	**a**	11 ± 2	**a**	14 ± 2	**a**
LSP	$\mu mol\ m^{-2}\ s^{-1}$	716 ± 61	**ab**	924 ± 60	**a**	620 ± 80	**b**
CO_2CP		0.060 ± 0.005	**ab**	0.077 ± 0.004	**a**	0.049 ± 0.005	**b**
PNUE	$\mu mol\ CO_2\ mol\ N^{-1}\ s^{-1}$	62.3 ± 7.4	**ab**	67.0 ± 4.4	**a**	40.0 ± 6.3	**b**
PPUE	$mmol\ CO_2\ mol\ P^{-1}\ s^{-1}$	4.7 ± 0.4	**a**	2.87 ± 0.4	**b**	2.4 ± 0.4	**b**
b) Mass-related							
$A_{maxmassTACA}$	$nmol\ g^{-1}\ s^{-1}$	83 ± 9	**a**	99 ± 12	**a**	42 ± 7	**b**
$A_{maxmassTAC33}$	$nmol\ g^{-1}\ s^{-1}$	83 ± 9	**a**	101 ± 12	**a**	50 ± 8	**b**
$A_{maxmassT25CA}$	$nmol\ g^{-1}\ s^{-1}$	91 ± 10	**a**	164 ± 18	**b**	94 ± 16	**a**
$A_{maxmassT25C33}$	$nmol\ g^{-1}\ s^{-1}$	91 ± 10	**a**	164 ± 17	**b**	106 ± 16	**a**
Dark respiration							
R_{DTA}	$\mu mol\ m^{-2}\ s^{-1}$	0.75 ± 0.07	**a**	0.62 ± 0.10	**a**	0.66 ± 0.07	**a**
R_{DT25}	$\mu mol\ m^{-2}\ s^{-2}$	0.83 ± 0.08	**a**	1.03 ± 0.15	**a**	1.48 ± 0.17	**b**
$\delta^{13}C$	‰	-31.9 ± 0.4	**a**	-29.2 ± 0.3	**b**	-28.9 ± 0.4	**b**

Results

Elevational change in leaf characteristics and photosynthetic capacity

Table 2 gives the community means of the three stands for about 30 parameters of photosynthetic capacity (at ambient and standardised low-elevation temperature and [CO_2] conditions), leaf dark respiration, and foliar morphology and nutrient concentrations. In addition, the species means of A_{max} at ambient conditions ($A_{maxTACA}$), leaf size, SLA, diameter growth and wood specific gravity of the 40 investigated tree taxa are given in Table A4 in the Appendix. While mean leaf size and SLA showed opposing elevation trends between 1000 and 2000 m for the 10-16 species from 9-12 families investigated per stand (increase in leaf size, decrease in SLA), a large and statistically significant decrease was observed for both traits between 2000 and 3000 m. Relative stem diameter increment measured in 9-11 species per stand tended to decrease in its community mean from the 1000-m to the 2000-m stand (difference not significant) but remained invariant higher upslope. Our data do not show significant elevational change in the community mean of wood specific gravity, even though the 3000-m stand exhibited the highest mean (0.56 g cm^{-3}, Table 2).

The mid-slope peak in leaf size was paralleled by maxima of mass-specific foliar N and P contents (N_m and P_m) and by a minimum of foliar N/P ratio at 2000 m; the 3000-m stand showed the smallest N_m mean along the transect (difference significant to the 2000-m stand). The area-specific N and P contents (N_a and P_a), in contrast, increased from 1000 to 2000 m and differed not significantly between the 2000-m and 3000-m stands (Table 2). When the six species of Melastomataceae or the four species of Lauraceae in the study region were analysed separately, similar elevational SLA, N_m and P_m trends appeared as for the whole tree species sample (Fig. 1A-C).

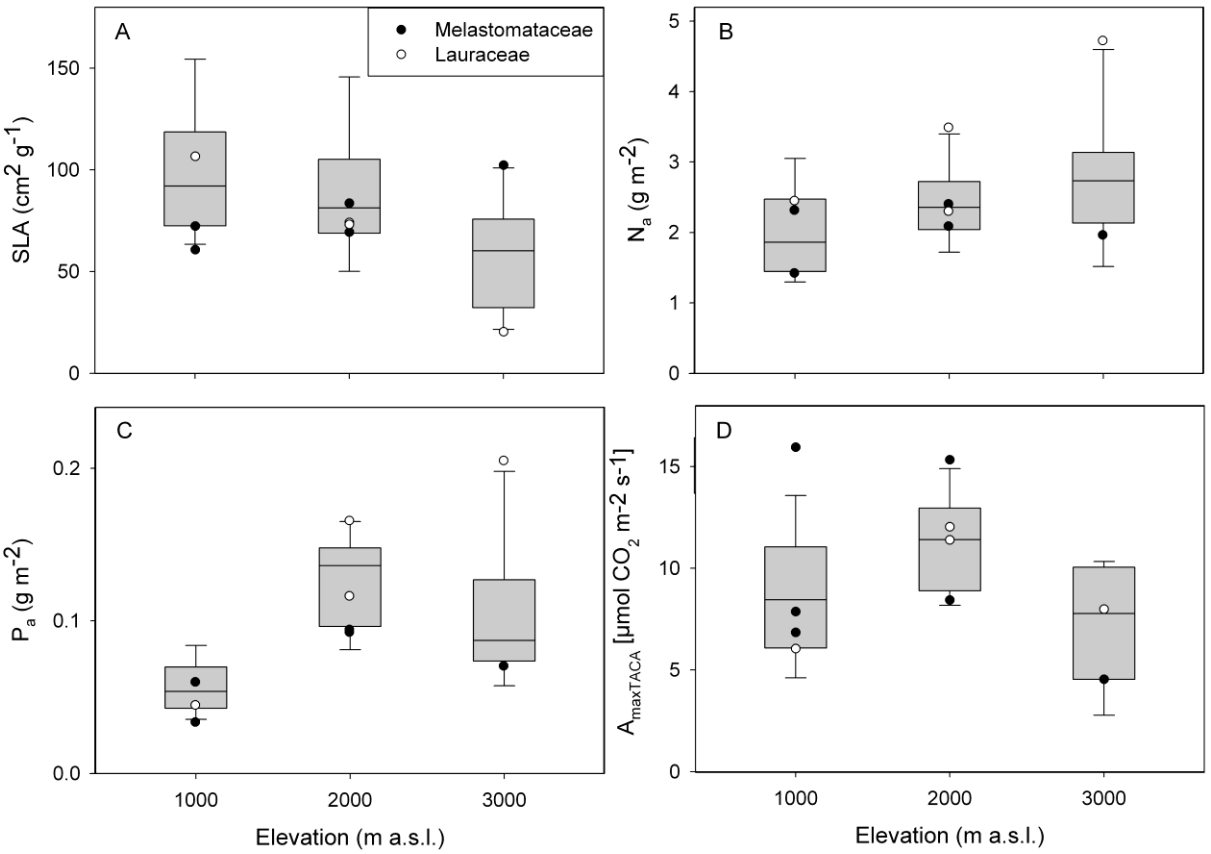

Figure 1. *Elevational change in SLA (A), area-based foliar N (N_a; B), area-based foliar P (P_a; C) and $A_{maxTACA}$ (D) in the species of Melastomataceae (filled dots) or Lauraceae (circles) in relation to the entire sample studied at 1000 m, 2000 m and 3000 m elevation (box-whisker-plots with median, 25- and 75-percent quartiles and 90th and 10th percentiles).*

The stand means of $A_{maxTACA}$ increased from 8.8 to 11.3 µmol m^{-2} s^{-1} between 1000 to 2000 m, but dropped again to 7.2 µmol m^{-2} s^{-1} at 3000 m (both differences significant; Table 2). This pattern was mirrored in the separate analysis of the Melastomataceae and Lauraceae taxa (Fig. 1D). When A_{max} was standardised to low-elevation temperature (25 °C) and [CO_2] (33 Pa) conditions, which characterise the sun-canopy conditions in the 1000-m stand around noon, photosynthetic capacity was higher than $A_{maxTACA}$ by 0.2, 7.6 and 7.7 µmol CO_2 m^{-2} s^{-1}, when local [CO_2] was increased to 33 Pa, the local temperature was adjusted to 25 °C, or both adjustments were applied, respectively (Table 2). At 3000 m, the adaptive increase in A_{max} relative to the 1000-m

conditions was even larger with 1.7 ($A_{maxTAC33}$), 9.1 ($A_{maxT25CA}$) and 11.6 µmol m^{-2} s^{-1} ($A_{maxT25C33}$) difference between A_{max} at ambient and simulated low-elevation conditions (Fig. 2 and Table 2). Thus, we found a more pronounced adaptation of A_{max} to the temperature reduction than to lowered [CO_2].

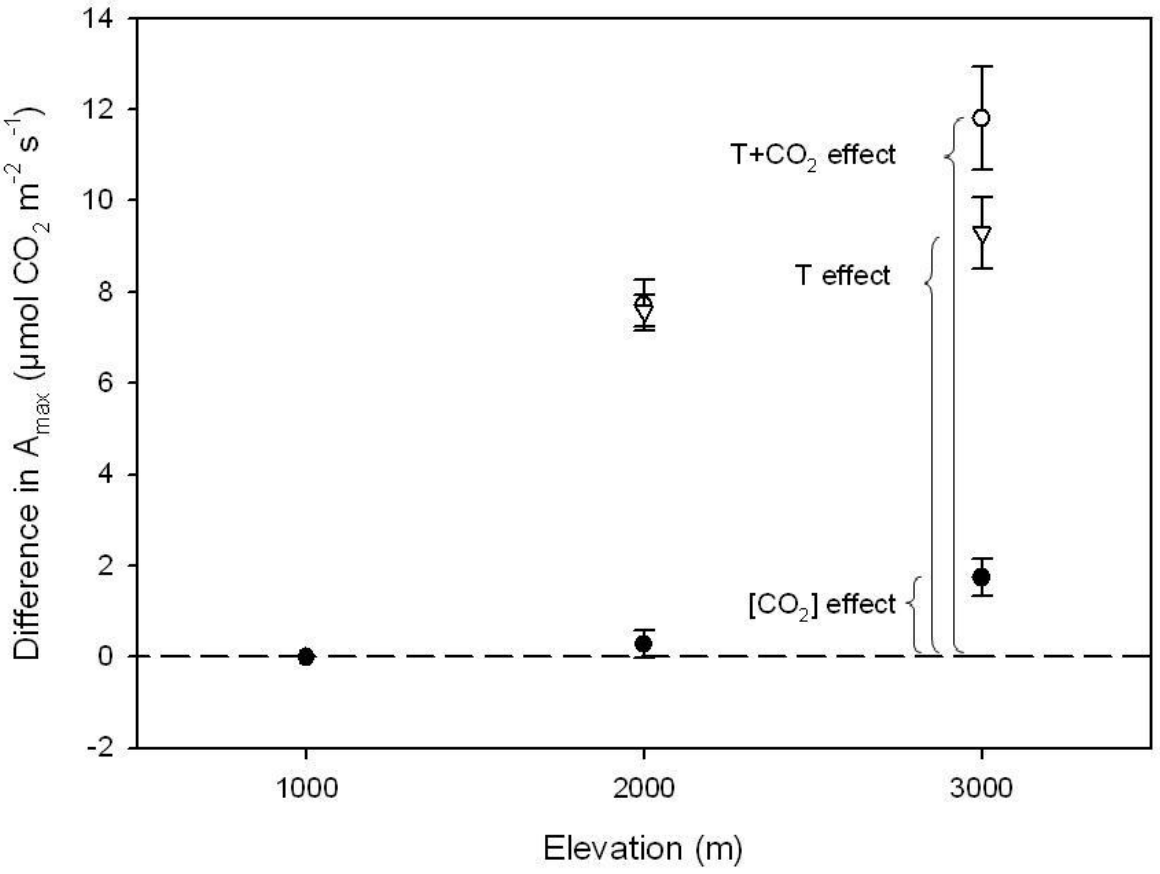

Figure 2. *Difference between $A_{maxTACA}$ (ambient T and [CO_2] conditions) and A_{max} standardised to low-elevation temperature (25 °C) and [CO_2] (33 Pa) in the stands at 2000 and 3000 m. The dotted line indicates the level of $A_{maxTACA}$. Means ± SE of 16 (2000 m) and 10 species (3000 m) per stand.*

The pronounced temperature adaptation of A_{max} is also evident from a comparison of maximum carboxylation rate (V_{cmax}) or maximum electron transport rate (J_{max})

measured either at ambient or standardised to low-elevation temperature (25 °C). V_{cmax} dropped significantly from 2000 to 3000 m when measured at ambient temperatures (Table 2). However, when V_{cmax} was corrected to 25 °C ($V_{cmaxT25}$) at 3000 m, maximum carboxylation rate increased to the same level as had been measured at 25 °C at 1000 m. In the 2000 m stand, the V_{cmax} mean increased to a rate significantly higher than the rates of the 1000 m and 3000 m stands when calculated for 25 °C. J_{max} measured at ambient temperature increased significantly from the 1000 m to 2000 m stand. When the J_{max} values were calculated for 25 °C, we found a significant increase from 1000 m to 2000 m and from 2000 m to 3000 m (Table 2).

Only minor (and mostly non-significant) elevational changes were detected for several parameters characterising the efficiency of light harvesting and carboxylation in tree sun leaves: quantum yield and the light compensation point of net photosynthesis showed no elevation trend in their community means between 1000 and 3000 m. Carboxylation efficiency (CE, the initial slope of the A/c_i curve) was not significantly different in its stand means at 1000 and 3000 m but peaked at 2000 m (difference significant; Table 2).

The shape of the light response curves was similar at the three elevations (averaged over 10-16 species) but the A/Q curves reflected the significantly higher $A_{maxTACA}$ mean in the 2000-m stand which likely is related to the relatively high foliar N and P concentrations (Fig. 3A). The A/c_i curves, averaged over all species of a stand, exhibited similar shapes and initial slopes (CE) at 1000 and 2000 m elevation. Yet, the mean A/c_i curve for the 3000-m stand showed a steeper increase at high c_i-concentrations (>500 µmol CO_2 mol air^{-1}) than the 1000- and 2000-m curves (Fig. 3B).

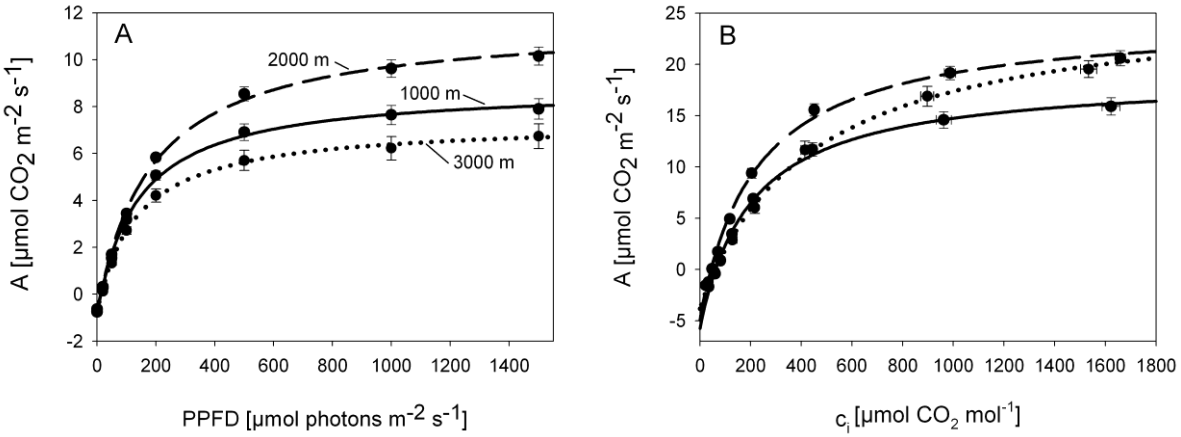

Figure 3. *Light (A) and CO_2 response curves (B) of net photosynthesis averaged over all sampled species for the stands at 1000, 2000, and 3000 m a.s.l. in southern Ecuador. Error bars give SE for 10 to 16 tree species examined with each three fully sun-exposed leaves measured per species.*

The stand-level means of instantaneous photosynthetic N or P use efficiencies ($A_{maxTACA}$ per unit foliar N or P) were significantly smaller at 3000 m than at lower elevation (PNUE: difference significant to the 2000-m stand, PPUE: difference significant to the 1000-m stand).

Photosynthetic capacity expressed per leaf mass ($A_{maxmass}$) was found to be highest at intermediate elevation (2000 m: mean of 99 nmol CO_2 g^{-1} s^{-1}) and lowest at 3000 m (42 nmol g^{-1} s^{-1}, difference significant to 1000 and 2000 m; Table 2), which broadly resembles the elevational patterns found for area-specific A_{max}. Standardised (low-elevation) temperature (25 °C) and [CO_2] conditions (33 Pa) led to large increases in $A_{maxmass}$ at 2000 and 3000 m, though to a lesser extent than in $A_{maxarea}$ due to the elevational SLA decrease.

Leaf dark respiration (R_D) at ambient temperature was similar at all three elevations. When R_D was corrected to 25 °C, respiration rate increased along the slope from 0.83 (1000 m) to 1.48 µmol CO_2 m^{-2} s^{-1} (3000 m).

The stand means of sun-leaf $\delta^{13}C$ decreased significantly from -31.9 ‰ at 1000 m to -29.2 ‰ at 2000 m and -28.9 ‰ at 3000 m (Table 2).

Table 3. Results of linear correlation analyses after Pearson between five gas exchange parameters and several biophysical and biotic variables with a possible influence on photosynthesis and leaf dark respiration (R_D). Given are the R and P values. Significant relationships ($P < 0.05$) in bold. All photosynthetic parameters are scaled to leaf area except for $A_{maxmassTACA}$.

	$A_{maxTACA}$	$A_{maxTAC33}$	$A_{maxT25C33}$	$A_{maxmassTACA}$	R_{DTA}
P_a	***R = 0.412***	***R = 0.460***	***R = 0.591***	*R = 0.085*	*R = 0.068*
	p < 0.001	***P = 0.003***	***P < 0.0001***	*P = 0.608*	*P = 0.682*
P_m	***R = 0.411***	*R = 0.294*	*R = 0.238*	***R = 0.759***	*R = 0.285*
	p < 0.001	*P = 0.065*	*P = 0.140*	***P < 0.0001***	*P = 0.071*
N_a	*R = 0.179*	*R = 0.278*	***R = 0.409***	*R = -0.243*	*R = -0.221*
	P = 0.277	*P = 0.091*	***P = 0.011***	*P = 0.136*	*P = 0.176*
N_m	*R = 0.172*	*R = 0.063*	*R = -0.084*	***R = 0.621***	*R = 0.125*
	P = 0.282	*P = 0.699*	*P = 0.606*	***P < 0.0001***	*P = 0.436*
SLA	*R = 0.029*	*R = -0.172*	***R = -0.383***	***R = 0.737***	*R = 0.267*
	P = 0.857	*P = 0.294*	***P = 0.016***	***P < 0.0001***	*P = 0.096*
Leaf size	*R = 0.294*	*R = 0.197*	*R = -0.022*	***R = 0.313***	*R = -0.020*
	P = 0.069	*P = 0.235*	*P = 0.894*	***P = 0.053***	*P = 0.904*
WSG	***R = -0.324***	***R = -0.338***	*R = -0.130*	***R = -0.413***	*R = -0.167*
	P = 0.041	***P = 0.035***	*P = 0.431*	***P = 0.010***	*P = 0.304*
DBH increment	*R = -0.042*	*R = -0.141*	*R = -0.216*	*R = 0.277*	*R = 0.062*
	P = 0.828	*P = 0.467*	*P = 0.260*	*P = 0.153*	*P = 0.750*

Controlling factors of photosynthetic capacity

Of the eight foliar and growth-related traits analysed in simple regressions, leaf phosphorus content was found to have the largest influence on area-specific A_{max} in the 40-species sample. P_a (and also P_m) correlated significantly with A_{max} at ambient and standardised conditions while the N influence was only of secondary importance (significant effect of N_a only on $A_{maxT25C33}$ but not on $A_{maxTACA}$, Table 3).

