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The vertical pattern of rooting and nutrient uptake at different altitudes of a south Ecuadorian montane forest

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Abstract The vertical pattern of root length densities (RLD) of fine roots (< 2 mm in diameter) and nitrogen (N) uptake potential were determined at different altitudes (1,900, 2,400, and 3,000 m a.s.l.) of a tropical montane forest in order to improve our knowledge about the depth distribution of nutrient uptake in this ecosystem. At higher altitudes, precipitation rate and frequency of fog were higher than at lower altitudes while mean annual air temperature decreased with increasing altitude. Soils were always very acid with significantly lower pH at a depth of 0.0–0.3 m in mineral soil at 3,000 m (2.8–2.9) than at 1,900 and 2,400 m (3.1–3.5). The vertical distribution of RLD was very similar both during the dry and the rainy season. During the dry season the percentage of root length in the organic layer increased from 51% at 1,900 m to 61% at 2,400 m and 76% at 3,000 m. At 3,000 m, RLD was markedly higher in the upper 0.05 m than in the remaining organic layer, whereas at 1,900 m and 2,400 m RLD were similar in all depths of the organic layer. In mineral soil, RLD decreased to a greater degree with increasing soil depth at the upper two study sites than at 1,900 m. The relative N uptake potential from different soil layers (RNUP) was determined by ^{15}N enrichment of leaves after application of ^{15}N enriched ammonium sulphate at various soil depths. RNUP closely followed fine root distribution confirming the shallower pattern of nutrient uptake at higher altitudes. RNUP was very similar for trees, shrubs and herbs, but shallower for saplings which obtained N only from the organic layer at both altitudes. Liming and fertilizing (N, P, K, Mg) of small patches in mineral soil had no significant impact on fine root growth. We conclude that the more superficial nutrient uptake ability at higher altitudes may be partly related to increased nutrient input from canopy by leaching. However, the specific constraints for root growth in the mineral soil of tropical montane forests warrant further investigations.

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Abbreviations
RLD root length density
RNUP relative nutrient uptake potential

Introduction

The spatial pattern of nutrient acquisition by plant roots has a large impact on nutrient fluxes within forest ecosystems. Both the acquisition of soil resources by plants and nutrient losses from the ecosystem by leaching strongly depend on the exploitation of the soil by roots (Stark and Jordan 1978). Horizontal and vertical root distribution in forest soils is much more heterogeneous than in arable land (George and Marschner 1996). The vertical distribution is related to soil and plant characteristics including nutrient availability (e.g. Leuschner et al. 1998; Sainju and Good 1993), soil pH (Godbold et al. 2003; Murach 1984), access to water (Bouillet et al. 2002; Lopez et al. 2001), waterlogging (Santiago 2000), bulk density (Carvalheiro and Nepstad 1996), stand age (Bouillet et al. 2002; John et al. 2001), species composition (Davis et al. 2004; Silva and Rego 2003; Göransson 2006), and the type of mycorrhizal symbiosis (Moreno-Chacón and Lusk 2004). Many forest ecosystems are characterized by a densely rooted surface layer (Claus and George 2005; Godbold et al. 2003; Yang et al. 2004). In other cases, e.g. more arid regions, fine root density is highest several centimetres below the soil surface (Lopez et al. 2001).

Most climatic zones are characterized by distinct seasons, which have great impact on root growth (John et al. 2001; Yang et al. 2004). Also the relative importance of understorey and tree layer for nutrient retention may differ throughout the year (Tessier and Raynal 2003). In the tropics, changes of climatic conditions during the year are less pronounced than in other regions and are usually characterized by dry and rainy seasons. Here, seasonal changes in soil water content may affect nutrient uptake activity of roots (Lehmann 2003; Lehmann et al. 2001; Roy and Singh 1995), and root distribution with soil depth (Lehmann 2003; Yavitt and Wright 2001). However, seasonal changes of nutrient uptake and rooting pattern have never been examined in moist tropical montane forests.