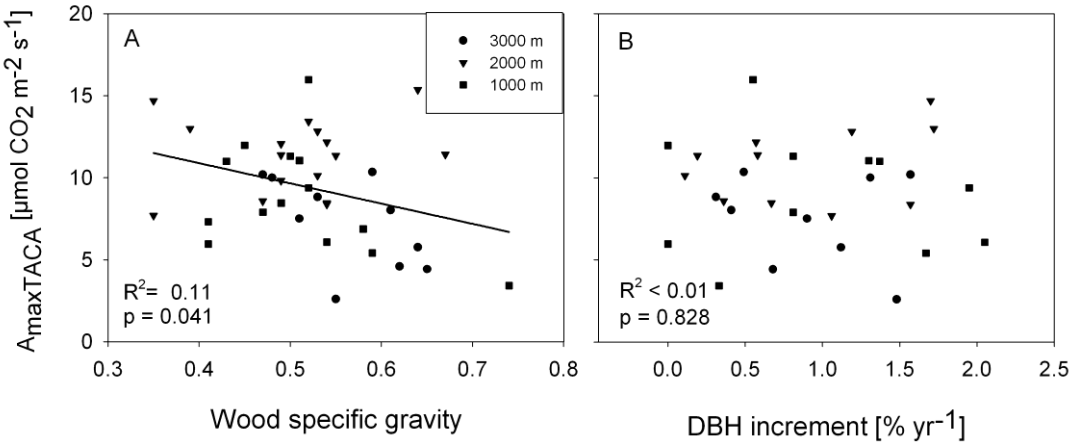

Figure 4. *Dependence of light-saturated net photosynthesis at ambient temperature and [CO_2] ($A_{maxTACA}$) of the 40 investigated species on A) wood specific gravity and B) relative stem diameter increment rate.*

We found a significant negative relationship between A_{max} and SLA (only in $A_{maxT25C33}$, not in $A_{maxTACA}$) in our sample. Further, $A_{maxTACA}$ increased with decreasing wood specific gravity of the stem, while no relation existed with relative stem diameter increment (Fig. 4A and B, and Table 3). As expected, the relationships between photosynthetic parameters and foliar and growth-related traits were much weaker when the analysis was conducted separately for the three stands (most relationships not significant; results not shown).

Leaf mass-specific photosynthetic capacity ($A_{maxmassTACA}$) showed very tight relationships to both P_m (R =0.76) and N_m (R =0.62) but not to foliar P and N per area. SLA and leaf size had a positive, wood specific gravity a negative influence on $A_{maxmassTACA}$. Leaf dark respiration was not related to any of the eight foliar and growth-related traits, neither when measured at ambient nor at standard (25 °C) temperature (Table 3).

A multiple regression analysis confirmed these results. For $A_{maxTACA}$, it indicated that P_m and SLA (in the order of significance) were the most important determinants of photosynthetic capacity in the 40-species sample while temperature, [CO_2] and wood specific gravity were only of secondary importance (only significant in factor combinations), and foliar nitrogen was not significant at all (Table 4). Leaf dark respiration was mainly influenced by foliar P but not N, similar to photosynthetic capacity. A significant temperature effect on respiration was not detected across the elevation gradient.

Table 4. Results of multiple regression analyses on the effects of the seven variables listed in Table 1 on photosynthetic capacity ($A_{maxTACA}$) and leaf dark respiration (R_D). Two models are presented in which either [CO_2] (a) or T (b) is excluded as variable due to their relatedness (see Table 1). Presented are the final models which were identified according to minimum AIC scores. The AIC values of the final models were 187.8 ($A_{maxTACA}$ (a)), 186.9 ($A_{maxTACA}$ (b)) and 20.3 (R_D). The final models include only the variables with largest influence on the dependent variable.

Dependent variable		Model R^2 (adjusted)	Independent variable	Parameter estimate	Partial regression coefficient	*p*-value
$A_{maxTACA}$	(a)	0.28	P_m	4.35	0.26	0.001
			SLA	-0.04	-0.03	0.035
			T	0.24	0.03	0.189
			WSG	-7.13	0.06	0.197
	(b)	0.30	P_m	4.30	0.26	0.001
			SLA	-0.04	-0.04	0.024
			CO_2	0.34	0.04	0.116
			WSG	-7.17	0.06	0.184
R_D	(a)	0.10	P_m	0.29	0.02	0.021
			P_a	-1.23	-0.01	0.356

Discussion

Photosynthetic performance of high-elevation trees

In the trees growing at 2000 and 3000 m, we found a considerable capacity to adapt to the less favourable temperature and [CO_2] conditions at high elevations by morphological or physiological modification, when referenced against the trees at 1000 m. This is evidenced by the comparison of photosynthetic capacity measured under ambient conditions and standardised to (low-elevation) [CO_2] and temperature levels. The photosynthetic capacity was on average higher by 2 and 24 % in the 2000-m and 3000-m stands, when the carbon dioxide concentration was increased from ambient to low-elevation conditions (33 Pa). The percental up-regulation of A_{max} was smaller than the relative decrease in CO_2 partial pressure (~ 30 %) from 1000 to

3000 m. The homeostatic A_{max} response was much larger to the 3 K- or 10 K-temperature decrease from 1000 to 2000 or 3000 m than to the [CO_2] decrease: A_{max} increased by 68 and 126 % at 2000 and 3000 m, respectively, when photosynthetic capacity was calculated for the low-elevation reference temperature of 25 °C instead of ambient temperature. The effectiveness of the homeostatic adjustment of A_{max} to the high-elevation environment is shown by the multiple regression analysis which revealed $A_{maxTACA}$ to be primarily controlled by foliar P and SLA in the 40-species sample while both the temperature and [CO_2] influences were small.

Homeostatic modification of A_{max} to the high-elevation environment may include leaf morphological change (increased leaf thickness with more layers of palisade mesophyll cells and a higher N content per leaf area; e.g. Benecke et al. 1981; Körner 2003; Wieser et al. 2012) but may also cover physiological modifications. Morecroft et al. (1992) and Cordell et al. (1999) found in Scotland and Hawai'i elevational increases in the carboxylation efficiency (CE) of trees and herbaceous plants. Such a response was not detected in the Ecuadorian transect, where the stand-level CE mean peaked at 2000 m, thus contradicting our second hypothesis. The mid-slope CE maximum coincided with the conspicuous maximum in foliar P concentration at this elevation pointing to a major influence of foliar P on photosynthetic capacity.

Maximum carboxylation rate per leaf area (V_{cmax}) did not decrease from 1000 to 3000 m when calculated for 25 °C, but it peaked at 2000 m probably reflecting the elevational change in P_a along the transect. Our average values of 49 µmol m^{-2} s^{-1} (1000 m) and 52 µmol m^{-2} s^{-1} (3000 m) are very similar to the V_{cmax} means found by Wullschleger (1993) (51 □ 31 µmol m^{-2} s^{-1}) and the literature survey of Van de Weg et al. (2012) for tropical rainforests. At ambient temperatures, however, V_{cmax} decreased in our transect between 1000 and 3000 m to the half pointing to a large temperature effect on rubisco activity at higher elevations (temperature difference: 7 K). The large decrease in mass-related A_{max} and also in photosynthetic N and P use efficiency with increasing elevation above 2000 m seems to support this interpretation.

Other than $V_{cmaxT25}$, the maximum rate of electron transport (J_{max}) significantly increased with elevation when standardised to 25° C; the elevational increase from 1000 to 2000 and 3000 m persisted when J_{max} was measured at ambient temperature. Van de Weg et al. (2012) also found indications for preferential investment in J_{max} as compared to V_{cmax} capacity in the canopy leaves of a Peruvian cloud forest at 3000 m a.s.l. They assumed this to be either a response to the reduced light availability at this elevation indicating a need for additional investment into electron transport capacity, or a consequence of the prevailing low vpd levels which promote higher leaf conductances and elevated average c_i values. This would reduce the need to invest in additional carboxylation capacity in this moist low-light environment. The high annual mean air humidity (>95 %) at 3000 m (see Table A1 in the Appendix) and the high cloud frequency throughout the year at 3000 m in the Ecuadorian transect (Bendix et al. 2008) seem to support both interpretations.

The widely observed tendency of reduced discrimination against $^{13}CO_2$ in the photosynthesis of high-elevation plants (Körner et al. 1988; Körner et al. 1991) is also evident from our data. Since carboxylation efficiency was not reduced at the highest elevation, this factor cannot explain the $\delta^{13}C$ increase in our transect. We assume that the effect is mainly caused by an elevational increase in the resistance to CO_2 diffusion from the intercellular spaces to the chloroplasts (Terashima et al. 1995) which would be plausible from the SLA decrease. A higher leaf internal resistance would decrease the c_i/c_a ratio leading to a less negative $\delta^{13}C$ signature. A lowered leaf-internal conductivity for CO_2 in thicker leaves could also explain, together with a putative temperature effect on photosynthetic capacity (and perhaps P limitation), the observed elevational reduction in A_{max} from 2000 to 3000 m.

It is not surprising that we did not find a relationship between A_{max} and wood productivity (annual relative diameter increment) across our species sample because leaf area, radiation interception and C allocation patterns may be more important determinants of wood growth rate than sun leaf photosynthetic capacity (Farmer 1980; Pallardy 2008). This implies that the negative relation found between A_{max} and wood specific gravity (WSG) cannot be explained by a causal relationship between

lower growth rate and reduced carbon demand in trees with high WSG. Rather, WSG, SLA and A_{max} might indirectly interact through plant nitrogen and/or phosphorus status or via WSG effects on hydraulic conductance and plant water status.

Our leaf dark respiration data indicate that the temperature decrease by about 13 K along the transect was fully compensated by a homeostatic response of leaf mitochondrial activity because we did not find significant differences between the area-related R_D means of the 1000 m- and the 3000 m-stands. Furthermore, temperature was not identified as a variable with a significant influence on leaf dark respiration in the multiple regression analysis. This finding is in agreement with earlier respiration studies which also observed a full or near-complete compensation of the effects of temperature reduction in high-elevation trees and herbaceous when compared to low-elevation plants (e.g. Larigauderie and Körner 1995; Atkin e al. 2000). Much of the apparent 'up-regulation' of respiratory activity per leaf area is explained by the large increase (by 80 %) in leaf mass per area between 1000 and 3000 m elevation, i.e. by morphological and not physiological adaptation to the high-elevation environment. This explanation is in agreement with results obtained by Cavaleri et al. (2008) in a lowland rainforest who found much of the within-canopy variation in R_D being caused by variation in leaf morphology. An unexpected finding was the dominant effect of P_a on area-related R_D in our species sample and the insignificant influence of foliar N. This relationship is best explained by our finding of a dominant P influence on photosynthetic capacity which should affect respiration through the close association between leaf respiratory activity and the rate of export of photosynthates from assimilating leaves.

In order to test for the influence of phylogenetic signal on the observed patterns of elevational change in leaf morphology and physiology (Losos 2008; Weber and Agrawal 2012), we examined whether closely related tree species within the families Melastomataceae and Lauraceae, two relatively abundant families with species at all elevations, differed in their trait patterns along the slope systematically from the rest of the species with more distant or no phylogenetic relatedness. From the observation that the elevational trends for $A_{maxTACA}$, SLA, N_a and P_a were similar for

the phylogenetically-related and unrelated species groups, it is unlikely that the observed elevational patterns are partly caused by phylogenetic niche conservatism (PNC). PNC would imply that related species show more similar photosynthetic properties along the slope than would be expected from a null model.

Influence of P and N nutrition on the photosynthetic capacity at high elevations

Our gas exchange measurements in 40 tree species did not reveal a significant elevational trend in A_{max} under ambient conditions (means of 8.8 vs. 7.2 µmol m^{-2} s^{-1} at 1000 and 3000 m), but a significantly higher stand mean at 2000 m. Some evidence suggests that this mid-slope A_{max} peak might be caused by the patterns of soil availability of P in this transect that seem to have a significant influence on the photosynthetic capacity of the trees.

From global surveys of the nutrient status of montane and alpine plants, it was concluded that nutrient shortage is limiting plant growth at high elevations only exceptionally (Körner 2003; Wieser et al. 2012), as for example at the alpine tree line in Colorado with assumed P limitation (Holtmeier and Broll 1992). Körner (2003) concluded for alpine herbaceous plants that they typically have higher N contents per leaf area than their relatives in the lowland which makes N limitation of growth and photosynthesis unlikely. However, extrapolation from the N and P status of alpine herbaceous plants to high-elevation trees is not feasible because forests generally have a higher nutrient demand than herbaceous vegetation. This is underpinned by the generally increasing foliar N concentrations with elevation in herbs (Körner 2003) while the foliar N_m values of tropical trees seem to decrease with elevation (see below).

In tropical trees, both foliar N and P contents per area (N_a and P_a) have been found to increase with elevation as a consequence of the general decrease in SLA (e.g. in Hawaiian and Bornean mountain forests, Cordell et al. 1999; Hikosaka *et al.* 2002). In support of our first hypothesis, our data from 40 Ecuadorian mountain forest species

show a N_a increase by about 30 % between 1000 and 3000 m, while Van de Weg et al. (2009) observed only a slight, but non-significant, N_a increase in the Peruvian Andes. Less information is available about elevational trends in foliar P per area in tropical trees. Van de Weg et al. (2009) reported no significant elevational change in P_a in the Peruvian Andes. As predicted in our first hypothesis, the stand mean of P_a nearly doubled in our transect from 57 to 104 mg m^{-2} between 1000 and 3000 m due to the large elevational SLA decrease. Thus, both N_a and P_a should principally support moderate to high photosynthetic activities per leaf area in the Ecuadorian high-elevation forests.

A different picture emerges when mass-specific instead of area-specific N and P contents are examined. While conifers at temperate alpine tree lines often have similar or even higher N concentrations per unit needle dry mass than trees at lower elevation (e.g. Richardson et al. 2001; Birmann and Körner 2009), this seems not to be the case in tropical trees. By summarising the existing information on tropical elevation transects, Tanner et al. (1998) and Benner et al. (2010) in most cases found decreases in N_m and P_m with elevation. However, exceptions from this rule do exist. For example, no significant trend in the N_m stand mean was found in a mountain forest transect in eastern Ecuador between 1000 and 2000 m a.s.l. (Wagner, Homeier & Leuschner, unpubl. data). Our data from southern Ecuador show a significant N_m decrease only at higher elevations (by ~30 % from 2000 to 3000 m) but not in the lower transect section which partly contradicts our hypothesis. This reduction relates to the decrease in N supply rates with increasing elevation in the Ecuadorian transect (Wolf et al. 2011). From the contrasting N_m and P_m gradients observed in different tropical mountains, it appears that the specific geological and soil fertility patterns have a considerable influence on the foliar nutrient status of tropical trees which could affect the physiology of mountain forest trees.

When regressing the investigated climatic and biotic parameters on photosynthetic capacity, we obtained three surprising results. First, neither temperature nor atmospheric CO_2 concentration were identified as significant influencing factors which indicates that selection has acted to reduce differences in the photosynthetic

capacity of species growing along the temperature and [CO_2] gradient along the slope. Second, foliar P concentration (P_m) was identified as the most important factor controlling A_{max} in the 40-species sample. This is surprising because it seems to show a large influence of nutrient availability on the photosynthetic capacity of these tropical mountain forest trees and it further indicates that P is much more relevant than N, as the nitrogen effect (N_a or N_m) was insignificant. This is supported by the mid-slope peak in area-related foliar P in our transect with foliar P being the environmental factor coinciding best with the conspicuous mid-slope A_{max} peak. Finally, it was unexpected that P_m and not P_a was the dominant factor because the dependent variable was area-related A_{max}. A direct effect of low foliar P on A_{max} might be caused by low concentrations of rubisco and other photosynthetic enzymes or feedback inhibition due to slow growth and low P_i concentrations in the cytosol (Lambers et al. 2008).

However, N availability might also influence the productivity of the Ecuadorian mountain forests. Several elevational trends reported in our study suggest that increasing N (perhaps together with P) shortage toward the highest elevations are negatively affecting forest productivity through indirect influences on leaf size, stand leaf area and tree carbon allocation patterns. First, the total amount of N and P allocated to stand leaf biomass is more than 60 % smaller at 3000 m than at 1000 m in the transect, which relates to a reduction in LAI from 6.0 to 2.2. Second, the mean amounts of N and P invested in the construction of a leaf decrease from 25 to 11 mg (N) or from 0.7 to 0.4 mg (P) from 1000 to 3000 m, in parallel with the reduction in mean leaf size. The less favourable high-elevation environment apparently forces the upper montane trees to allocate the N and P, which is available for leaf production and photosynthesis, to a smaller total leaf area consisting of small, thick and scleromorphic and long-lived leaves. Third, *Moser et al.* (2011) found a large increase in the root:shoot ratio of the trees from 1000 to 3000 m in this transect which was best explained by the large decrease in N availability (but not P availability) along the slope.

From our data, a complex picture of the role of N and P nutrition for the

photosynthesis and productivity of the Ecuadorian mountain forest trees emerges. While N shortage is likely one of the causes of the large leaf area reduction along the slope (Moser et al. 2007), the A_{max} decrease by 30 % from 2000 to 3000 m is probably a consequence of lowered temperature (and perhaps reduced P availability) and not caused by N shortage, because A_{max} decreased (from 11.3 to 7.2 µmol m^{-2} s^{-1}) while N_a increased (from 2.4 to 2.7 g m^{-2}; P_a decreased from 128 to 104 mg m^{-2}). Since P_m was also the principal factor determining leaf dark respiration in our species sample, we assume that the prominent role of foliar P concentration for A_{max} might relate to the role of energy availability (ATP supply) for leaf metabolism which should increase with decreasing temperatures at higher elevations.

Further studies must show whether our results regarding the likely P effect on tree A_{max} in tropical high-elevation forests are more generally valid. Elevational A_{max} patterns may not only differ between mountains with contrasting geology and climate but might also depend on the tree genera and families present in a region which might have different adaptive potentials. From our results, we hypothesize that the photosynthetic capacity should decrease with elevation in mountains with an upslope decrease in P availability, while montane and sub-alpine forests at sites with a more favourable P supply should show only small or no elevational reduction in area-specific A_{max} rates. This could be tested by comparing elevational transects in tropical mountains on allophane-rich (volcanic) and P-impoverished acidic soils.

Conclusion

In support of our third hypothesis, the gas exchange measurements showed a substantial up-regulation of leaf photosynthetic and mitochondrial activity in montane and upper montane trees under the less favourable temperature and [CO_2] conditions close to the alpine tree line. However, a full compensation was detected only for leaf dark respiration but not for A_{max}. The homeostatic adjustment is mainly caused by changes in leaf morphology, i.e. a large reduction in SLA and the resulting increase in N and P content per leaf area. In contrast, we found no indication of a higher carboxylation efficiency at high elevations as was reported, for example, in Hawaiian montane forests. In fact, V_{cmax} at ambient temperature was lower at 3000 than at 1000 m while J_{max} was higher. This indicates a shift toward increased investment in electron transport capacity with increasing elevation as physiological response to the cloudy and moist environment. The multiple regression analysis identified mass-related foliar P as the most important environmental factor influencing the variation in A_{max} between the three elevation levels and among the 40 tree species examined. This highlights the role of P supply for the photosynthetic capacity of tropical trees which was much more important than N. However, from the large decrease in soil N availability with increasing elevation, the low foliar N concentration in the uppermost stand, and the marked LAI decrease with elevation, we assumed that N shortage may reduce the carbon gain of trees at high elevations mainly through limiting the assimilating surface area. Our findings are not only important for achieving a mechanistic understanding of elevational change in tree carbon gain and climate warming response, but they may also contribute to the debate on the causes of alpine tree lines in tropical mountains.

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CHAPTER 4

Ammonium, nitrate and glycine uptake of six Ecuadorian tropical montane forest tree species: an *in situ* pot experiment with saplings

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Abstract

Not much is known about the nitrogen (N) uptake capacity and N-form preference of tropical trees. In a replicated labelling experiment with ^{15}N-ammonium, ^{15}N-nitrate and dual-labelled glycine applied to saplings of six tree species from southern Ecuadorian montane forests, we tested the hypotheses that (1) the saplings of tropical trees are capable of using organic N even though they are forming arbuscular mycorrhizas, and (2), with increasing elevation, tree saplings increasingly prefer ammonium and glycine over nitrate due to reduced nitrification and growing humus accumulation. Three- to 5-yr-old saplings of two species each from 1000, 2000 and 3000 m elevation were grown in pots inside the forest at their origin and labelled with non-fertilizing amounts of the three N forms; ^{15}N enrichment was detected 5 d after labelling in fine roots, coarse roots, shoots and leaves. The six species differed with respect to their N-form preference, but neither the abundance of ammonium and nitrate in the soil nor elevation (1000-3000 m asl) seemed to influence the preference. Two species (those with highest growth rate) preferred NH_4^+ over NO_3^-, while the other four species took up NO_3^- and NH_4^+ at similar rates when both N forms were equally available. After ^{13}C-glycine addition, ^{13}C was significantly accumulated in the biomass of three species (two species with exclusively AM and one species with ECM and AM symbionts) giving a first hint toward organic N use by tropical montane forest trees irrespective of the type of their mycorrhiza.

Key words: dual-labelled glycine, elevational gradient, *Graffenrieda harlingii, Hedyosmum translucidum, Hedyosmum purpurascens, Hedyosmum sprucei*, *Myrcia* sp. nov., nitrogen uptake, *Pouteria torta*, ^{15}N tracer study

Introduction

Nitrogen (N) and phosphorus (P) are thought to be the principal growth-limiting nutrient elements in tropical rainforests (Tanner *et al.* 1998) but their relative importance is not entirely clear and seems to vary with site conditions. While P likely is limiting the productivity of many tropical lowland forests, N shortage may be more decisive in tropical montane forests on younger soils and under lowered temperatures (Paoli *et al.* 2005, Tanner 1981, Vitousek & Sanford 1986). Studies along elevation gradients in tropical mountains found marked decreases in ammonification rate with elevation and even steeper decreases in nitrification rate, because the activity of autotrophic nitrifiers is particularly sensitive to the cool and often acidic soil conditions at higher elevations (Jones *et al*. 2009, Marrs *et al.* 1988, Wolf *et al.* 2011). Thus, not only the relative importance of N and P shortage may vary with elevation, but the supply rates of NH_4^+ and NO_3^- and the relative availability of the two N forms as well. Moreover, the depth of organic layers on top of the soil was found to greatly increase with elevation in tropical mountains (Moser *et al.* 2011, Wolf *et al.* 2011) suggesting that organic N forms should be more readily available for plant use at high elevations. As a consequence, N supply should vary greatly across elevation and exposition gradients in tropical mountain forests. Given that many tropical mountain forests are rich in tree species, one might assume that the heterogeneity in N supply patterns is associated with plurality in N acquisition strategies in the trees. Studies in temperate and boreal forests suggest that trees with apparent preference of ammonium are more abundant in cold environments and that the importance of organic N forms for tree nutrition increases with decreasing decomposition and N mineralization rate (Finzi & Berthrong 2005, Kielland 1994, 1997, Näsholm *et al.* 1998). If these patterns are also valid in tropical mountains, we predict a higher abundance of nitrate-preferring trees at lower elevation and a dominance of trees with preference of organic N and/or ammonium at higher elevation. However, in contrast to temperate and boreal forests, the large majority of tropical tree species seem to form arbuscular mycorrhizas (AM) (Kottke *et al.* 2004)

and not ectomycorrhizal associations (ECM).

So far, information on the preference of different N forms by tropical trees is lacking. N acquisition strategies in tropical forest plants have been studied for hemi-epiphytic Clusiaceae (Arndt *et al.* 2002, Wanek *et al.* 2002), understorey palms (Andersen & Turner 2013), and rain-forest bryophytes (Wanek & Pörtl 2008), but not for trees. The first study showed that hemi-epiphyte *Clusia minor* can use ammonium, nitrate and also glycine under greenhouse conditions, but three other *Clusia* species seem to prefer NH_4^+ or glycine over NO_3^- under field conditions (Wanek *et al.* 2002). Andersen & Turner (2013) found seedlings of understorey palms to be able to use organic nitrogen with no preferences for chemical forms of N but an overall acquisition pattern of glycine ≥ NO_3^- ≥ NH_4^+. It is not known whether N form preferences differ among co-occurring dicotyledonous tree species in species-rich tropical forests and whether preferences change with alteration in inorganic and organic N availability along mountain slopes.

In this study, we examined the uptake of ammonium, nitrate and glycine by seedlings of six native tree species from three Ecuadorian montane forests that were grown under natural conditions in pots inside the forest at 1000, 2000 and 3000 m asl (two species per elevation). NH_4^+, NO_3^- and glycine were added at low doses (5 kg N ha^{-1}) with ^{15}N-labelled solutions (or dual-labelled glycine solution) and the plants were harvested after 5 d. For measuring N uptake under conditions as close to nature as possible, we used intact plants instead of excised roots and grew the plants under the characteristic low-light conditions on the forest floor where they received natural rainfall. The main objectives of the study were (1) to search for a significant elevation effect on the N form preference in tropical tree species, and (2) to examine the role of organic nitrogen (glycine) for the nutrition of trees in tropical mountain forests. More specifically, we tested the hypotheses that (1) the saplings of tropical trees are capable of using organic N even though they form arbuscular mycorrhiza, and (2), with increasing altitude, tree saplings increasingly prefer ammonium and glycine over nitrate due to a lowered nitrification rate and increased humus accumulation.

Methods

Study sites

This study took place in tropical montane forests on the eastern slope of the South Ecuadorian Andes along a 2000-m elevation transect. Three study sites were selected at ca. 1000, 2000 and 3000 m asl in Podocarpus National Park and in the Reserva San Francisco in the Provinces of Loja and Zamora-Chinchipe (Figure 1). The study area has a tropical humid climate with a very wet season in April-July and experiences a less humid period from September to December (Bendix *et al.* 2006). Regularly occurring longer dry periods do not exist. Table 1 gives further details on the climatic conditions at the study sites.

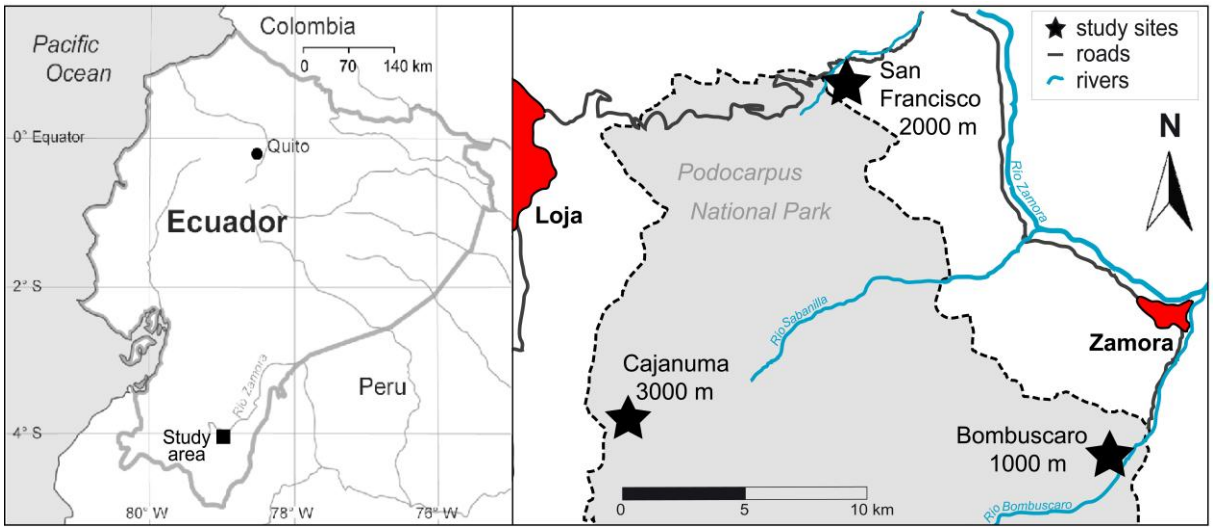

***Figure 1**. Location of the study area in southern Ecuador with the three stands at 1000, 2000 and 3000 m asl.*

According to Homeier *et al*. (2008), the forests at the study sites can be classified as follows: At 1000 m (4°7´S, 78°58´W), in the transition zone between tropical lowland and lower montane forest, evergreen forest with tree heights of up to 40 m is present. Common tree families of this forest type are Fabaceae, Melastomataceae, Moraceae, Myristicaceae, Rubiaceae and Sapotaceae. The evergreen lower montane forest at 2000 m (3°58'S, 79°04'W) achieves a canopy height of 18-22 m. Characteristic tree families are Euphorbiaceae, Lauraceae, Melastomataceae and Rubiaceae. At 3000 m (4°7´S, 79°11´W), evergreen upper montane forests and elfin-forests are found that extend up to the tree line; canopy height rarely exceeds 8-10 m. Dominant tree families are Aquifoliaceae, Clusiaceae, Cunoniaceae, Lauraceae and Melastomataceae.

All soils are acidic with a progressive pH decrease toward higher elevation. With increasing elevation, soil nutrient availability also decreases along the elevation transect. Both N net mineralization and nitrification rate decreased with elevation, as does the mineral N concentration of the topsoil (Table 1, after Wolf *et al.* 2011). The $\delta^{15}N$ signature of the mineral topsoil material and the organic layer decreases in general from 1000 to 3000 m by about 1.5‰ (difference significant between 2000 and 3000 m). The mean $\delta^{15}N$ value of tree sun leaves increased from +1.1‰ at 1000 m to c. +2.4‰ at 2000 m and rapidly dropped to -1.5‰ at 3000 m; foliar N concentration followed this pattern with a mid-slope peak at 2000 m (Wittich *et al.* 2012) (Figure 2). Kottke *et al.* (2004) investigated the mycorrhizal status of the tree species in the montane forests of southern Ecuador and found more than 95% of the species to be colonised by AM fungi without the formation of ECM. In our stand at 2000 m, five of ca. 300 abundant tree species including the genus *Graffenrieda* were found to form ECM (I. Haug, pers. comm.).

Table 1. Characteristics of the studied forest stands in the elevation transect in southern Ecuador (climate data from Moser *et al.* (2007); soil data from Wolf *et al.* (2011) and A. Baldos, unpubl. data). C/N ratio, pH, net nitrification and net N mineralization rate (in situ buried bag method) refer to the topsoil (0-10 cm), organic N concentrations to 0-5 cm.

Elevation (m asl)	1000	2000	3000
Rainfall (mm y^{-1})	c. 2230	c. 1950	c. 4500
Air temperature (°C)	19	16	9
Air humidity (%)	86	91	94
pH (H_2O)	4.9 ± 0.2	4.4 ± 0.2	3.9 ± 0.1
C/N	17.6 ± 0.8	14.8 ± 0.7	18.2 ± 0.9
Net N mineralization (kg N ha^{-1} per 10d)	2.5 ± 0.6	1.5 ± 0.3	0.1 ± 0.2
Net Nitrification (kg N ha^{-1} per 10d)	1.97 ± 0.73	0.89 ± 0.30	0.01 ± 0.01
KCl-extractable NO_3^- (kg N ha^{-1})	0.43 ± 0.10	0.24 ± 0.05	0.02 ± 0.01
KCl-extractable NH_4^+ (kg N ha^{-1})	1.8 ± 0.3	0.9 ± 0.1	0.7 ± 0.1
K_2SO_4-extractable organic N (mg kg^{-1})	36.3 ± 2.3	138.6 ± 10.6	127.6 ± 4.4

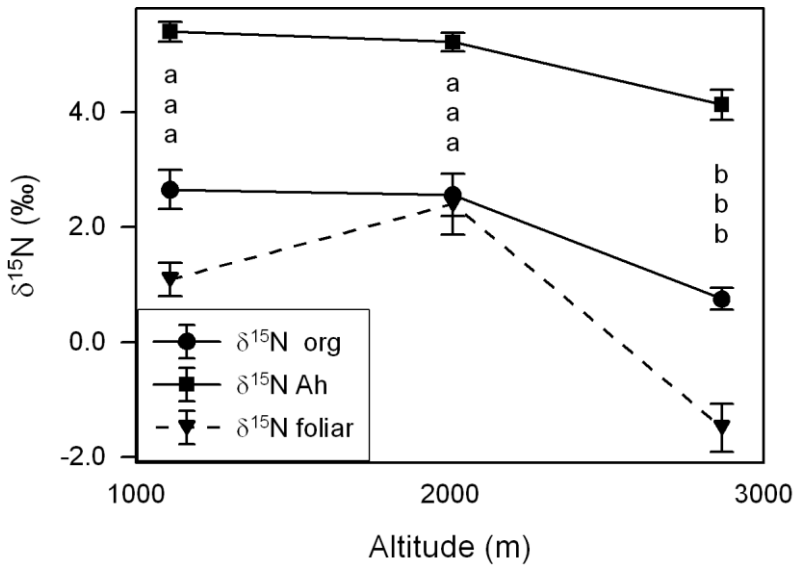

Figure 2. *$\delta^{15}N$ signatures (mean ± SE; 18 plots per elevation) in canopy leaves (mean of various tree species per elevation) and in the soil (organic layer and 0-30 cm of mineral soil) at 1000, 2000 and 3000 m asl in the study transect after data from Wolf et al. (2011) and unpublished data of K. Wolf.*

Plant material

The forests in the study area are extremely diverse with ≥800 tree species present (J. Homeier, unpubl. data) and no tree species was found to be abundant at all three study sites (1000, 2000 and 3000 m). We selected six tree species (two each per site) that were considered to be representative for the sites because they occurred more frequently in the stands than elsewhere: *Pouteria torta* (Mart.) Radlk. (Sapotaceae) and *Hedyosmum sprucei* Solms (Chloranthaceae) at 1000 m asl, *Myrcia* sp. nov (undescribed species, Myrtaceae) and *Hedyosmum translucidum* Cuatrec. (Chloranthaceae) at 2000 m, and *Graffenrieda harlingii* Wurdack (Melastomataceae) and *Hedyosmum purpurascens* Todzia (Chloranthaceae) at 3000 m. *Hedyosmum* is one of the very few genera found from 1000 to 3000 m asl in the study area. Characteristics of the six species are summarized in Table 2.

Some 4-6 mo before the start of the experiment, saplings of all species were collected from the three stands and planted into plastic pots. According to sapling monitoring studies in the field (Homeier *et al*., umpubl.), the plants were approximately 3-5 y in age at the time of the experiment. Average shoot height was 27 cm. The pots of 25 cm diameter and 25 cm height were filled with local forest soil from the sites where the saplings had been collected. We used mineral topsoil (upper 25 cm) from patches of undisturbed primary forest. The pots with each one sapling growing in it were placed on wooden tables at the three study sites in the interior of the local stands under closed forest canopy. Photosynthetically active radiation at the height of the pots was on average 4.5% of incident flux density (range = 2.04-7.14%; measured with a LI-1000 Quantum Sensor, Licor Biosciences, Lincoln, NE, USA). By placing two layers of fine-meshed polypropylene net on the soil surface of the pots, we prevented water logging after strong rainfall events.

Table 2. Characteristic parameters of the tree species investigated in this study. Given are ranges for mature trees growing in the study region (unpublished data) and means (± SE) for the seedlings studied for the tracer study. AM: arbuscular mycorrhiza; ECM: ectomycorrhiza (after I. Haug, pers. commun.). Number of replicates per treatment and per date (*three harvest dates for *P. torta* and *H. purpurascens*). The number of replicate measurements in brackets.

Elevation	1000 m asl		2000 m asl		3000 m asl	
Species	*Pouteria torta*	*Hedyosmum sprucei*	*Myrcia sp. nov*	*Hedyosmum translucidum*	*Graffenrieda harlingii*	*Hedyosmum cf. purpurascens*
Mycorrhizal status	AM	AM	AM	AM	ECM / AM	AM
Successional status	Late successional	Early successional	Late successional	Early successional	Early successional	Early successional
Mature trees						
Leaf N concentration (mg g^{-1})	14.1-17.8 (11)	18.4 (1)	8.5-11.4 (6)		13.4-15.3 (5)	13.9-16.9 (8)
SLA (cm^2 g^{-1})	66.6-99.8 (8)	126.5 (1)	32.4-36.8 (6)		49.4-62.5 (5)	64.5-87.2 (8)
Saplings						
Shoot height (cm)	27 ± 1	23 ± 1	12 ± 1	58 ± 3	22 ± 1	22 ± 1
Leaf N concentration (mg g^{-1})	20 ± 1	17 ± 1	15 ± 0	22 ± 1	17 ± 2	20 ± 1
Number of replicates per treatment	3*	5	4	4	5	4*

^{15}N and ^{13}C tracer application

For determining the optimal time of harvest in the ^{15}N labelling experiment, we conducted a preliminary study with *Pouteria torta* saplings at 1000 m and with *Hedyosmum purpurascens* saplings at 3000 m. We harvested the leaves of selected plants at seven different time steps (2 h–18 d) after adding ^{15}N-ammonium, ^{15}N-nitrate or ^{15}N-glycine solution and calculated the temporal development of ^{15}N accumulation into leaf biomass. This preliminary experiment with all three N forms indicated a measurable increase in the first 6 d and a very slow further increase (or even a decrease) in the ^{15}N values in the leaves when more than 6 d (up to 18 d) had passed after application. The harvest times were chosen according to these results and they are also based on the time lag of response found by Graefe *et al.* (2011) who conducted an experiment on the stimulation of tree fine-root growth by locally adding N, P or K at the study sites.