In tropical montane forests, nutrient uptake by roots is improved by the high mycorrhizal abundance (Kottke et al. 2004). However, it may be hampered by acid soil reaction (Stewart 2000; Wilcke et al. 2001) and anaerobic soil conditions (Santiago 2000). Precipitation in tropical montane forests can reach several thousand mm per year (Richter 2003). Hence, nutrients are subjected to leaching.

With increasing altitude, temperature decreases, while rain and fog precipitation usually increases in tropical montane ecosystems (e.g. Holder 2003). The depth of the organic surface horizon that stores high amounts of nutrients is often considerably larger at higher than at lower altitudes (Schrumpf et al. 2001; Wilcke et al. 2002). Thus, the depth distribution of rooting and nutrient acquisition may change along altitudinal gradients.

Determination of root distribution in tropical montane forests was mostly restricted to the organic horizons and upper horizons of mineral soil (Cavalier 1992; Hertel et al. 2003; Vance and Nadkarni 1992). We are aware of only one study in tropical mountains assessing root length distribution down to the parent soil material (Ostertag 2001). No data are available on direct measurements of nutrient uptake by fine roots in tropical montane forests, although these are necessary for the understanding of nutrient fluxes.

Nutrient uptake depends on nutrient supply to the root surface and active absorption by root cells (Chapin 1980). It is influenced by various factors, such as exploitation of soil by roots, nutrient concentrations in soil solution, soil water content, soil temperature, soil compaction or mycorrhizal symbiosis (Arvidsson 1999; Chapin 1980; Engels 1993; Lehmann 2003). The ability for nutrient uptake can be assessed directly, e.g. by tracer experiments (Lehmann and Muraoka 2001; Lehmann et al. 2001; Rowe et al. 1999). Root length densities (RLD) (Lehmann 2003; Lopez et al. 2001), i.e. root length per unit soil volume, provide an estimate of the exploitation of the soil by roots and may be closely related to the nutrient uptake ability.

The first objective of this study was to assess rooting pattern and the ability for nutrient uptake by fine roots at three different altitudes of a South Ecuadorian montane forest. Therefore, RLD were determined in two seasons to a maximum mineral soil depth of 1.1 m or down to a depth where root growth was confined by the parent soil

material. The nutrient uptake activity of roots growing in situ was assessed by measuring ^{15}N enrichment in leaves after application of ^{15}N-labelled N fertilizer at different depths. As the nutrient concentration of the soil solution was increased by the fertilizer application, this method gives an estimate of the N uptake potential, i.e. the nutrient uptake under a non-N-limiting situation. The second objective of the present study was to investigate possible causes for the rooting pattern. Chemical soil properties of soil cores were modified by liming and fertilization, and *in situ* root growth in these soil cores was measured.

It was hypothesised that root length distribution and N uptake potential becomes more superficial with increasing altitude. Shallow rooting at high altitude may be caused by high subsoil acidity due to increased precipitation and by enhanced accumulation of nutrients at the soil surface in comparison with lower altitudes. Furthermore, it was hypothesised that RLD in mineral soil increases in the dry season due to low water availability in the organic layer and decreases in the rainy season due to oxygen deficiency in mineral soil.

Materials and methods

Study sites

The investigations were done at three study sites on the northern and north western fringes of the Podocarpus National Park on the eastern Andes. The two lower study sites (1,900 m and 2,400 m a.s.l.) were situated in the Reserva San Francisco (RSF) (Table 1). The highest site (3,000 m) was located in Cajanuma near the north western entrance of the Podocarpus National Park. The sites were all situated on slopes (27–31°) facing north-east or north-west.

Plant communities represented typical vegetation types of montane forests and changed with increasing altitude. Species composition is listed in Röderstein et al. (2005). Maximum tree height decreased from 19 m at 1,900 m to 12 m at 2400 m and 9 m at 3,000 m. The upper site was located close to the timber line and was a typical elfin forest with crooked stem forms. The soils were classified as gleyic Cambisols (1,900 and 2,400 m) and Podzols (3,000 m) according to FAO taxonomy (S. Iost, pers. comm.). The average depth of the organic layer increased from 0.15 m at 1,900 m and 0.16 m at 2,400 m to 0.31 m at 3,000 m. The average depth of the upper mineral horizon accumulated with humus (Ah) decreased from 0.70 m at 1,900 m to 0.15 m at 2,400 m and 0.20 m at 3,000 m. Bedrock was similar at all sites, varying on a small scale between metamorphic shale, quartzite and sandstone. Bedrock was between 0.7 and > 1.1 m depth at 1,900 m, at about 0.6 m at 2,400 m and between 0.4 and 0.6 m at 3,000 m.

Annual rainfall increased from 1,900 m to the upper two sites (Table 1). Within the RSF, annual fog water input increased from 55 lm^{-2} at 1,800 m to 2,747 lm^{-2} at 3,185 m (Fabian et al. 2005). Rainfall input was higher from April to August 2002 than from September 2001 to March 2002. At a weather station at 1,950 m lowest precipitation was recorded in January 2002 (58 mm) and a rainfall peak in July 2002 (299 mm). At this altitude 57% of annual rainfall was recorded in the rainy season from April to August and 43% in the dry season from September to March (P. Emck, pers. comm.). Mean annual temperature decreased markedly with increasing altitude (Table 1). Seasonality in temperature was low (difference of 1.9–2.4°C) with the warmest month in November 2001 during the dry season and the coldest in August 2002 at the end of the rainy season (Röderstein et al. 2005).

Table 1 Location and climatic characteristics of the study sites

Altitude (m)	Location	Rainfall (mm y^{-1})[a]	Mean air temperature (°C)[a]
3,000	S 04°06′ W 79°10′	4,500	8.6
2,400	S 03°59′ W 79°04′	5,000	12.3
1,900	S 03°58′ W 79°04′	1,950	14.9

[a]Data by Röderstein et al. (2005)

For studies of root abundance and soil properties, one 20 m × 20 m plot in forest with closed canopy was established at each altitude. Tracer experiments were performed in representative places of a more widespread area around the plots.

Determination of root length densities

Root length densities were determined during the dry season in November/December 2001 and at the end of the rainy season in July/August 2002. At each altitude, samples were taken from 20 locations using a random block design. From the organic layer, a sample with a surface area of 100 mm × 100 mm was taken from the upper 0.05 m (O1) and the rest (O2). A soil corer with a diameter of 80 mm was used to sample mineral soil in the layers 0–0.1, 0.1–0.3, 0.3–0.5, 0.5–0.7, 0.7–0.9 and 0.9–1.1 m. When parent soil material was reached above 1.1 m, coring was stopped earlier.

Roots within soil samples were dissected with scissors to homogenize the samples. Afterwards, roots < 2 mm were sorted from a sub-sample of 20–50 g (organic layer) or 100–200 g (mineral soil) using a 0.6-mm mesh sieve for washing and tweezers for the separation of roots and remaining soil residue. In a previous test it was shown that the variation of root length between subsamples from one homogenized sample was much less than the variation between samples. Under a binocular living and dead roots were separated by colour, root elasticity and the degree of cohesion of cortex, periderm and stele (Persson 1978). Roots were deep frozen for storage. In Germany, length densities of living roots were determined using WinRhizo software (Régent Instruments, Quebec, Canada).