For every tree species, four treatments with three- to fivefold replication (Table 2, depending on plant availability) were established: (1) control (only water added), (2) addition of labelled nitrate ($NH_4{}^{15}NO_3$, 98 atom-%), (3) addition of labelled ammonium ($^{15}NH_4NO_3$; 98 atom-%), and (4) addition of ^{15}N ^{13}C dual-labelled glycine ($H_2{}^{15}N{}^{13}CH_2{}^{13}CO_2H$; 98 atom-%). Thus, the experiment consisted of c. 32 pots each (2 species × 4 treatments × 4 (3-5) replicates) at the three elevations. Since exclusive uptake of ammonium or nitrate leads to acidification or alkalinisation of the rhizosphere, we applied ammonium-nitrate with specific labelling of only one of the components (NH_4^+ or NO_3^-) in order to exclude soil pH effects on uptake kinetics. The ^{15}N tracer was added on 13 April 2010 to all pots (except for the control) at 1000 m asl and on 14 April 2010 to the pots at 2000 and 3000 m asl as 50 ml solution in a dose of 5 kg N ha^{-1} (0.3 g N per pot) calculated on the basis of the pot surface area.

In order to avoid losses of the added ^{15}N ammonium through nitrification, we added the nitrification inhibitor dicyandiamide (DCD, AlzChem Trostberg GmbH, Trostberg, Germany) to all ^{15}N-ammonium pots (20 mg 14 atom%-DCD-N); DCD is widely used in agriculture and decomposes in soil into non-toxic components (Di & Cameron

2004, Zacherl & Amberger 1990). The concentration used was shown to inhibit nitrification for 6-10 d in tropical soils (Verma *et al.* 2007).

Harvest and analysis

All investigated plants were harvested either 5 d after nutrient application (*H. sprucei* (1000 m), *Myrcia*, *H. translucidum* (2000 m), *Graffenrieda* (3000 m)), or 2, 5 and 8 d after application (*Pouteria* (1000 m), *H. purpurascens* (3000 m)) to document the temporal course of ^{15}N acquisition in plant biomass. In the latter two species, a three times larger number of experimental plants was cultivated.

Plants were cut into leaves, shoot and roots. The roots were washed immediately to remove all soil. The plant material was dried at 70°C for 48 h and transferred to Germany. Roots were separated into coarse roots and fine roots (diameter of dried fine roots < 1.5 mm) and all plant material was weighed.

The ^{15}N and ^{13}C concentrations and the total concentrations of N and C in the plant biomass were determined with an elemental analyser (NA 1108, Fisons-Instruments, Rodano, Milano, Italy) coupled with an isotope mass ratio spectrometer (Delta plus, Finnigan MAT, Bremen, Germany) in the Laboratory for Stable Isotope Research at Göttingen University (KOSI). The ^{15}N concentration in the dry mass of the organs of all treatments including the control was calculated as atom% ^{15}N of total N. Percental recovery of ^{15}N in a given organ is the total amount of ^{15}N (minus the background level, i.e. untreated control plants) detected in the organ's biomass related to the ^{15}N amount added to the pot at the experiment's start.

In the case of dual-labelled glycine, we calculated the ^{15}N concentration of the sample after $^{15}N^{13}C$-glycine addition with two different approaches. The first enrichment value was derived directly from the ^{15}N values measured with the mass ratio spectrometer (termed glycine-^{15}N approach hereafter). This calculation should include all ^{15}N that is accumulated in that plant organ (the balance of influx into minus efflux out of the organ) from the labelled glycine either through uptake of intact

glycine or glycine deaminated prior to plant uptake. The second approach (glycine-^{13}C) corrects this figure by considering the accumulation of ^{13}C based on the following equation:

$$^{15}N(glycine\text{–}^{13}C) = \frac{0.5(A_{CG} - A_{CC})T_{CG}B_G M(N)}{T_{NG}B_G M(C)} + A_{NC}$$

with A_{CG} being the ^{13}C concentration of the glycine-treated plants (in atom-%), A_{CC} the mean ^{13}C concentration of the control plants (in atom-%), T_{CG} the total C concentration of the glycine-treated plants (in g g^{-1}), B_G the biomass of the glycine-treated plants (in g), T_{NG} the total N concentration of the glycine-treated plants (in g g^{-1}), M(N) the molar mass of ^{15}N (in g mol^{-1}), M(C) the molar mass of ^{13}C (in g mol^{-1}), and A_{NC} the ^{15}N concentration of the control (in atom-%).

This calculation assumes that ^{13}C enrichment is a reliable indicator of the synchronous uptake of the C skeleton and the amino group of the glycine molecule. The glycine-^{15}N approach may overestimate the amount of glycine taken up by the plant due to deamination in the soil prior to plant uptake, while the glycine-^{13}C approach may underestimate the amount due to ^{13}C loss in the form of $^{13}CO_2$ respired after plant uptake (Näsholm & Persson 2001). By plotting the $^{13}C_{excess}$ values against the corresponding $^{15}N_{excess}$ values, we tested for glycine uptake in intact form which would show a 2:1 line.

Data analysis

Data analysis focused on treatment differences within a species, i.e. acquisition of NH_4^+, NO_3^- or glycine relative to the untreated control which served for obtaining the ^{15}N background levels. We refrained from analysing for species differences because the six species differed largely in growth rate. Analysis of variance (Scheffé`s test) was used to conduct multiple comparisons among the means of the three stands. If the data were not normally distributed according to a Shapiro-Wilk test, the Mann–Whitney two-sample test (Wilcoxon U test) was used instead of Scheffé`s test. All

calculations were conducted with SAS software (version 9.1; SAS Institute, Cary, NC, USA). A significance level of 5% was used throughout the analysis.

Results

Uptake of different N forms

Five d after tracer application, we found a characteristic pattern of tracer enrichment in the plants with generally highest ^{15}N concentrations in fine roots and a decrease in the sequence coarse roots – shoot – leaves in all six species and all three N forms (Table 3). High atom-% ^{15}N values were found in the fine roots of H. sprucei (7.53 atom-%) while leaves typically did not exceed 1 atom-% (except in H. sprucei and G. harlingii). In the two treatments with inorganic N addition (ammonium-nitrate), in general more ^{15}N label was accumulated when NH_4^+ was labelled as compared to NO_3^- labelling which indicates higher ammonium uptake when both N forms were equally available (Table 3). However, the difference between the two treatments was only significant in certain species and biomass fractions (H. sprucei: leaves; H. translucidum; fine roots, coarse roots and shoot).

Figure 3 gives the temporal development of ^{15}N accumulation in two species, P. torta at 1000 m asl and H. purpurascens at 3000 m, 2, 5 and 8 d after application. In H. purpurascens at the high-elevation site (3000 m), ^{15}N recovery increased from day 0 to day 2 and further to day 5 (slight increase in fine roots, marked increase in total biomass) in all treatments, and decreased from day 5 onwards (except for the ammonium treatment which showed further increase). In P. torta, the accumulation patterns were in general similar but ^{15}N recovery in the biomass was much lower in this species. In contrast to H. purpurascens, the ^{15}N content in the glycine treatment of the P. torta saplings increased strongly between day 5 and day 8 when calculated as glycine-^{15}N.

Table 3. ^{15}N concentration (in atom-%) in the saplings of six tree species grown in pots outdoor inside three tropical montane forests in southern Ecuador at 1000, 2000 and 3000 m asl that were harvested 5 d after the application of labelled nitrate, ammonium or glycine (means ± SE, N = 3-5). The ^{15}N enrichment in the glycine treatment is presented either as uncorrected value (glycine-^{15}N) or corrected to the amount of ^{13}C accumulated which may indicate uptake of intact glycine molecules (glycine-^{13}C). Some saplings (such as all *Myrcia* saplings) had no coarse roots; consequently, some values and statistics are missing. Different letters indicate significant differences between treatments.

Species	Organ	^{15}N concentration (atom-%)									
		Control		Nitrate		Ammonium		Glycine(^{15}N)		Glycine(^{13}C)	
1000 m asl											
Pouteria torta	Fine roots	0.44 ± 0.03	a	1.00 ± 0.26	a	1.10 ± 0.36	a	0.93 ± 0.15	a	0.46 ± 0.01	a
	Coarse roots	0.44 ± 0.01	a	0.54 ± 0.02	b	0.67 ± 0.02		0.48 ± 0.01		0.49 ± 0.02	a
	Shoot	0.42 ± 0.02	a	0.43 ± 0.02	a	0.52 ± 0.08	a	0.42 ± 0.01	a	0.45 ± 0.00	a
	Leaves	0.38 ± 0.00	a	0.39 ± 0.00		0.39 ± 0.01	a	0.39 ± 0.00	a	0.39 ± 0.00	a
Hedyosmum sprucei	Fine roots	0.48 ± 0.03	a	3.93 ± 1.30	ab	6.28 ± 0.77	b	7.53 ± 1.27	b	0.55 ± 0.04	a
	Coarse roots	0.59 ± 0.08	a	1.72 ± 0.18	ab	3.82 ± 0.42	bc	4.24 ± 0.67	c	1.77 ± 0.56	ab
	Shoot	0.43 ± 0.02	a	2.43 ± 0.97	b	4.12 ± 0.53	b	3.60 ± 0.72	b	0.51 ± 0.01	c
	Leaves	0.39 ± 0.01	a	0.69 ± 0.08	b	1.56 ± 0.16	c	1.76 ± 0.46	c	0.41 ± 0.01	a
2000 m asl											
Myrcia sp. nov	Fine roots	0.60 ± 0.10	a	1.05 ± 0.06	a	1.26 ± 0.32	a	1.26 ± 0.26	a	0.80 ± 0.11	a
	Coarse roots										
	Shoot	0.56 ± 0.13	a	0.79 ± 0.06	a	0.81 ± 0.04	a	1.12 ± 0.38	a	0.75 ± 0.17	a
	Leaves	0.42 ± 0.02	a	0.69 ± 0.19	a	0.58 ± 0.06	a	0.59 ± 0.06	a	0.42 ± 0.01	a

		Control		Nitrate		Ammonium		Glycine(^{15}N)		Glycine(^{13}C)	
Hedyosmum translucidum	Fine roots	0.43 ± 0.03	a	1.22 ± 0.11	b	2.44 ± 0.22	c	2.03 ± 0.12	c	0.58 ± 0.03	a
	Coarse roots	0.43 ± 0.02	a	1.10 ± 0.11	bc	2.45 ± 0.20	d	1.56 ± 0.15	c	0.59 ± 0.04	ab
	Shoot	0.38 ± 0.00	a	0.69 ± 0.05	b	1.28 ± 0.19	c	1.17 ± 0.21	bc	0.41 ± 0.01	d
	Leaves	0.37 ± 0.00	a	0.43 ± 0.01	b	0.59 ± 0.06	b	0.43 ± 0.02	b	0.38 ± 0.00	a
3000 m asl											
Graffenrieda harlingii	Fine roots	0.69 ± 0.05	a	1.68 ± 0.25	ab	2.06 ± 0.52	ab	2.78 ± 0.29	b	0.91 ± 0.12	a
	Coarse roots	0.49 ± 0.03	a	1.02 ± 0.09	bc	1.24 ± 0.21	bc	1.72 ± 0.41	b	0.79 ± 0.17	c
	Shoot	0.40 ± 0.01	a	0.70 ± 0.04	b	1.18 ± 0.26	b	2.20 ± 0.18	c	0.47 ± 0.01	d
	Leaves	0.40 ± 0.01	a	0.60 ± 0.06	b	1.31 ± 0.38	bcd	1.47 ± 0.18	c	0.40 ± 0.00	ad
Hedyosmum cf. purpurascens	Fine roots	0.53 ± 0.04	a	2.89 ± 0.75	ab	3.47 ± 0.69	b	2.92 ± 0.62	ab	0.68 ± 0.08	
	Coarse roots	0.54 ± 0.02	a	2.27 ± 0.62	a	2.45 ± 0.19		1.89 ± 0.98		0.82 ± 0.01	
	Shoot	0.40 ± 0.01	ac	1.78 ± 0.82	b	1.90 ± 0.83	ab	0.92 ± 0.24	b	0.46 ± 0.00	c
	Leaves	0.39 ± 0.01	a	0.66 ± 0.18	b	0.63 ± 0.11	bc	0.90 ± 0.27	bc	0.39 ± 0.00	ac

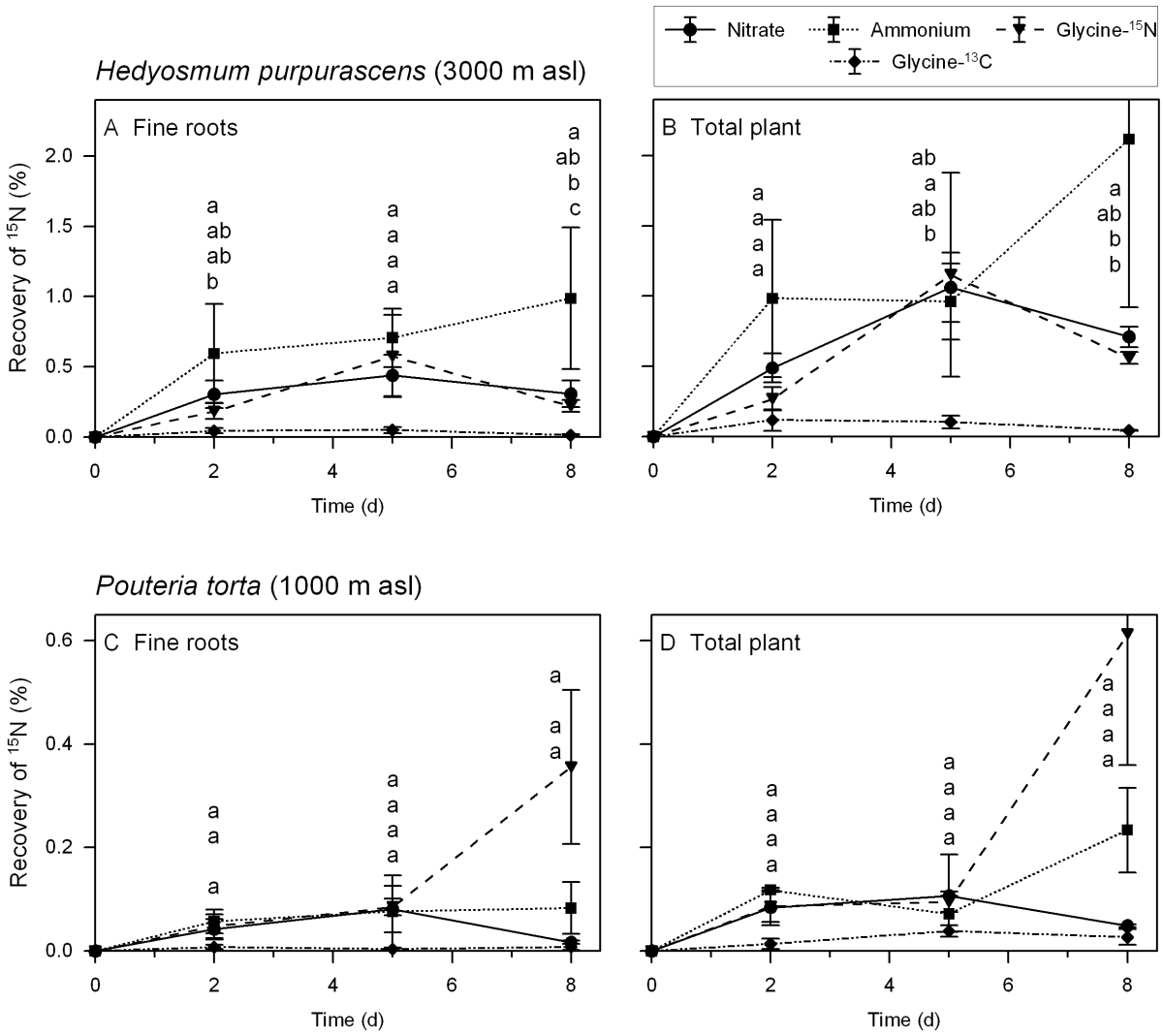

***Figure 3**. Temporal development of the ^{15}N content in the fine-root biomass or total plant biomass of saplings of Hedyosmum purpurascens (3000 m asl, upper row) and Pouteria torta (1000 m asl, lower row) 2, 5 and 8 d after application of labelled fertilizer to the soil. The saplings were cultivated in pots and grown outdoor inside the natural forest under a closed forest canopy. The ^{15}N enrichment in the glycine treatment is presented either as uncorrected value (glycine-^{15}N) or corrected to the amount of ^{13}C accumulated which may indicate uptake of intact glycine molecules (glycine-^{13}C). Some saplings (such as all Myrcia saplings) had no coarse roots; consequently, some values and statistics are missing. Different letters indicate significant differences between treatments. N = 3-4.*

The distribution to different organs of the ^{15}N accumulated in the plants after 5 d revealed a considerable variation in N allocation patterns among the six species and also for the different treatments but no clear elevational trend (Table 3, Figure 4). The species with very low ^{15}N accumulation, P. torta, accumulated a relatively large proportion of the N tracer in the fine or coarse roots (nitrate and glycine-^{15}N vs. ammonium treatment) with 85-95% of the ^{15}N remaining in the below-ground organs. These differences are partly related to species differences in carbon allocation patterns; P. torta and Graffenrieda saplings had a particularly large root biomass (35% of total, Table 4).

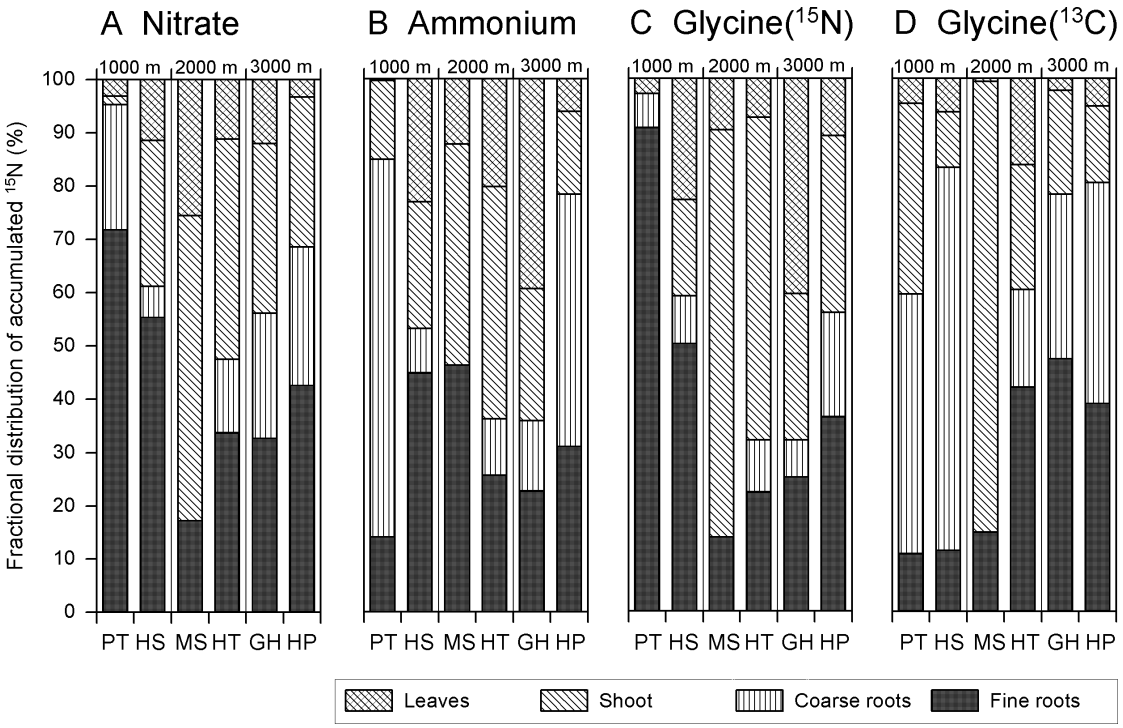

Figure 4. *Distribution to leaves, shoot, coarse and fine roots of ^{15}N taken up by the plant from labelled nitrate, ammonium or glycine solution (in % of total ^{15}N uptake). The ^{15}N enrichment in the glycine treatment is presented either as uncorrected value (glycine-^{15}N) or corrected to the amount of ^{13}C accumulated which may indicate uptake of intact glycine molecules (glycine-^{13}C). Some individuals (such as all Myrcia seedlings) had no coarse roots; consequently, this category is missing here.*

Table 4. Biomass of the saplings at the date of harvest (in g per plant and in % of total biomass). Some individuals (such as all *Myrcia* seedlings) had no coarse roots; consequently, values are missing.