Measurement of the nitrogen uptake potential at different soil depths

Nitrogen (N) uptake potential from different soil depths was assessed at altitudes of 1,900 and 3,000 m. Five plots (3 × 3 m) per altitude were established for every soil depth (surface of the organic layer, 0.05 and 0.40 m depth of the mineral soil). These plots were obtained randomly with a minimal distance of 10 m. Tracer was applied during the dry season in December 2003. In the organic layer, tracer solution was applied with a syringe, and in 0.05 m and 0.40 m depth of mineral soil using a syringe and plastic tubes installed in holes pre-augered to the requested depth. Tracer solution, corresponding to 1.7 kg ^{15}N ha^{-1} (17 kg total N ha^{-1}), was placed in a grid of 41 regularly arranged application points (8 ml per point) within the plots to obtain uniform distribution of ^{15}N within the respective soil depth. The solution contained 216 g l^{-1} ammonium sulfate (10 atom % ^{15}N) and 850 g l^{-1} glucose (corresponding a C:N ratio of 7.4:1). In a preceding experiment that was conducted at 1,900 m following a protocol of Rowe et al. (1999), the addition of glucose diminished ^{15}N leaching in soil during the uptake study. In another experiment it had been established that approximately 60 days were necessary to achieve appropriate ^{15}N enrichment in leaves of all plant groups (data not shown). Thus, 60 days after tracer application 20 young leaves (i.e. the youngest fully developed leaves or younger leaves) per plot were collected from trees (woody, >3 m height), shrubs (woody, 0.5–3 m height), saplings (woody, < 0.5 m height) and herbs (non woody), respectively. Therefore, twigs from trees and shrubs were harvested from different sides of the crown and one leaf per twig was collected. To address the problem that leaf sizes differed between species, several leaves per twig were sampled from microphyllous species and only parts of leaves (from the edge to the center of one leaf) were sampled from macrophyllous species. For herbs and saplings, often less than 20 young leaves were available. Leaves were dried at 50°C and ground with a flint mill (Type MM2, Retsch-GmbH & CoKG, Haan, Germany). The ^{15}N enrichment in plant tissue was determined with a mass spectrometer (DELTA E/NA 1500, Finnigan MAT, Thermo Electron Corporation, USA). ^{15}N enrichment is expressed in $\delta^{15}N$, calculated as:

$$\delta^{15}\mathrm{N}(‰) = 1000 \times \frac{(\mathrm{atom}\%_{\mathrm{sample}} - \mathrm{atom}\%_{\mathrm{reference}})}{\mathrm{atom}\%_{\mathrm{reference}}}$$

where atom% is the proportion of ^{15}N in total N and atom%$_{\mathrm{reference}}$ is 0.3662%. For the calculation of ^{15}N excess, natural ^{15}N levels of each plant group at each altitude were used as background levels:

$$\delta^{15}N_{excess}(‰) = \delta^{15}N_{sample}(‰) - \delta^{15}N_{reference}(‰)$$

where $\delta^{15}N_{reference}$ is the natural ^{15}N level. The relative ^{15}N uptake potential (RNUP) from a specific soil depth in relation to N uptake potential from the surface of organic layer was calculated by:

$$^{15}N_{Xi}(\%) = 100 \times \frac{\delta^{15}N_{excessXi}(‰)}{\delta^{15}N_{excessOL}(‰)}$$

where $^{15}N_X$ is the RNUP from soil depth X (surface of organic layer, 0.05 m or 0.40 m depth of mineral soil), *i* is *i*th replication from this soil depth, and $\delta\ ^{15}N_{excessOL}$ is the average ^{15}N excess obtained by tracer application in the organic layer at the same altitude.

Soil analyses

The concentrations of mineral nitrogen (N_{min}) and soil pH were measured at the same soil depths as RLD. For the determination of N_{min} (the sum of ammonium and nitrate), soil samples were taken by soil coring in February 2003 (end of dry season). Twelve randomly distributed replicate sub-samples were taken at 1,900 m, and 9 at 2,400 m and 3,000 m, respectively. Three subsamples each were combined to one sample. Fresh soil samples were extracted by 12.5 mM $CaCl_2$ (ratio soil:solution = 1:2 for mineral soil and 1:4 for the organic layer). Nitrate concentrations in the extracts were assessed with a spectral photometer (Lambda 2S, Perkin Elmer, Milano, Italy) from the difference of the extinction at 210 and 275 nm. Ammonium was measured photometrically at 636 nm after staining with the indophenol blue procedure (Bundy and Meisinger 1994). The soil pH was determined electrometrically on three replicate air-dried samples per soil depth and altitude using a 1:2.5 (w:w) mixture of soil and a 0.01 M $CaCl_2$ solution.

Measurement of root growth into soil cores with altered chemical properties

An ingrowth core experiment was performed at 1,900 m and 3,000 m between October 2002 and October 2003. Natural substrate from mineral soil (Ah and B horizon) from each altitude was sieved with a mesh of 7 mm for homogenization and removal of fine roots. The soil was divided into four quarters. One quarter was limed with $CaCO_3$ until pH 5 was reached. One quarter was fertilized with H_2KO_4P, NH_4NO_3 and $MgSO_4$. Hereby, N and Mg were applied at 30 mg kg^{-1}, K at 34 mg kg^{-1} and P at 27 mg kg^{-1} dry soil. One quarter was both limed and fertilized. One quarter was left as a control. Per altitude and treatment, ten gauze tubes with a length of 0.4 m and a diameter of 35 mm were filled with these substrates. To position these tubes vertically in the upper 0.4 m of mineral soil, a soil corer with a diameter of 35 mm was used at 1,900 m. At 3,000 m, a more rigid soil corer with a diameter of 80 mm had to be used because of the rocky mineral soil. The space between gauze tubes and the soil was filled with untreated soil. After insertion of the gauze tubes, the mineral soil was covered with the original substrate from the organic layer. After 12 months, the gauze tubes were removed from the mineral soil. All living roots were sorted out and RLD were determined as described above.

Statistical analyses

Significant differences of RLD between altitudes and of differences in RNUP between plant groups were assessed by ANOVA. For RLD, Scheffé test for unequal numbers of replicates was used as a post-hoc test. Differences of RLD between seasons and between the O1 and O2 layer were determined by the Student's *t*-test. The non-parametric *H*-test for not normally distributed replicates was used to assess differences of different soil treatments in ingrowth cores. Differences in N_{min} concentrations and pH values between altitudes were assessed by ANOVA. Scheffé post-hoc test was used for uneven numbers of replicates (N_{min}). Tukey post-hoc test was used for even numbers of replicates (pH).

Results

Root length densities

At all altitudes, RLD was highest in the organic layer (Fig. 1). Root length densities at 1,900 and

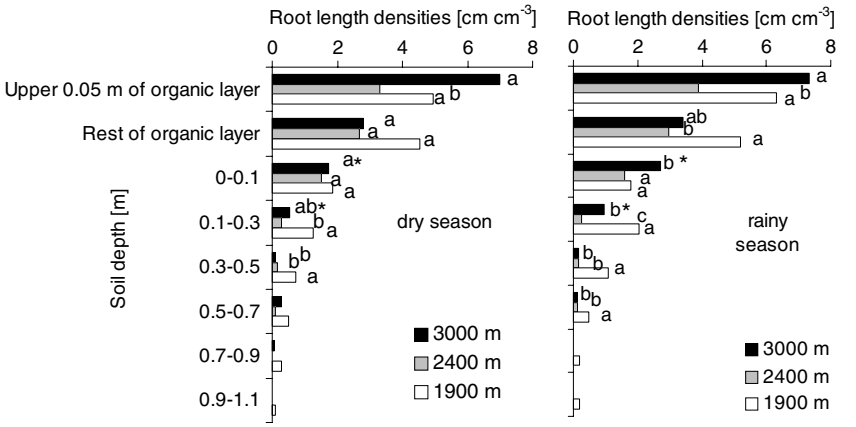

Fig. 1 Vertical distribution of RLD at different altitudes and seasons. Lower case letters indicate significant differences of RLD between altitudes (Scheffé-test; $P < 0.05$; $n = 8$–20); asterisks indicate significant differences between seasons (student's t-test; $P < 0.05$)

2,400 m were distributed evenly in the two organic layers, showing similar values in the O1 and O2 layer ($P > 0.05$). At 3,000 m, RLD was significantly higher in the O1 than in the O2 layer ($P < 0.001$). With increasing depth in mineral soil, RLD decreased more sharply at 3,000 m and 2,400 m than at 1,900 m. At 2,400 m and 3,000 m, RLD decreased to values lower than 0.5 cm cm^{-3} at soil depths below 0.1 and 0.3 m, respectively. At 1,900 m, RLD fell below 0.5 cm cm^{-3} at a soil depth of 0.7–0.9 m. Root length density was significantly higher at 1,900 m, compared to other sites for the lower mineral soil. This was true in 0.3–0.5 m depth during the dry season and in 0.3–0.7 m depth of mineral soil during the rainy season. Below these depths low numbers of replicates did not allow any statistical analysis. At 3,000 m, 76% of fine root length was located in the organic layer during the dry season, as compared to 61% at 2,400 m, and 51% at 1,900 m. Thus, fine root distribution was shallower at higher altitudes.