Elevation	1000 m		2000 m		3000 m	
Species	*Pouteria torta*	*Hedyosmum sprucei*	*Myrcia* sp. nov.	*Hedyosmum translucidum*	*Graffenrieda harlingii*	*Hedyosmum purpurascens*
Leaves	0.37 (21%)	1.02 (43%)	0.08 (22%)	6.63 (42 %)	0.42 (21%)	0.79 (42%)
Shoots	0.78 (44%)	0.65 (28%)	0.25 (67 %)	6.83 (44%)	0.86 (43%)	0.57 (30%)
Coarse roots	0.45 (26%)	0.34 (14%)		1.17 (7%)	0.43 (21%)	0.29 (15%)
Fine roots	0.16 (9%)	0.35 (15%)	0.04 (11 %)	1.00 (6%)	0.29 (14%)	0.23 (12%)
Plant total	1.76 (100%)	2.35 (100%)	0.37 (100 %)	15.64 (100%)	2.00 (100%)	1.87 (100%)

Glycine incorporation

After adding dual-labelled $^{15}N^{13}C$-glycine, much more ^{15}N was accumulated in the biomass than ^{13}C which resulted in the calculation of much higher apparent glycine uptake rates when considering the ^{15}N enrichment (glycine-^{15}N calculation) than when calculating with the accumulation of ^{13}C (glycine-^{13}C approach); the ^{15}N enrichment was often twofold higher than the corresponding ^{13}C enrichment. The difference between the glycine-^{15}N and glycine-^{13}C values was significant in H. sprucei (all organs), H. translucidum (all organs), G. harlingii (all organs), and H. purpurascens (shoots). An extreme case was the ^{15}N concentration in the fine-root biomass of H. sprucei which exceeded the ^{13}C-concentration nearly ten-fold (Table 3). In contrast, P. torta reached slightly higher glycine uptake rates according to the ^{13}C approach in the shoots and the coarse roots than when calculated through ^{15}N (glycine-^{15}N approach), but all values were very low in this species. All values of apparent glycine uptake according to the ^{13}C approach (glycine-^{13}C) were lower than the ^{15}N content after ^{15}N-nitrate and ^{15}N-ammonium addition. The glycine^{13}C values were only in a few cases significantly higher than those of the respective control treatment (in H. sprucei and H. translucidum in the shoot, in G. harlingii in the coarse roots and the shoot, and in H. purpurascens in the shoot). Thus, two Hedyosmum species and Graffenrieda exhibited a significantly higher ^{13}C label in at least one plant organ after addition of dual-labelled glycine. Plotting the $^{13}C_{excess}$ values against the corresponding $^{15}N_{excess}$ values showed much lower slopes (typically <0.4) than expected for the case of complete glycine incorporation as intact molecule (slope = 2.0) (Table 5 in the Appendix).

A simple addition of the whole-plant uptake rates from the respective ammonium, nitrate and glycine (glycine-^{13}C approach) experiments may be used for estimating the relative importance of the three N forms for the nitrogen nutrition of the six species, given that all N forms were available at similar abundances (Figure 5). Accordingly, 50-60% would have been taken up as NH_4^+, 20-50% as NO_3^- and 5-20% as glycine in the six species.

Tracer recovery

Between 0.02% and 6.28% of the added amount of ^{15}N was recovered in the biomass of the saplings 5 d after application (Figure 6 and 7). The total amount of ^{15}N recovered showed no significant preference for either ammonium or nitrate in any of the species. However, NH_4^+ tended to reach a higher accumulation in the total biomass than nitrate in H. sprucei and H. translucidum and in the fine root biomass of all three Hedyosmum species. Similar to the ^{15}N atom% values, the mean recovery of ^{15}N in total biomass was always lower in the glycine-^{13}C than the glycine-^{15}N approach (significant in H. sprucei and G. harlingii).

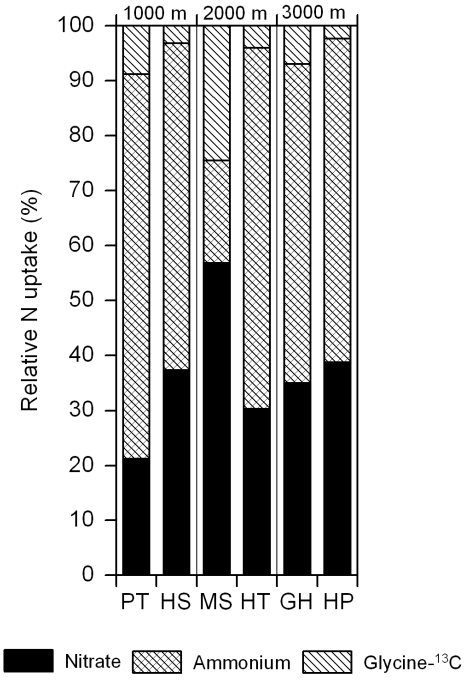

Figure 5. *Relative importance of nitrate, ammonium and glycine (calculated with the glycine-^{13}C calculation approach) in assumed total N uptake of the six species if all N forms were equally available. This calculation is a simple addition of the ^{15}N incorporation data for ammonium, nitrate and glycine and does not consider interactions among the uptake of the three N forms.*

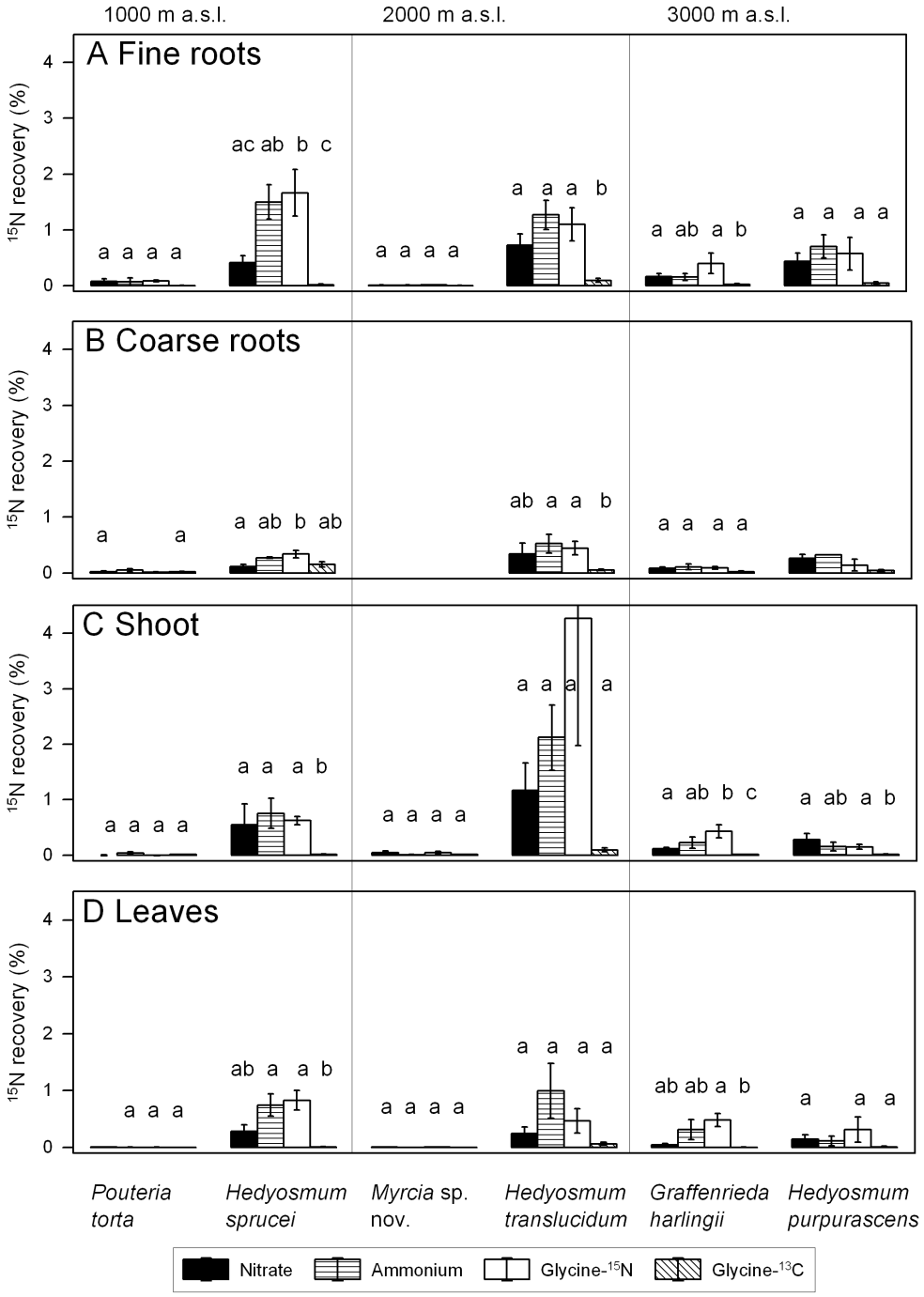

***Figure 6**. Recovery of ^{15}N in the biomass of saplings in % of the ^{15}N added for the six species in the fine roots (A), coarse roots (B), shoot (C) and leaves (D) five days after labelling with ^{15}N-nitrate, ^{15}N-ammonium, or $^{15}N^{13}C$-glycine (means ± SE). The ^{15}N enrichment in the glycine treatment is presented either as uncorrected value (glycine-^{15}N) or corrected to the amount of ^{13}C accumulated which may indicate uptake of intact glycine*

molecules (glycine-^{13}C). Some saplings (all Myrcia plants) had no coarse roots; consequently, some values and statistics are missing. Different letters indicate significant differences between treatments within a species.

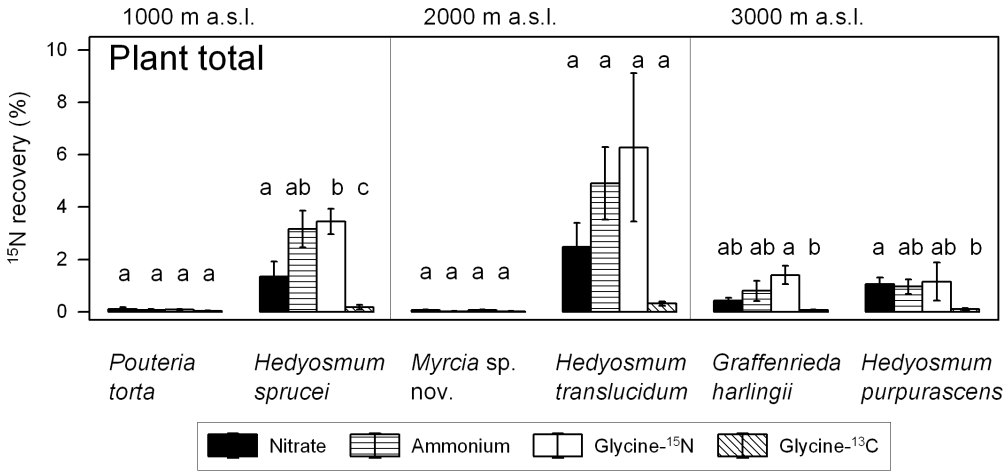

***Figure 7**. Recovery of ^{15}N in the biomass of saplings in % of the ^{15}N added for the six species in the total plant biomass five days after labelling with ^{15}N-nitrate, ^{15}N-ammonium, or ^{15}N^{13}C-glycine (means ± SE). The ^{15}N enrichment in the glycine treatment is presented either as uncorrected value (glycine-^{15}N) or corrected to the amount of ^{13}C accumulated which may indicate uptake of intact glycine molecules (glycine-^{13}C). Different letters indicate significant differences between treatments within a species.*

Discussion

The elevation transect in southern Ecuador is characterized by a large decrease in net N mineralization rate and an even steeper decrease in nitrification rate from 1000 to 3000 m (Wolf *et al.* 2011). With mineralization and subsequent nitrification, the upper montane forest receives less than 5% of the NH_4^+ and less than 1% of the NO_3^- of the pre-montane forest. At 1000 m, about 80% of the NH_4^+ released through mineralization is oxidized to NO_3^-, while it is only c. 10% at 3000 m resulting in increasing dominance of ammonium over nitrate on the cation or anion exchangers in the soil with increasing elevation (c. 80% of the exchangeable mineral N pool at 1000 m and c. 98% at 3000 m consists of NH_4^+). Data on the concentration of dissolved organic N (DON) show a marked increase from 1000 to 2000 m with growing humus layer thickness. At 2000 m, Goller *et al.* (2006) found 50%-70% of the soil solution N to be DON and 27%-43% NH_4^+; only 3%-5% referred to NO_3^-. As DON is released from soil organic matter mainly by microbial degradation (Guggenberger *et al.* 1994, Michalzik *et al.* 2001, Uselman *et al.* 2012), the DON fraction should increase in importance with increasing organic matter content of the soil. Thus, we expected that the relative abundance of organic N compounds and of ammonium both should increase with elevation at the expense of nitrate.

The great dominance of DON and NH_4^+ over NO_3^- in the soils at 2000 and at 3000 m is only partly reflected in the N form preference of the investigated tree species. Only one of the four species from 2000 and 3000 m (*H. translucidum*) took up ammonium more rapidly than nitrate when both N forms were equally available. Another species (*G. harlingii*) showed a tendency for NH_4^+ preference but the ^{15}N accumulation from added ammonium was not significantly higher than that from nitrate in any of the organs examined. At 1000 m with a higher abundance of nitrate in the soil, one species (*H. sprucei*) seemed to prefer NH_4^+ over NO_3^-, but the other species showed no difference in the uptake of nitrate and ammonium. Thus, our data from six relatively abundant montane forest tree species indicate that there seem to be species-specific differences in the N form preference but they were not related to the

abundance of ammonium and nitrate in the soil and thus apparently independent from elevation. While no species seemed to prefer NO_3^- over NH_4^+, we found apparent ammonium preference in a minority of tree species, in particular the species with highest sapling growth rates (unpubl. data). It must be kept in mind that experiments adding different N forms at equal concentrations (as done here) may not reflect actual N form preferences in the stands because the three forms occurred at very different abundances which could influence root uptake kinetics. Nevertheless, it appears that balanced uptake of NH_4^+ and NO_3^- seems to be preferred by the majority of species when both N forms are equally available. A similar conclusion was drawn from ^{15}N-uptake experiments in a five-species temperate broad-leaved forest by Jacob & Leuschner (2014). The existing two N-uptake studies for tropical rain-forest plants reported a higher ammonium than nitrate uptake in three hemiepiphytic *Clusia* species (Wanek *et al.* 2002) and no preferences for ammonium or nitrate for understorey palms (Andersen & Turner 2013).

Indirect evidence for differences in the use of ammonium or nitrate in tropical forests may be derived from the $\delta^{15}N$ signature of foliage and soil. Brearley (2012) concluded that the trees of a montane forest on acidic soil in Jamaica must prefer NH_4^+ over NO_3^- due to the isotopic similarity between the leaf and bulk soil signatures. In our transect, the altitudinal decrease in bulk soil $\delta^{15}N$ from 1000 to 3000 m matches well with the measured decrease in mineralization and nitrification rates along the slope and the very low nitrate availability at high elevations. However, low foliar $\delta^{15}N$ values in the trees at 3000 m in our study should not be mistaken as indication of NH_4^+ preference; in fact, the uptake experiments showed that nitrate and ammonium were incorporated at roughly similar rates by the two species from this elevation. It should be noted that the soil chemical conditions measured at 1000, 2000 and 3000 m in the stands (see Table 1) are not necessarily exactly those established in the pots, even though we used local soil.

Our data from dual-labeling provide some evidence that intact glycine molecules are used as an additional N source by certain montane forest species and that this capability is not restricted to ECM species. According to the ^{13}C incorporation data of

the glycine-^{13}C calculation approach, glycine skeletons showed a significant accumulation relative to the control in at least one plant organ in three species (*H. sprucei, H. translucidum* and *G. harlingii*), with the first two species having exclusively AM, the latter ECM and AM symbionts.

The amount of ^{15}N incorporated in the biomass after feeding with labelled glycine was in most cases similar to the ^{15}N accumulation after ammonium addition and typically exceeded the ^{15}N incorporation from glycine as derived from the ^{13}C data by factors of two to four. This is expressed by very small slopes (typically <0.4) of the regression line $^{13}C_{excess}$ vs. $^{15}N_{excess}$ values in the biomass of the plants (see Table 5 in the Appendix). This discrepancy suggests that much of the glycine has been deaminated in the soil in the 5 d before harvest and that most ^{15}N was subsequently taken up as NH_4^+ or NO_3^-. The glycine^{15}N calculation should therefore largely overestimate glycine uptake while the ^{13}C-based calculation must reflect the minimum uptake of intact glycine because part of the accumulated C skeletons may already have been lost via respired $^{13}CO_2$ in the days after the start of the experiment. Our results suggest that ^{15}N tracer studies on the uptake of organic N in the tropics using single-labelled glycine as the study on understorey palms in a lower montane forest in Panama by Andersen & Turner (2013) might largely overestimate the actual uptake of organic N. Studies with dual-labeled amino acids as conducted here also have been criticized for possible shortcomings such as possible uptake of labelled inorganic C through the roots (e.g. Rasmussen & Kuzyakov 2009). Therefore, our findings can only be a first step towards a proof of the use of intact organic N sources in tropical trees. The decreasing ^{13}C content in the biomass of *H. purpurascens* after day 5 of the experiment (Figure 4) may relate to respirative C losses. Our data are not comprehensive enough to prove an altitudinal increase in the use of glycine as it was found in the tree species of a temperate mountain by Averill & Finzi (2011).

One of the factors that could lead to contrasting N uptake rates and differences in N-form preference among the co-occurring tree species of a species-rich tropical forest is phenology. Two species of our sample (*P. torta* at 1000 m and the unnamed *Myrcia*

species at 2000 m) showed only poor sapling growth in the experiment and the plants accumulated only very small amounts of ^{15}N from the added tracer which made it impossible to detect preferences for certain N forms. *Pouteria torta* shows leaf flushing in January and February and reduces growth thereafter with presumably reduced N demand. This species and also the *Myrcia* species are typical late-successional trees with normally slower growth than more light-demanding species. The small fine- and coarse-root systems of the two species may be related to the generally slow growth rates which are a likely explanation of the low N uptake of these species.