There was no change in the vertical distribution of RLD from the dry season to the rainy season, with the exception of the upper 0.3 m in mineral soil at 3,000 m, where RLD was significantly higher during the rainy season. Accordingly, the percentage of root length in the organic layer decreased to 69% during the rainy season.

There was no consistent trend of total fine root length with increasing altitude. Total fine root length in the dry season was 15.5 km m^{-2} at 1,900 m, 7.4 km m^{-2} at 2,400 m and 13.9 km m^{-2} at 3,000 m. In the rainy season, fine root lengths were 20.2 km m^{-2}, 7.6 km m^{-2} and 16.8 km m^{-2} at 1,900, 2,400 and 3,000 m, respectively.

Relative N uptake potential from different soil layers

Sixty days after tracer injection, marked ^{15}N enrichment was detected in young leaves of all plant groups (Table 2). ^{15}N excess increased in the order trees < shrubs < herbs < saplings and was always higher in plants growing at 3,000 m than at 1,900 m.

The RNUP was highest in the organic layer (Fig. 2). At 3,000 m it decreased more sharply with increasing soil depth than at 1,900 m. On average of all plant groups, 43% of the N obtained from the organic layer was acquired from 0.05 m depth at 1,900 m, but only 19% at 3,000 m. From 0.4 m depth 32% of the N obtained from the organic layer was acquired at 1,900 m, in comparison to 2% at 3,000 m.

Due to the high standard errors, the pattern of N uptake potential did not significantly differ between plant groups. At both altitudes, however, saplings tended to have the most shallow distribution of RNUP. Different to other plant groups, saplings obtained nearly all N from the organic

Table 2 ^{15}N excess (in ‰) of young leaves of different plant groups at two altitudes 60 days after tracer injection in the organic layer. Values are means ± standard errors ($n = 5$)

Altitude (m)	Plant group			
	Trees	Shrubs	Herbs	Saplings
1,900 m	163 ± 61	211 ± 89	407 ± 107	920 ± 473
3,000 m	374 ± 122	545 ± 108	821 ± 284	1033 ± 470

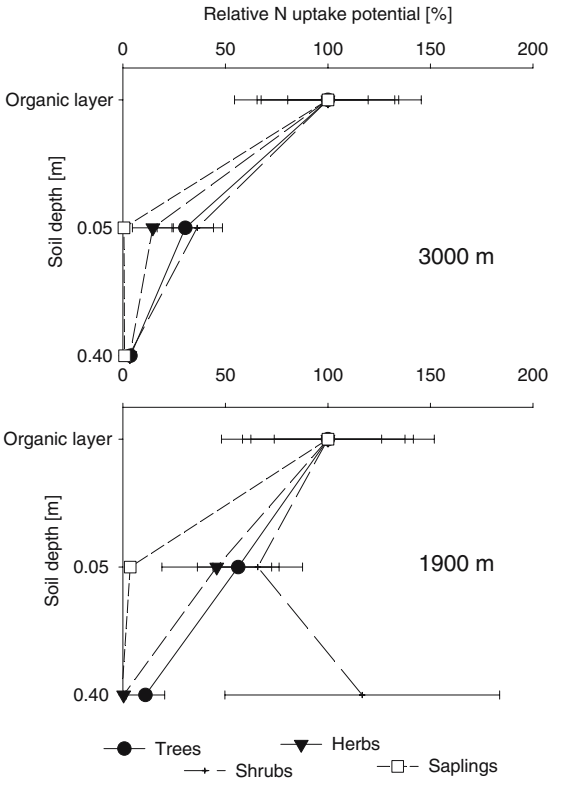

Fig. 2 Vertical pattern of the RNUP of different plant groups at 1,900 and 3,000 m. Error bars represent standard errors ($n = 5$). Differences between RNUP of different plant groups within one soil depth were not significant (H-test, $P < 0.05$)

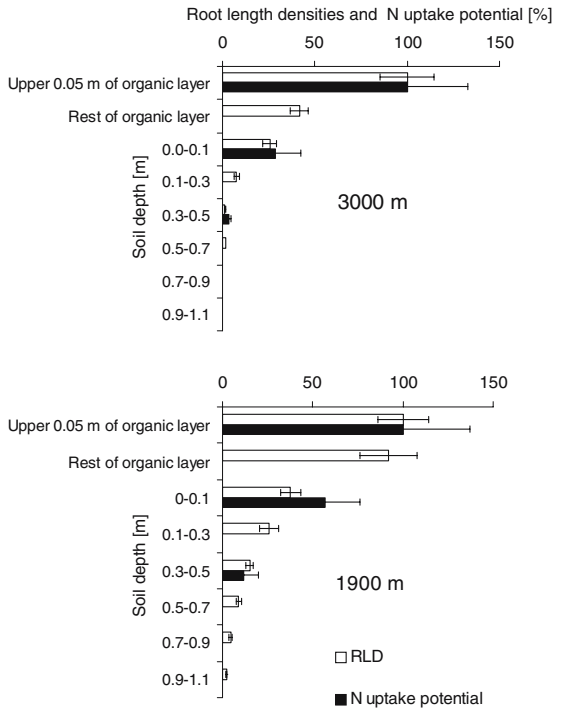

Fig. 3 Effect of soil depth on the relative ability for nutrient acquisition as assessed by RNUP of trees and the relative exploitation of the soil by fine roots as assessed by RLD

layer. At 1,900 m, shrubs obtained considerable amounts of ^{15}N from 0.4 m depth of mineral soil.

The relative distribution of N uptake potential of trees was very similar to the relative distribution of RLD (Fig. 3). This implies that for the site conditions in this study, N uptake potential can be reasonably well estimated from RLD.

Soil chemical parameters

N_{min} concentrations were always very low. Within one soil depth, N_{min} concentrations did not differ significantly ($P > 0.05$) between altitudes (Fig. 4). As an exception, N_{min} concentrations in 0.3–0.5 m soil depth were significantly higher at 1,900 m than at 2,400 m. Pools of N_{min} in the organic layer and the upper 0.7 m of mineral soil were 9.1 kg ha^{-1} at 1,900 m, 5.5 kg ha^{-1} at 2,400 m and 5.7 kg ha^{-1} at 3,000 m. Due to high variations between replicates, these differences were not significant ($P > 0.05$). The percentage of nitrate as a fraction of total N_{min} ranged between 24 and 84% and was not dependent on altitude and soil depth.

Soil reaction was very acid at all altitudes and in all soil layers (Fig. 5). At 1,900 m and 2,400 m, pH values were lowest in the O2 layer and increased again with increasing depth in mineral soil to values near 4. At 3,000 m, pH values up to a soil depth of 0.3 m were even significantly lower than at 1,900 m. Below that depth, pH values also increased to about 4.

Effect of altered soil properties on fine root growth in mineral soil

At 1,900 m, RLD in ingrowth cores of all treatments were similar in all soil depths, whereas at 3,000 m, there was a sharp decrease in RLD with increasing depth (Fig. 6). At 3,000 m, RLD was always markedly lower than at 1,900 m (note the differences in scale). This difference may be attributed to the described discrepancies in the