Future studies on N uptake patterns in species-rich tropical forests should examine possible relationships between light demand, growth rate, type of mycorrhiza and N uptake capacity and N-form preference among the co-occurring species. Relationships between these traits may only become visible when a much larger number of species is investigated. Further, the study of organic N use should be extended to include other larger and charged amino acid species as well.

Conclusions

Our knowledge about the nitrogen uptake capacity and N form preference of tropical montane forest trees is rudimentary. This study with six tree species provides some of the first information on uptake rates into fine roots and the whole plant under field conditions, on possible preferences for ammonium or nitrate, and on the role of organic N (glycine) for the N nutrition of trees. Despite the large decrease in N supply rate from 1000 to 3000 m asl, we found no indication of an altitudinal shift in N-form preference. Future studies in a larger number of tree species should search for more profound evidence (e.g. through triple-labeling of amino acids) that organic N indeed is playing a significant role in the N nutrition of these forests on humus-rich cool soils, and how uptake rates are dependent on tree functional traits and mycorrhiza type. In

addition, this could lead to a better understanding of the importance of phylogeny versus elevation in the N nutrition of tropical trees.

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CHAPTER 5

Synthesis

Background

In tropical moist forests, nitrogen availability is an important factor regarding forest productivity but, in the lowlands, often not limiting. Most tropical lowland forests are supposed to be phosphorus limited (Paoli et al., 2005; Tanner et al., 1998). In montane tropical forests, nitrogen limitation should become more crucial with elevation, mainly, due to reduced decomposition and mineralization rates and, therefore, lower availability of nitrogen for plants at higher altitudes. Moreover, reduced decomposition rates at higher altitudes lead to thicker organic soil layers and a change in available nitrogen forms from mostly inorganic at low elevations to mostly organic at high elevations (Wolf et al., 2011). However, the knowledge about the preference of tropical trees for different nitrogen forms is very limited and, to date, it remains an unanswered question, if tropical trees are able to take up significant amounts of organic nitrogen and how they adapt to different nitrogen forms being available at different altitudes.

Nitrogen availability along elevational gradients in tropical montane forests should also have an effect on the photosynthetic capacity of trees which is related to foliar nitrogen content mainly because of the high nitrogen demand for the proteins of the Calvin cycle and thylakoids which represent the majority of foliar nitrogen (Evans, 1989). Other possible factors controlling photosynthetic capacity which change along altitudinal gradients are the availability of phosphorus, temperature, the partial pressure of CO_2 [CO_2], VPD and radiation.

The study was conducted in three forest stands at 1000, 2000 and 3000 m elevation and aimed at answering the following main questions:

(1) How is the photosynthetic capacity of tropical trees affected by altitude?

(2) Which factors are controlling the photosynthetic capacity of tropical trees along altitudinal transects?

(3) Does the preference for different nitrogen forms of tropical tree saplings change with altitude?

Photosynthetic capacity as affected by altitude

With increasing elevation trees have to cope with changes in environmental conditions that should lead to decreasing photosynthetic capacities, mainly reduced temperatures, reduced [CO_2], reduced availability of nutrients and reduced VPD and radiation due to increased cloudiness (Angert, 2006; Körner, 2007). Trees adapt to these environmental conditions by changes in leaf morphology and physiology (Cordell et al., 1999) to maintain photosynthetic capacities as high as under the more favourable conditions in the lowlands. In a study on gas exchange of mature trees, we found light saturated net photosynthesis at ambient temperatures and [CO_2] (A_{sat}) in the lower, pre-montane stand to be only slightly decreased compared to tropical lowland forests (e.g. Reich et al., 1999; Santiago and Wright, 2007; Santiago et al., 2004) with a mean A_{sat} of 8.8 µmol CO_2 m^{-2} s^{-1} for 15 tree species. When moving 2000 m in upslope direction, we measured a slightly lower mean A_{sat} of 7.2 µmol CO_2 m^{-2} s^{-1} for 10 tree species at 3000 m. Hence, the trees in the studied montane forests seem to nearly have compensated for the decreasing temperatures (- 10 K) and other changes in environmental conditions along the elevational gradient and to have maintained a photosynthetic capacity at 3000 m elevation almost as high as that at 1000 m. Similar to A_{sat}, no significant altitudinal trend was observed for leaf dark respiration (R_D).

However, A_{sat} measured at mid-elevation did not fit into this pattern of a constant or slightly decreasing photosynthetic capacity with elevation due to less favourable environmental conditions and compensation strategies of the tree species. In contrast, A_{sat} at 2000 m elevation was significantly increased (mean A_{sat} of 11.3 µmol CO_2 m^{-2} s^{-1} for 16 tree species) compared to both, the premontane forest at 1000 m and the upper montane forest at 3000 m.

The mid-elevation site of the elevational gradient studied in this thesis is characterized by high nitrogen and phosphorus availabilities and high foliar phosphorus and nitrogen contents per mass (Wolf et al., 2011). In addition, the very high foliar phosphorous and nitrogen contents of the tree individuals measured in this study might be a consequence of choosing many fast growing tree species with good nutrient supply at this site due to access restrictions.

Phosphorus, as well as nitrogen, is needed by plants to build the photosynthetic apparatus and to guarantee the availability of energy in form of ATP for leaf metabolism (Warren, 2011). Hence, a high photosynthetic capacity of leaves demands high foliar nitrogen and phosphorus contents. The mid-elevation site has furthermore the lowest annual precipitation of all three sites and a soil pH that tends to be higher compared to the other sites (Moser et al., 2007; Wolf et al., 2011). It can be assumed that site-specific conditions added to the influence of altitude on photosynthetic capacity, leading to an increased A_{sat} at mid-elevation.

We put the data on photosynthesis as affected by altitude acquired in a South Ecuadorian montane moist forest into the broader context of a pan-tropical literature survey for A_{sat} of mature tropical trees of c. 170 species in 25 stands located at elevations between 100 and 3700 m. According to the literature survey, A_{sat} decreases by 1.3 µmol CO_2 m^{-2} s^{-1} per km altitude increase. A_{sat} decreased in spite of decreasing SLA with altitude, indicating that tropical trees are not able to compensate completely for less favourable environmental conditions with increasing elevation by changes in leaf morphology and physiology.

The data on photosynthetic rates of the leaves at ambient temperatures and [CO_2] combined with data on leaf area index (LAI) (Moser et al., 2007) could be used to calculate annual gross photosynthesis at stand-level (Leuschner et al., 2013). Gross photosynthesis was estimated at 12.5 to 27.2 Mg C ha^{-1} yr^{-1} and was considerably reduced from 2000 to 3000 m elevation due to markedly decreasing photosynthetic capacities and LAI. This was accompanied by a decrease of autotrophic respiration, which may be linked to reduced growth rates, and a decrease of heterotrophic

respiration, slowing down decomposition and mineralisation. Leuschner et al. (2013) concluded that canopy carbon gain decreases largely with elevation in this tropical montane forest.

However, it should be considered that A_{sat} for shade leaves was not measured directly but estimated from the A_{sat} values of sun leaves. Furthermore, the measured sun leaves were accessed from the ground and, even if we measured only leaves that were exposed to full sun light at least part of the day, we cannot exclude the possibility that measurements of sun leaves from the upper part of the canopies would lead to higher A_{sat} values. Measurements of clear sun and additionally of clear shade leaves should improve the estimates of stand level gross photosynthesis, especially at 1000 and 2000 m elevation, were shade leaves constitute the biggest part of the canopies.

Moreover, the coefficients of among-species variation in A_{sat} and R_D of our study in South Ecuador accounted for 20 to 53 %. The 40 studied species represent only 5 % of the c. 800 tree species abundant in the study region and even the c. 170 species of the literature survey represent only a tiny number of the approximately 37000 woody taxa in the tropics (Odegaard, 2000). For a better understanding of the photosynthesis of tropical trees as affected by altitude, more species at different elevations should be studied. Choosing the studied tree species according to their dominance in a stand and differentiating between early successional and late successional species should give us a more accurate and detailed picture of the photosynthetic capacity of tropical trees.

Effect of temperature, partial pressure of CO_2 and nutrient availability on photosynthesis

A variety of environmental factors that change along elevational gradients, may limit photosynthetic capacity (A_{max}) in tropical montane forests (Letts et al., 2011). With increasing elevation, trees must try to maintain sufficient rates of photosynthesis in

spite of less favourable abiotic conditions such as decreasing temperature and [CO_2] and biotic factors as varying specific leaf area (SLA), foliar nitrogen and phosphorus content and wood specific gravity. Gas exchange measurements on 40 species of mature forest trees showed no altitudinal trend of A_{max} and R_D at ambient conditions, but A_{max} tended to be lowest in the stand at the highest elevation. When standardized to 25° C at all sites, A_{max} and R_D increased with elevation. Thus, A_{max} showed partial and R_D full homeostatic adjustment to adverse environmental conditions at higher elevations. Surprisingly, the maximum electron transport rate J_{max} increased with elevation even at ambient conditions possibly as a response to increased cloudiness at high elevations (Van de Weg et al., 2009). Along the elevational gradient, mass based foliar phosphorous content (P_m) was the most important factor influencing variations in A_{max} while foliar nitrogen, temperature and partial pressure of CO_2 had no effect on A_{max}. However, foliar nitrogen and temperature are probably influencing A_{max} indirectly through reduced leaf area. At high elevations, we saw a pronounced reduction in SLA but no increase in carboxylation efficiency. Hence, carbon gain at high elevations in tropical montane forests is probably influenced to a greater extend by reduced SLA and canopy leaf area than by adaptive modifications of leaf physiology and the photosynthetic apparatus.

Leaf dark respiration per leaf area seemed to remain constant with elevation due to the decrease of SLA. In contrast, stem and coarse root respiration decreased with elevation along the same altitudinal gradient in South Ecuador (Zach et al., 2008). Leuschner et al. (2013) concluded that temperature is influencing gross primary production and net primary production along the altitudinal gradient in South Ecuador directly and indirectly, with a decrease of autotrophic respiration, which may be linked to reduced growth rates, and a decrease of heterotrophic respiration, slowing down decomposition and mineralization. Nitrogen availability seems to influence gross primary production and net primary production mainly through a restriction of leaf area expansion with elevation (reduced N supply with decreasing temperature) which limits carbon gain in high altitudes (Leuschner et al., 2013).

Letts et al. (2011) studied A_{max} and various environmental factors along an elevational

gradient in Colombia. They assumed cloudiness to be the most important factor influencing A_{max} with increased cloudiness reducing the photosynthetic capacity of tropical tree species by decreased radiation and increased surface wetness of the leaves.

The effect of environmental factors on photosynthetic capacity might be dependent on geology, climate and the abundant tree species of a stand. From the results of our study on gas exchange in South Ecuador, we would expect A_{max} to decline with increasing elevation along elevational gradients with reduced phosphorous availabilities at high elevations and to show little or no altitudinal decline at slopes with good phosphorous supply at high elevations. Further studies should address the question if the effect of phosphorous on A_{max} is valid for other tropical mountains than the slope monitored in South Ecuador as well as the direct and indirect effects of nitrogen availability and temperature on A_{max}. A possibility would be the comparison of the development of A_{max} with elevation on slopes with young volcanic ash soils that are rich in allophanes and on slopes with acidic soils which have a low phosphorous availability. Gas exchange measurements on tropical montane forest trees in nutrient manipulation experiments could lead to a better understanding of the influence of nutrients on variations in A_{max}.

Altitude effects on the preference for different nitrogen forms

As tropical montane forests accumulate thick organic layers with increasing elevation (Moser et al. 2011; Wolf et al., 2011), one would expect that tropical tree species adapt to the high abundance of organic nitrogen forms at high elevations by developing the ability to take up organic nitrogen. However, most tropical tree species are supposed to form arbuscular mycorrhiza (AM), with ectomycorrhyzal (ECM) species being rather rare (Kottke et al., 2004). AM fungi are generally thought to lack the proteolytic capacity that was found in ECM species (Chalot and Brun, 1998) and thus to be more effective in capturing inorganic than organic nitrogen. A ^{15}N-^{13}C-

tracer study, conducted with tree sapling of two species each at 1000, 2000 and 3000 m elevation fertilized with labelled ammonium, nitrate and glycine, confirmed this assumption, as three of the six species were able to take up organic nitrogen in form of glycine, one being a ECM species (3000 m), two being AM species (1000 and 2000 m). As the ^{13}C enrichment in a plant after fertilization with double-labelled glycine indicates the synchronous uptake of the C skeleton and the amino group of the glycine molecule, the ^{13}C-enrichment in the saplings can be regarded as a good measurement for the minimum uptake of glycine.

The preference for a certain nitrogen form seems to be altitude-independent and thus independent of the abundance of different nitrogen forms in the soil. Of the six tree species, two species preferred ammonium over nitrate (at 1000 and 2000 m), and four species took up nitrate and ammonium at similar rates. In spite of a very steep decrease of net nitrogen mineralization rate and nitrification rate along the elevational gradient (Wolf et al., 2011) and a generally very low nitrate content of the soil solution nitrogen (3-5% at the mid-elevation site, Goller et al., 2006), species at all elevations seem to take up nitrate to a rather high extent when supplied with it. Tree seedlings of tropical lowland *Clusia* species have been shown to take up nitrogen from nitrate, ammonium and glycine as well, but to prefer ammonium (Arndt et al., 2002; Wanek et al., 2002).

The analysis of the $\delta^{15}N$-contents in the leaves of mature trees and in the organic soil layers and the mineral soil at the three sites yielded decreasing $\delta^{15}N$-values with elevation in all three compartments but most pronounced in the leaves, leading to an increasing $\Delta\delta^{15}N$- value between leaves and soil (difference $\delta^{15}N_{plant}$ - $\delta^{15}N_{soil}$) that could be indicating a more pronounced uptake of ammonium compared to nitrate (Amundson, 2003; Craine et al., 2009). As our tracer study showed no increasing preference for ammonium with altitude, increasing $\Delta\delta^{15}N$- values probably are a consequence of decreasing nitrate availability with elevation instead.

Leuschner et al (2013) concluded from an up-slope reduction of fine root respiration in spite of a large increase in standing fine root biomass at the altitudinal gradient in

South Ecuador that trees at high altitudes should have much reduced nutrient uptake activities of their fine roots compared to the trees at lower altitudes. Reduced nitrogen uptake activities with altitude were not visible in our experiment with the lowest uptake of nitrogen per biomass at 1000 m (*Pouteria)* and 2000 m (*Myrcia*) and the highest uptake of nitrogen at 1000 m elevation (*H. sprucei)*. In a nutrient manipulation experiment at the study site at 2000 m elevation, mature trees of *Myrcia sp.* tended to reduce growth upon nitrogen and phosphorus fertilization (Homeier et al., 2012).

The six studied tree species represent less than 1% of the c. 800 tree species abundant in the study area. For a better understanding of nitrogen uptake and preferences for different nitrogen forms, tracer studies with more species at different elevations should be conducted, especially for getting a clearer picture of the uptake capabilities of organic nitrogen by AM tree species in tropical montane forests. Furthermore, variation in natural abundance foliar $\Delta\delta^{15}$N-values along elevational gradients could help investigating the use of different nitrogen forms at the stand level even if some uncertainties of interpretation exist (e.g. Averill and Finzi, 2011 in temperate forests). Apart from studying nitrogen uptake in more tree species, one could additionally investigate the different forms of nitrogen in detail. Glycine, the amino acid we used in this study, is frequently used in studies on the uptake of soluble organic nitrogen by plants because it is small and charged neutral and, hence, taken up easily. However, glycine is only one of many organic nitrogen compounds that might be taken up by trees. Analysing additionally bigger amino acids charged negatively or positively, could help getting more accurate results on organic nitrogen uptake. It should be taken into account for future ^{15}N tracer studies in tropical montane forests, with 2 to 8 days between application and harvest, that it is necessary to use double-labelled instead of single-labelled glycine. In our experiment, the calculation of uptake rates from ^{15}N single-labelled glycine would have led to a more than 10-fold overestimation for some species. Dicyandiamide, the nitrification inhibitor we used in the ammonium treatment, worked well at 2000 and 3000 m elevation but came to its limits after 5 days at 1000 m elevation.

Suggestions for future research

As part of the Tropical Andes, the Andean forests of Ecuador belong to one of the world's hotspots of biodiversity (Myers et al., 2000). Brummitt and Lughadha (2003) even described the Tropical Andes as the "hottest hotspot" worldwide. In this thesis, we studied 6 tree species in a ^{15}N tracer study and forty tree species in a gas exchange study in South Ecuador, representing only less than 5 % of the c. 800 tree species in the study area. Globally, approximately 37000 woody taxa exist in the tropics (Odegaard, 2000). Consequently, more species should be studied in the future. Pooling the abundant species of one site instead of replicating the same species at a site is one possibility of including more species in a study and getting more representative results.

We measured the photosynthesis of sun-leaves of mature trees of representative species along the altitudinal gradient. To be more accurate in calculating canopy carbon gain, in a next step, light response curves of shade leaves and at various temperatures should be measured. It would be good, to get better access to the sun-canopy of the trees to be able to choose the abundant species without restrictions because of poor accessibility from the ground. Better access and bigger species numbers could make it possible to distinguish the photosynthetic capacity of different tree functional groups.

One outcome of the ^{15}N tracer study that we conducted at three different elevations was the importance of the use of a nitrification inhibitor (DCD for maximum 5 days) when studying the uptake of ammonium and the use of a double-labelled amino acid when studying the uptake of organic nitrogen. Apart from examining more species, the analysis of foliar $\delta^{15}N$ values could be a possibility to extend our knowledge of the acquisition of different nitrogen forms to the stand level. However, the interpretation of the foliar $\delta^{15}N$ values is often complicated. The best approach seems to be a combination of tracer study paying special attention to the mycorrhiza and the analysis of foliar $\delta^{15}N$ values (e.g. Averill and Finzi, 2011).

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CHAPTER 6

Summary / Zusammenfassung

Summary

With increasing elevation, the growth conditions of trees in tropical mountains become generally more adverse in terms of decreasing nutrient availabilities, decreasing temperatures and decreasing atmospheric concentration of carbon dioxide (CO_2). In tropical montane forests, reduced decomposition rates at higher altitudes lead to thicker organic layers and together with reduced mineralization and nitrification rates to a change in available nitrogen forms and nitrogen has been shown to limit productivity in these forests. How photosynthetic capacity (A_{max}) of tropical trees on the one hand, and nitrogen uptake capacity and nitrogen form preference on the other hand adapt to variation in environmental conditions along elevation gradients, is not precisely known.

The present study was conducted in three tropical montane forest stands along an elevational transect at 1000, 2000 and 3000 m asl in South Ecuador. It aimed (1) to quantify the photosynthetic capacity of adult tropical trees along the elevational transect by means of gas exchange measurements and to analyse the possible controlling effects of temperature, partial pressure of CO_2 and nutrient availability on photosynthesis and (2) to investigate altitudinal changes in the use of nitrate, ammonium and organic nitrogen sources by tropical forest trees by means of a stable isotope tracer study with seedlings.

Stand-level means of light-saturated net photosynthesis (A_{sat}) were 8.8, 11.3 and 7.2 µmol CO_2 m^{-2} s^{-1}; those of dark respiration (R_D) 0.8, 0.6 and 0.7 µmol CO_2 m^{-2} s^{-1} at 1000, 2000 and 3000 m elevation, respectively, with no significant altitudinal trend. Examining our data in the context of a pan-tropical A_{sat} data base for mature tropical trees (c. 170 species from 18 sites at variable elevation) revealed that area-based A_{sat} decreases in tropical mountains by, on average, 1.3 µmol CO_2 m^{-2} s^{-1} per km altitude increase (or by 0.2 µmol CO_2 m^{-2} s^{-1} per K temperature decrease). The A_{sat} decrease occurred despite an increase in leaf mass per area with altitude. Lowered A_{sat} together with a reduced stand leaf area decrease canopy carbon gain with elevation in tropical mountains.

The P content per leaf mass was the principal factor determining A_{max} across the altitudinal gradient while the effects of foliar N, temperature and [CO_2] were insignificant. A_{max} was subject to partial, and R_D to full homeostatic adjustment to the reductions in temperature and [CO_2] at higher elevations, mainly through a large reduction in SLA and the resulting increase in foliar N and P per leaf area, while no altitudinal increase in carboxylation efficiency was detected. We conclude that the altitudinal decrease in both SLA and canopy leaf area are more important determinants of carbon gain in tropical high-elevation forests than adaptive physiological modifications in the photosynthetic apparatus.

The seedlings of six tree species differed with respect to their nitrogen form preference but neither the abundance of ammonium and nitrate in the soil nor altitude seemed to influence the preference. Two species (those with highest growth rate) preferred ammonium over nitrate while the other four species took up nitrate and ammonium at similar rates when both nitrogen forms were equally available. After $^{15}N^{13}C$-glycine addition, ^{13}C was significantly accumulated in the biomass of three species (two species with arbuscular and one species with ectomycorrhizal symbionts) in addition to a siginficant ^{15}N accumulation indicating that trees in tropical mountain forests can use organic nitrogen sources irrespective of the type of their mycorrhiza.

Zusammenfassung

Mit zunehmender Meereshöhe werden die Wachstumsbedingungen in tropischen Bergregionen im Allgemeinen ungünstiger, was sich in einer sinkenden Nährstoffverfügbarkeit, sinkenden Temperaturen und sinkendem CO_2-Partialdruck zeigt. In tropischen Bergregenwäldern führen verminderte Abbauraten in größeren Höhen einerseits zu dicken organische Auflageschichten und andererseits in Kombination mit verminderten Mineralisierungs- und Nitrifizierungsraten zu Veränderungen in der Verfügbarkeit der verschiedenen Stickstoffformen, und es gibt Nachweise einer Limitierung der Produktivität dieser Wälder durch Stickstoff. Auf welche Weise sich die Photosynthesekapazität (A_{max}) tropischer Bäume einerseits und die Stickstoffaufnahmekapazität und Präferenz für einzelne Stickstoffformen andererseits an die veränderten Umweltbedingungen entlang von Höhengradienten adaptieren ist nicht genau bekannt.

Die vorliegende Untersuchung wurde in drei tropischen Bergregenwäldern durchgeführt, die entlang eines Höhengradienten auf 1000, 2000 und 3000 m ü. NN in Südequador liegen. Das Ziel war es, (1) die Photosynthesekapazität ausgewachsener tropischer Bäume entlang eines Höhengradienten mit Hilfe von Gaswechselmessungen zu bestimmen und die Effekte von Temperatur, CO_2-Partialdruck und Nährstoffverfügbarkeit auf die Photosynthese zu quantifizieren und (2) Veränderungen in der Verwendung von Nitrat, Ammonium und organischen Stickstoffquellen durch tropische Waldbäume mit der Meereshöhe mittels einer Tracer-Untersuchung mit stabilen Isotopen an Jungpflanzen zu untersuchen.

Mittelwerte der lichtgesättigten Photosyntheserate (A_{sat}) auf Bestandeseben betrugen 8.8, 11.3 und 7.2 µmol CO_2 m^{-2} s^{-1}, die der Dunkelatmung (R_D) 0.8, 0.6 und 0.7 µmol CO_2 m^{-2} s^{-1} jeweils auf 1000, 2000 and 3000 m Meereshöhe, ohne einen signifikanten Höhentrend. Die Einordnung unserer Daten in den Kontext eines pantropischen A_{sat}-Datensatzes von tropischen Bäumen (c. 170 Arten an 18 Standorten unterschiedlicher Meereshöhe) zeigte, dass das flächenbezogene A_{sat} in tropischen Bergen im Mittel 1.3 µmol CO_2 m^{-2} s^{-1} pro km Höhenzunahme abnimmt

(bzw. 0.2 µmol CO_2 m^{-2} s^{-1} pro K Temperaturabnahme). Die Abnahme von A_{sat} trat auf, obwohl die Blattmasse je Fläche mit der Höhe zunahm. Eine verminderte Photosyntheserate und eine reduzierte Bestandesblattfläche bewirken gemeinsam eine Verringerung der Kohlenstoffaufnahme des Kronenraums mit der Meereshöhe in tropischen Bergregionen.

Der Phosphorgehalt pro Blattmasse war der Faktor, der A_{max} entlang des Höhengradienten hauptsächlich beeinflusste, während die Effekte von Blattstickstoff, Temperatur und CO_2-Partialdruck nicht signifikant waren. A_{max} erfuhr einen teilweisen und R_D einen vollständigen homöostatischen Ausgleich als Reaktion auf die Verminderung von Temperatur und CO_2-Partialdruck in größeren Höhen, was hauptsächlich durch eine stark reduzierte spezifische Blattfläche (SLA) und die daraus entstehende Zunahme von Blattstickstoff und -phosphor je Blattfläche bedingt war, während keine Zunahme der Karboxylierungseffizienz festgestellt wurde. Wir schlussfolgern, dass die Verminderung von SLA und Gesamtblattfläche mit der Meereshöhe die Kohlenstoffaufnahme von tropischen Wäldern in großen Meereshöhen deutlich stärker bestimmen als adaptive physiologische Modifizierungen des Photsyntheseapparates.

Jungpflanzen von sechs Baumarten unterschieden sich hinsichtlich ihrer Präferenz für verschieden Stickstoffformen, allerdings schienen weder das Amonium- und Nitratvorkommen im Boden noch die Meereshöhe die Präferenz zu beeinflussen. Zwei Arten (jeweils die, mit den höchsten Wachstumsraten) bevorzugten Amonium gegenüber Nitrat, während die restlichen vier Arten Nitrat und Amonium mit ähnlichen Raten aufnahmen, wenn beide Stickstofformen verfügbar waren. Nach der Gabe von $^{15}N^{13}C$-Glyzin zeigte sich bei drei Arten eine signifikante Akkumulierung von ^{13}C in der Biomasse (zwei Arten mit arbuskulären Mykorrhiza und eine Art mit Ektomykorrhiza) zusätzlich zu einer signifikanten Akkumulierung von ^{15}N, was darauf hindeutet, dass Bäume in tropischen Bergregenwäldern organische Stickstoffverbindungen unabhängig vom Typ ihrer Mykorrhizierung aufnehmen können.

CHAPTER 7

Appendix

Supplementary material of Chapter 2

Table 5. Light-saturated net photosynthesis at ambient temperature and [CO_2] (A_{sat}), foliar N and P concentrations (N_m, P_m) and leaf mass per area (LMA) of ca. 169 tree species (or morphotypes) of lowland to upper montane tropical forests according to various authors. For A_{sat}, area- or mass-based species means (± SE) are given. Only measurements referring to mature or pre-mature tree individuals (no seedlings and saplings) of non-pioneer stands were considered. A_{sat} values obtained at irradiances <1200 µmol photons m^{-2} s^{-1} were only included if that flux density was explicitly identified as being saturating. If information was available, T and PPFD refer to the temperature and irradiance conditions during the measurements; otherwise, MAT is given. only data referring to sun-lit, fully expanded leaves of the upper canopy were included. Wet season data were preferred over dry season data if both were given. The sequence of locations mainly follows the altitudinal gradient. For comparison in the leaf chemical analyses, N_m, P_m and LMA data of additional 35 species from a transect in Peru are included in the list, even though A_{sat} data are not available.

Location	Elevation	Temperature	Rainfall	Species	A_{sat}	SE	A_{sat}	N_m	P_m	LMA	Reference
	m a.s.l.	*°C*	*mm yr^{-1}*		*µmol m^{-2} s^{-1}*		*nmol g^{-1} s^{-1}*	*mg g^{-1}*	*mg g^{-1}*	*g m^{-2}*	
Lowland											
Venezuela	100	26	3565	*Cecropia ficifolia*	17.7	--	252.2	24.7	--	69.16	(1)
	100	26	3565	*Vismia lauriformis*	13.8	--	159.3	17.9	--	87.49	
	100	26	3565	*Clidemia sericea*	9.1	--	115.5	16.5	--	79.11	
	100	26	3565	*Vismia japurensis*	12.8	--	140.4	16.4	--	94.88	

100	26	3565	*Belinda grossularioides*	14.0	--	111.5	16.9	--	128.04
100	26	3565	*Goupia glabra*	7.8	--	99.0	15.1	--	76.98
100	26	3565	*Neea obovata*	6.4	--	65.0	18.4	--	96.99
100	26	3565	*Miconia dispar*	10.3	--	91.8	15.9	--	110.74
100	26	3565	*Retiniphyllum truncalum*	5.6	--	46.5	6.8	--	120.77
100	26	3565	*Rhodognaphalopsis humilis*	6.2	--	47.2	7.8	--	132.98
100	26	3565	*Protium sp.*	4.0	--	34.7	8.6	--	115.47
100	26	3565	*Aspidosperma album*	6.5	--	52.2	10.7	--	121.07
100	26	3565	*Protium sp.*	8.2	--	67.2	17.7	--	121.95
100	26	3565	*Caraipa heterocarpa*	5.7	--	38.8	9.3	--	147.49
100	26	3565	*Ocotea costulata*	5.9	--	44.9	15.0	--	130.21
100	26	3565	*Licania heteromorpha*	8.0	--	53.8	13.0	--	148.59
100	26	3565	*Eperua purpurea*		--	55.1	15.2	--	
100	26	3565	*E. leucantha*	3.7	--	35.1	12.5	--	105.15
100	26	3565	*Leguminosae sp.*	6.7	--	47.7	21.4	--	138.69
100	26	3565	*Micrandra sprucei*	7.4	--	43.3	10.8	--	171.23

	100	26	3565	*Micropholis maguirei*	--	--		--	8.0	--	180.83		
	100	26	3565	*Protium sp*	5.7	--		33.0	10.3	--	172.12		
Panama	130	31	3100	*Vochysia ferruginea*	18.3	--	--	--	--	--		(2)	
	130	31	3100	*Simarouba amara*	17.5	--	--	--	--	--			
	130	31	3100	*Miconia borealis*	16.8	--	--	--	--	--			
	130	31	3100	*Pourouma bicolor*	13.7	--	--	--	--	--			
	130	31	3100	*Virola sebifera*	13.5	--	--	--	--	--			
	130	31	3100	*Tapirira guianensis*	12.9	--	--	--	--	--			
	130	31	3100	*Ocotea ira*	12.6	--	--	--	--	--			
	130	31	3100	*Dussia munda*	12.3	--	--	--	--	--			
	130	31	3100	*Guatteria dumentorum*	12.2	--	--	--	--	--			
	130	31	3100	*Trattinickia aspera*	12.2	--	--	--	--	--			
	130	31	3100	*Poulsenia armata*	11.8	--	--	--	--	--			
	130	31	3100	*Humiriastrum diguense*	11.2	--	--	--	--	--			
	130	31	3100	*Nectandra purpurascens*	11.1	--	--	--	--	--			

	130	31	3100	*Manilkara bidentata*	10.3	--	--	--	--	--		
	130	31	3100	*Marila laxiflora*	9.9	--	--	--	--	--		
	130	31	3100	*Aspidosperma cruenta*	9.7	--	--	--	--	--		
	130	31	3100	*Apeiba membranacea*	16.1	--	--	--	--	--		
	130	31	3100	*Simarouba amara*	15.7	--	--	--	--	--		
	130	31	3100	*Vochysia ferruginea*	15.2	--	--	--	--	--		
	130	31	3100	*Jacaranda copaia*	14.6	--	--	--	--	--		
	130	31	3100	*Aspidosperma cruenta*	14.2	--	--	--	--	--		
	130	31	3100	*Manilkara bidentata*	11.8	--	--	--	--	--		
	130	31	3100	*Brosimum utile*	10.4	--	--	--	--	--		
Panama	140	26	3100	*Apeiba membranaceae*	14.9	--	133.3	25.8	1.12	111.86	(3)	
	140	26	3100	*Aspidosperma cruentum*	10.2	--	65.9	20.4	0.84	155.04		
	140	26	3100	*Brosimum utile*	11.4	--	77.6	18.3	1.04	146.20		
	140	26	3100	*Calophyllum longifolium*	11.2	--	43.4	12.1	0.72	258.40		
	140	26	3100	*Carapa guianensis*	10.1	--	49.3	13.7	0.86	205.34		

140	26	3100	*Cecropia insignis*	15.7	--	143.1	17.1	1.24	110.01
140	26	3100	*C. obtusifolia*	13.2	--	350.8	41.6	3.04	37.48
140	26	3100	*Dussia munda*	11.9	--	105.8	21.1	1.14	112.11
140	26	3100	*Jacaranda copaia*	16.3	--	157.8	25.5	1.21	103.52
140	26	3100	*Lonchocarpus latifolius*	14.8	--	102.9	18.4	1.06	144.09
140	26	3100	*Manilkara bidentata*	13.4	--	78.0	16.5	0.83	172.12
140	26	3100	*Miconia borealis*	15.3	--	158.3	22.0	1.30	96.34
140	26	3100	*Nectandra purpurea*	10.9	--	73.6	17.8	0.77	147.71
140	26	3100	*Ochroma pyrmidale*	14.5	--	224.7	21.4	1.49	64.68
140	26	3100	*Oenocarpus mapora*	7.6	--	67.9	20.5	1.27	112.23
140	26	3100	*Pourouma bicolor*	15.8	--	110.5	20.0	1.24	142.65
140	26	3100	*Simarouba amara*	13.8	--	78.6	17.4	0.65	176.06
140	26	3100	*Socratea exorrhiza*	11.2	--	76.7	19.9	1.24	146.20
140	26	3100	*Tapirira guianensis*	13.0	--	92.2	15.4	0.88	141.24
140	26	3100	*Trema micrantha*	13.4	--	169.7	20.9	0.87	78.93
140	26	3100	*Vochysia ferruginea*	15.9	--	138.7	17.3	1.00	114.29

Malaysia (Sabah)	150	28	3150	*Macaranga hypoleuca*	14.2	--	--	--	--	--	(4)
	150	28	3150	*Dinochloa trichogona*	11.6	--	--	--	--	--	
	150	28	3150	*Chisocheton macranthus*	9.5	--	--	--	--	--	
	150	28	3150	*Glochidion rubrum*	8.8	--	--	--	--	--	
	150	28	3150	*Dryobalanops lanceolata*	7.3	--	--	--	--	--	
	150	28	3150	*Acacia mangium*	6.3	--	--	--	--	--	
	150	28	3150	*Parashorea tomentella*	6.1	--	--	--	--	--	
	150	28	3150	*Shorea seminis*	6.0	--	--	--	--	--	
	150	28	3150	*S. xanthophylla*	5.9	--	--	--	--	--	
	150	28	3150	*Pentace adenophora*	5.6	--	--	--	--	--	
	150	28	3150	*Dipterocarpus caudiferus*	5.0	--	--	--	--	--	
Malaysia (Sarawak)	200	30	2100-3300	*Shorea beccariana*	17.9	--	--	--	--	--	(5)
	200	30	2100-3300	*S. acuta*	10.7	--	--	--	--	--	
	200	30	2100-3300	*Dryobalanops aromatica*	10.2	--	--	--	--	--	

	200	30	2100-3300	*Dipterocarpus globosus*	9.5	--	--	--	--	--	--	
	200	30	2100-3300	*Shorea macroptera*	6.9	--	--	--	--	--	--	
Peru	220	26.4	2730	*Leonia glycocarp.*	--	--	--	21.4	1.50	63.90	(6)	
	220	26.4	2730	*Mabea nitida.*	--	--	--	27.1	1.20	63.10		
	220	26.4	2730	*Pourouma cecropiifolia*	--	--	--	23.3	1.30	62.50		
	200	26.4	2730	*Rinorea viridifolia*	--	--	--	23.4	1.20	48.20		
	220	26.4	2730	*Symphonia globulifera*	--	--	--	22.9	0.80	66.90		
	220	26.4	2730	*Iryanthera juruensis*	--	--	--	29.0	1.10	77.20		
	220	26.4	2730	*Bixa arborea*	--	--	--	26.0	1.00	62.60		
	220	26.4	2730	*Brosimum guianense*	--	--	--	28.0	1.10	66.50		
	220	26.4	2730	*Micropholis guyanensis*	--	--	--	18.9	0.60	130.00		
	220	26.4	2730	*Neea divaricata.*	--	--	--	19.6	1.20	53.00		
S China	570	21.7	1560	*Anisoptera costata*	13.7	1.40	210.0	26.8	1.51	65.00	(7)	
	570	21.7	1560	*Dipterocarpus alatus*	20.3	0.70	191.0	22.1	1.27	106.00		

570	21.7	1560	*D. intricatus*	14.7	1.40	105.0	16.2	1.08	152.00
570	21.7	1560	*D. retusus*	14.0	0.70	132.0	19.4	1.40	106.00
570	21.7	1560	*D. tuberculatus*	18.9	0.70	177.0	18.5	1.12	107.00
570	21.7	1560	*D. turbinatus*	16.1	0.70	158.0	19.0	1.17	101.00
570	21.7	1560	*Hopea chinensis*	9.9	0.80	124.0	22.0	1.21	80.00
570	21.7	1560	*H. hainanensis*	10.1	0.60	102.0	20.0	1.38	98.00
570	21.7	1560	*H. hongayensis*	6.1	0.40	118.0	19.8	1.32	52.00
570	21.7	1560	*H. mollissima*	7.2	0.20	82.0	19.6	1.33	88.00
570	21.7	1560	*Parashorea chinensis*	8.3	0.50	159.0	21.7	1.66	52.00
570	21.7	1560	*Shorea assamica*	10.7	0.60	165.0	19.3	1.17	65.00
570	21.7	1560	*S. robusta*	17.2	1.00	184.0	19.2	1.19	94.00
570	21.7	1560	*Shorea sp.*	9.7	0.60	89.0	18.9	0.99	112.00
570	21.7	1560	*Vatica guangxiensis*	5.1	0.30	64.0	17.0	0.86	81.00
570	21.7	1560	*V. mangachapoi*	9.3	0.70	97.0	17.8	1.16	96.00
570	21.7	1560	*V. xishuangbannaensis*	7.3	0.40	89.0	18.6	1.01	82.00

Malaysia	600	24.3		*	5.4	--	116.0	23.0		50.80	(8)
(Sabah)											
Cameroon	640	23	1522	*Amphimas pterocarpoides*	11.0	--	--	23.8	0.90	83.33	(9)
	640	23	1522	*Celtis adolfi-friderici*	10.3	--	--	26.3	1.02	60.98	
	640	23	1522	*Musanga cecropioides*	13.9	--	--	24.0	0.98	76.34	
	640	23	1522	*Staudtia stipitata*	6.8	--	--	18.0	0.97	65.36	
	640	23	1522	*Trichilia sp*	6.3	--	--	30.7	0.90	49.50	

Pre-montane/montane

Ecuador	1000	19	2230	*Saurauia spec.1*	11.0	0.8	107.9	18.26	0.81	101.94	(10)
	1000	19	2230	*Guatteria pastazae*	11.3	0.3	142.9	18.15	0.46	79.05	
	1000	19	2230	*Hedyosmum sprucei*	12.0	0.5	--	18.41	0.78	--	
	1000	19	2230	*Inga spec.*	9.4	2.5	78.8	25.73	0.66	119.33	
	1000	19	2230	*Lozania klugii*	9.4	0.3	109.0	18.58	0.47	86.21	

	1000	19	2230	*Licaria cf terminalis*	6.1	1.0	65.1	26.21	0.48	93.72	
	1000	19	2230	*Mollia cf gracilis*	5.4	0.4	92.8	20.80	0.65	58.21	
	1000	19	2230	*Centronia laurifolia*	7.9	0.7	57.3	10.34	0.25	137.93	
	1000	19	2230	*Miconia spec.*	6.9	0.5	41.9	14.10	0.37	164.47	
	1000	19	2230	*Tibouchina ochipetala*	16.0	1.0	--	16.05	0.54	--	
	1000	19	2230	*Ficus cervantesiana*	7.3	0.6	48.1	12.75	0.56	151.75	
	1000	19	2230	*Ficus spec.*	8.5	0.6	--	11.06	0.49	--	
	1000	19	2230	*Neea cf divaricata*	6.0	0.6	82.3	42.44	0.74	72.94	
	1000	19	2230	*Cinchona spec.*	11.1	0.5	92.6	13.90	0.43	119.90	
	1000	19	2230	*Pouteria torta*	3.4	1.7	33.3	14.09	0.44	102.15	
Indonesia	1100	20	1840	*Cananga odorata*	13.2	--	--	--	--	--	(11)
	1100	20	1840	*Bischofia javanica*	9.6	--	--	--	--	--	
	1100	20	1840	*Semecarpus forstenii*	9.1	--	--	--	--	--	
	1100	20	1840	*Aglaia argentea*	8.5	--	--	--	--	--	
	1100	20	1840	*Litsea spec.*	5.4	--	--	--	--	--	

	1100	20	1840	*Siphonodon celastrineus*	5.3	--	--	--	--	--		
	1100	20	1840	*Pimelodendron amboinicum*	5.1	--	--	--	--	--		
	1100	20	1840	*Meliosma sumatrana*	3.6	--	--	--	--	--		
Ecuador	2000	16	1950	*Saurauia spec.2*	8.6	1.3	71.6	20.41	1.23	120.19	(10)	
	2000	16	1950	*Piptocoma discolor*	12.8	1.0	165.9	26.24	1.90	77.16		
	2000	16	1950	*Critoniopsis zamorensis*	8.4	0.8	113.7	23.36	1.85	73.86		
	2000	16	1950	*Clethra revoluta*	13.0	1.6	66.6	12.98	0.52	195.31		
	2000	16	1950	*Vismia cf tomentosa*	12.2	0.4	72.8	15.50	0.92	167.50		
	2000	16	1950	*Aniba spec.*	12.1	1.3	89.8	17.10	1.23	134.77		
	2000	16	1950	*Rhodostemonodaphne kunthiana*	11.4	1.0	83.6	25.62	0.85	136.43		
	2000	16	1950	*Heliocarpus americanus*	14.7	1.0	249.6	33.32	2.51	58.89		
	2000	16	1950	*Meriania hexamera*	8.5	1.3	71.1	17.51	0.79	119.47		
	2000	16	1950	*Tibouchina lepidota*	15.4	1.2	107.0	16.74	0.64	143.88		
	2000	16	1950	*Ficus pertusa*	11.3	1.6	95.7	19.08	1.15	118.06		
	2000	16	1950	*F. citrifolia*	10.1	1.8	69.4	21.42	0.91	145.56		

	2000	16	1950	*Isertia laevis*	13.4	2.1	63.7	15.96	0.78	210.53	
	2000	16	1950	*Ladenbergia acutifolia*	9.8	2.0	102.7	17.87	0.79	95.42	
	2000	16	1950	*Siparuna aspera*	11.4	1.7	119.9	29.09	1.51	95.06	
	2000	16	1950	*Cecropia andina*	7.7	1.6	61.1	17.62	0.66	125.94	
Peru	1000	20.7	3087	*Ficus sanguinosa*	--	--	--	18.20	1.30	131.00	(6)
	1000	20.7	3087	*Virola cf. elongata.*	--	--	--	22.10	1.20	66.90	
	1000	20.7	3087	*Pourouma minor*	--	--	--	22.20	1.10	80.40	
	1000	20.7	3087	*Pouteria sp.*	--	--	--	19.70	1.50	82.70	
	1500	18.8	2631	*Mollinedia simulans*	--	--	--	24.00	1.30	83.70	
	1500	18.8	2631	*Guatteria sp.*	--	--	--	27.30	1.10	124.00	
	1500	18.8	2631	*Tachigali cf setifera*	--	--	--	27.30	1.30	75.20	
	1500	18.8	2631	*Miconia sp.*	--	--	--	24.50	1.40	94.10	
	1855	18.0	2472	*Cyathea lechler*	--	--	--	18.70	0.80	123.00	
	1855	18.0	2472	*Clethra revoluta*	--	--	--	12.10	0.60	159.00	
	1855	18.0	2472	*Myrcia sp.*	--	--	--	15.30	0.70	103.00	

	1855	18.0	2472	*Hedyosmum racemosum*	--	--	--	18.20	1.00	97.10	
	2350			*Clusia sp.*	--	--	--	15.30	0.90	131.00	
	2350			*Prunus sp.*	--	--	--	20.90	1.10	105.00	
	2350			*Hedyosmum scabrum*	--	--	--	19.70	0.90	90.80	
	2350			*Miconia sp.*	--	--	--	24.30	1.10	115.00	
Malaysia	1700	18.15		*	5.5	--	64.0	14.00		90.20	(8)
Colombia	1445	18	3600	*Cecropia garciae*	8.7	--	--	--	--	--	(12)
	1445	18	3600	*Miconia sp.*	8.1	--	--	--	--	--	
	1450	18	3600	*Psychotria race*	10.6	--	--	--	--	--	
	1480	18	3600	*Clusia sp.*	8.8	--	--	--	--	--	
	2160	11	7000	*Clusia pentandra*	8.2	--	--	--	--	--	
	2160	11	7000	*Psychotria cuat*	9.2	--	--	--	--	--	
	2145	11	7000	*Cecropia bullata*	10.2	--	--	--	--	--	
	2160	11	7000	*Miconia sp.*	5.4	--	--	--	--	--	

Venezuela	2400	13.6	2200	*Clusia multiflora*	6.6	--	200.2	11.10		304.30	(13)
	2400	13.6	2200	*Guettarda steyermarkii*	6.3	--	92.9	18.40		148.60	
	2400	13.6	2200	*Sapium stylare*	7.6	--	41.4	31.40		54.20	
	2400	13.6	2200	*Lycianthes ferruginea*	4.5	--	24.4	23.60		54.00	
	2400	13.6	2200	*Miconia resimoides*	6.7	--	46.8	36.90		69.70	
Upper montane											
Ecuador	3000	9	4500	*Ilex teratopis*	2.6	0.2	8.0	9.17	0.25	325.66	(10)
	3000	9	4500	*Hedyosmum purpurascens*	7.5	1.0	54.0	15.82	0.53	138.89	
	3000	9	4500	*Clethra revoluta*	10.0	0.4	59.0	16.15	0.74	169.49	
	3000	9	4500	*Clusia alata*	4.4	1.2	35.6	9.52	0.47	123.63	
	3000	9	4500	*C. elliptica*	5.8	1.5	35.4	13.45	0.58	163.93	
	3000	9	4500	*Weinmannia pubescens*	8.8	1.1	78.3	12.97	0.50	112.36	
	3000	9	4500	*Persea ferruginea*	8.0	1.2	16.5	9.72	0.42	486.37	
	3000	9	4500	*Miconia spec.*	4.6	1.3	46.9	20.15	0.72	98.04	

	3000	9	4500	*Myrica pubescens*	10.3	0.6	66.3	21.97	0.51	155.40	
	3000	9	4500	*Styrax foveolaria*	10.2	0.9	33.5	10.00	0.40	304.46	
Peru	2990	12.5	1706	*Clusia cretosa*	--	--	--	12.30	1.10	127.00	(6)
	2990	12.5	1706	*Weinmannia crassifolia*	--	--	--	13.10	1.10	134.00	
	2990	12.5	1706	*Schefflera allocotantha*	--	--	--	14.00	1.20	138.00	
	2990	12.5	1706	*Clethra cuneata*	--	--	--	8.60	0.60	226.00	
	3600			*Polylepis pauta .*	--	--	--	19.80	1.20	147.00	
	3600			*Gynoxis sp.*	--	--	--	16.80	1.30	170.00	
	3600			*Budleja sp.*	--	--	--	13.80	0.90	220.00	
	3600			*Pluchea*	--	--	--	17.60	1.60	172.00	
Malaysia	3400	8.8		*	4.3	--	32.0	16.00		160.70	(8)
	3700	7.2		*	3.1	--	29.0	20.00		141.60	

References: (1) (Reich and others (1999), (2) Santiago and others (2004); (3) Santiago and Wright (2007); (4) Eschenbach and others (1998); (5) Kenzo and others (2004); (6) van de Weg and others (2009); (7) Zhang and Cao (2009); (8) Hikosaka and other (2002); (9) Meir and others (2007); (10) this study; (11) Hölscher and others (2006); (12) Letts and Mulligan (2005); (13) Rada and others (2009). (*) Values are means over different species of a location.

Supplementary material of Chapter 3

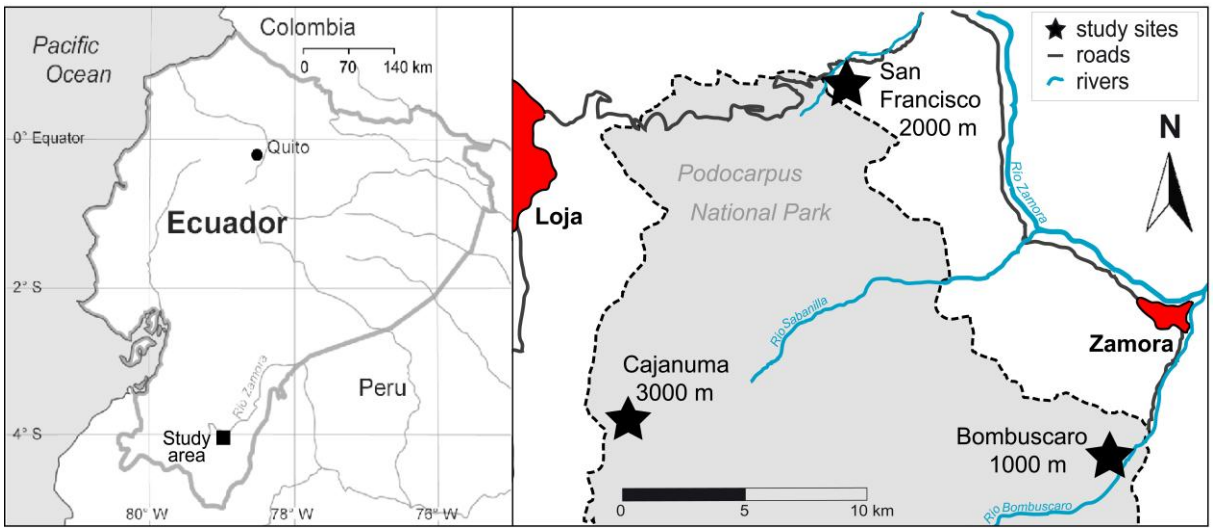

Figure A1. *Location of the study area in southern Ecuador with the three stands at 1000 m (Bombuscaro), 2000 m (San Francisco Reserve) and 3000 m a.s.l (Cajanuma).*

Table A1. Climatic characteristics of the stands at 1000, 2000 and 3000 m a.s.l. (after T. Peters, University of Erlangen, unpublished). Rainfall, mean annual air temperature and relative air humidity according to measurements in gaps close to the three forest sites (2-year means, 2008-2009). [CO_2] is the CO_2 concentration of the air above the boundary layer as estimated from air pressure and by assuming a constant mixing ratio of 370 µmol CO_2 mol air^{-1} along the slope (after Wittich *et al.* 2012).

Elevation *m asl*	**Rainfall** *mm yr^{-1}*	**Air temperature** *°C*	**Air humidity** *%*	**[CO_2]** *Pa*
2960	c. 2580	7	97	27
1950	c. 1790	13	86	30
1000	c. 2200	20	92	33

Table A2. Chemical characteristics of the organic layer and upper mineral soil in the three stands at 1000, 2000 and 3000 m (after Wolf 2010 and Wittich *et al.* 2012). Means ± SE of soil profiles dug in six plots at mid-slope position in the stands at a maximum distance to the studied trees of 500 m. P_{av} – available P was determined by combining a modified Hedley fractionation with an extraction by anion exchange resins and $NaHCO_3$ percolation. N mineralization and nitrification rates were measured by the in situ buried bag method. Different small letters indicate significant differences between elevations ($P < 0.05$).

Elevation	*m asl*	**1000 m**		**2000 m**		**3000 m**	
Organic layer							
pH (H_2O)		4.3 ± 0.6	**a**	4.8 ± 0.5	**a**	3.7 ± 0.1	**a**
C/N		19.0 ± 2.5	**ab**	15.6 ± 0.6	**a**	23.9 ± 1.4	**b**
P_{tot}	*kg ha^{-1}*	46.8 ± 17.3		70.3 ± 26.5		15.1 ± 1.4	
P_{av}	*kg ha^{-1}*	11.8 ± 5.3	**a**	12.5 ± 4.9	**a**	5.1 ± 0.6	**a**
Mineral soil							
pH(H_2O) (Ah)		4.3 ± 0.3	**ab**	4.7 ± 0.4	**a**	3.7 ± 0.1	**b**
C/N (Ah)		15.6 ± 0.1	**a**	14.1 ± 1.5	**a**	18.0 ± 1.1	**a**
P_{tot} (0-10 cm)	*kg ha^{-1}*	123.8 ± 9.7		241.3 ± 47.7		62.6 ± 12.6	
P_{av} (0-10 cm)	*kg ha^{-1}*	12.4 ± 0.7	**a**	34.6 ± 7.1	**b**	14.9 ± 3.0	**a**
Topsoil (0-5 cm)							
Extractable NH_4^+	*kg N ha^{-1} 10d^{-1}*	2.7 ± 0.3	**a**	0.9 ± 0.3	**b**	0.7 ± 0.1	**b**
Extractable NO_3^-	*kg N ha^{-1} 10d^{-1}*	0.5 ± 0.2	**a**	0.3 ± 0.1	**a**	0.02 ± 0.01	**b**
Net N mineralization	*kg N ha^{-1} 10d^{-1}*	4.0 ± 1.6	**a**	1.4 ± 0.7	**ab**	0.6 ± 0.4	**b**
Net nitrification	*kg N ha^{-1} 10d^{-1}*	1.3 ± 2.7	**ab**	1.8 ± 0.6	**a**	0.04 ± 0.04	**b**

Table A3. Stand structural characteristics of the three stands at 1000, 2000 and 3000 m (after Wittich *et al.* 2012 and Homeier *et al.*, unpublished; LAI, leaf life span, BGB and leaf biomass: Moser *et al.* 2007, 2011). Abbreviations: AGB, aboveground biomass; BGB, belowground biomass (coarse and fine roots); DBH, diameter in breast height; LAI, leaf area index. Given are means ± SE for each elevation. Means of tree DBH, stem density, basal area and AGB were calculated for 9-18 permanent plots (400 m² each) covering the whole range of topographic positions at the respective elevations (trees > 10cm dbh). Estimates for nutrient pools in canopy leaf biomass were calculated from leaf biomass data (Moser *et al.* 2007) and mean foliar N and P concentrations according to Homeier *et al.* (unpublished). Different small letters indicate significant differences between elevations ($P < 0.05$).

Elevation	*m asl*	**1000**		**2000**		**3000**	
Canopy height	*m*	25-30		16-20		8-10	
DBH	*cm*	19 ± 1	**a**	20 ± 1	**a**	18 ± 1	**a**
Stem density	*n ha^{-1}*	822 ± 50	**a**	900 ± 62	**a**	1061 ± 84	**a**
Basal area	*m^2 ha^{-1}*	29 ± 4	**a**	34 ± 3	**a**	30 ± 3	**a**
AGB	*Mg ha^{-1}*	177 ± 28	**a**	158 ± 22	**a**	89 ± 10	**b**
BGB	*Mg ha^{-1}*	32.1		26.1		62.8	
LAI	*m^2 m^{-2}*	6 ± 0.4	**a**	5.7 ± 0.5	**a**	2.2 ± 0.2	**b**
Leaf life span	*months*	16 ± 2.6	**a**	24 ± 2.3	**b**	25 ± 2.3	**b**
Leaf biomass	*Mg ha^{-1}*	6.8	**a**	9.7	**b**	3.6	**c**
Leaf biomass N pool	*kg ha^{-1}*	123		202		46	
Leaf biomass P pool	*kg ha^{-1}*	3.8		7.9		1.6	

Supplementary material of Chapter 4

Table 5. Slope b, R^2, adjusted R^2 and p value of the regression of $^{13}C_{excess}$ values (in µmol g^{-1}) on the corresponding $^{15}N_{excess}$ values (in µmol g^{-1}) in different organs of the six tree species 5 d after the application of dual-labelled glycine. A slope of 2.0 would indicate 100% uptake of glycine-derived N in form of intact molecules. Note that the slope is always much smaller than 2 but none of the regressions are significant at $p < 0.05$.

		b	R^2	R^2 adj.	*p*	n
1000 m asl						
Pouteria torta	*Fine roots*	0.12	0.51	0.02	0.25	3
	Coarse roots	0.31	0.90	0.79	0.11	3
	Shoot	-1.83	0.97	0.93	0.06	3
	Leaves	-0.48	0.01	-0.97	0.46	3
Hedyosmum sprucei	*Fine roots*	0.03	0.28	0.04	0.18	5
	Coarse roots	0.16	0.01	-0.32	0.44	5
	Shoot	0.01	0.07	-0.24	0.33	5
	Leaves	0.03	0.64	0.52	0.05	5
2000 m asl						
Myrcia sp. nov	*Fine roots*	0.24	0.12	-0.33	0.33	4
	Coarse roots					
	Shoot	-0.32	0.16	-0.26	0.30	4
	Leaves	-0.02	0.01	-0.49	0.46	4
Hedyosmum translucidum	*Fine roots*	0.42	0.53	0.30	0.13	4
	Coarse roots	0.57	0.79	0.69	0.05	4
	Shoot	0.00	0.00	-0.50	0.49	4
	Leaves	0.08	0.04	-0.44	0.40	4
3000 m asl						
Graffenrieda harlingii	*Fine roots*	-0.36	0.22	-0.04	0.22	5
	Coarse roots	-0.01	0.00	-0.33	0.49	5
	Shoot	-0.03	0.29	0.05	0.17	5
	Leaves	0.01	0.03	-0.29	0.38	5
Hedyosmum cf. purpurascens	*Fine roots*	0.13	0.97	0.93	0.06	3
	Coarse roots					(2)
	Shoot	-0.14	0.16	-0.27	0.30	4
	Leaves	0.08	0.61	0.41	0.11	4

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