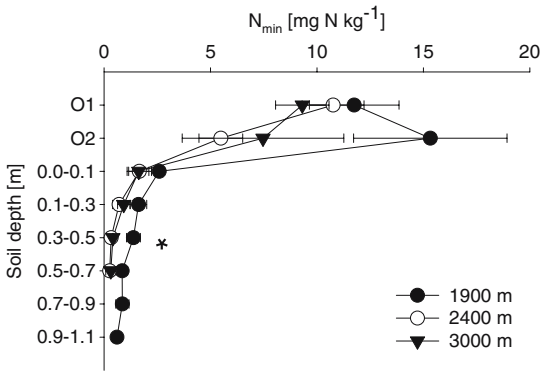

Fig. 4 N_{min} concentrations (mg N kg^{-1}) at different soil depths at three altitudes. Error bars represent standard errors ($n = 4$ at 1,900 m, $n = 3$ at 2,400 and 3,000 m). Asterisk indicates significant difference in N_{min} concentration between altitudes (Scheffé-test, $P < 0.05$). O1 = upper 0.05 m of organic layer, O2 = rest of organic layer

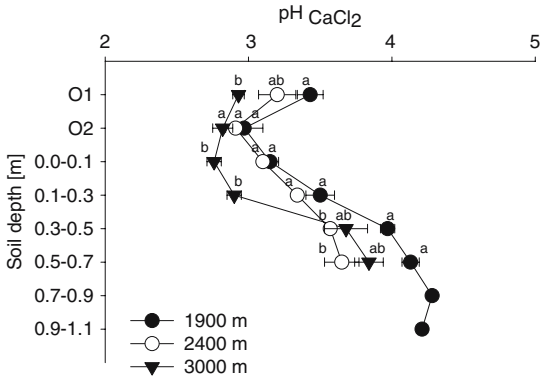

Fig. 5 Values of pH_{CaCl2} at different soil depths at three altitudes. Error bars represent standard errors ($n = 3$); lower case letters indicate significant differences between altitudes within one layer (Tukey-test, $P < 0.05$). O1 = upper 0.05 m of organic layer, O2 = rest of organic layer

methodological procedure of ingrowth core installation. Differences of RLD between the treatments were not significant at any altitude (H-test, $P > 0.05$).

Discussion

Seasonal changes of the nutrient uptake ability

The vertical rooting pattern in the present study was not affected by season. As an exception, RLD in mineral soil at 3,000 m showed an unexpected increase in the rainy in comparison to the dry season (Fig. 1). Seasonal changes in root densities in a lowland forest in Panama were explained by changes in soil water contents and nutrient availability (Yavitt and Wright 2001). However, in the lowland forest the dry period was much more pronounced than in the forest of the present study. In a study by Huxley et al. (1974), low topsoil water availability during the dry in comparison to the wet season in Kenya was associated with a decrease of tracer uptake from the topsoil by coffee trees, whereas root mass distribution was similar in both seasons. This indicates that tracer uptake is more sensitive to seasonal changes of water availability than fine root distribution. The lack of substantial seasonal effects on root distribution in the present study is presumably due to the fact that the seasonal fluctuations of temperature, precipitation and soil water contents were only small in the studied tropical montane forests.

Vertical pattern of nutrient uptake ability at different altitudes

In the present study, RNUP as a measure for the ability for nutrient acquisition from different soil depths was similar to the vertical pattern of RLD (Fig. 3). Length densities of living fine roots are primarily an estimate for the spatial exploration of the soil by plant roots, and thus, for the ability of plants to access nutrients at different soil depths (Bouillet et al. 2002; Lehmann 2003). It should be noted, however, that the spatial availability of nutrients is also dependent on soil characteristics regulating nutrient transport to the root surface by diffusion (Arvidsson 1999), and on root characteristics such as root hair formation (Wissuwa and Ae 2001) or mycorrhization (Muthukumar et al. 2003). Furthermore, RLD is commonly poorly correlated with the ability for nutrient acquisition under conditions where the physiologically based ability of roots for nutrient uptake is impaired by factors such as lack of soil moisture (Buljovcic and Engels 2001; Lehmann 2003) or oxygen deficiency (Morard et al. 2000) or where it is altered by differences in the functionality of roots (Göransson 2006). ^{15}N enrichment in leaves after application of ^{15}N

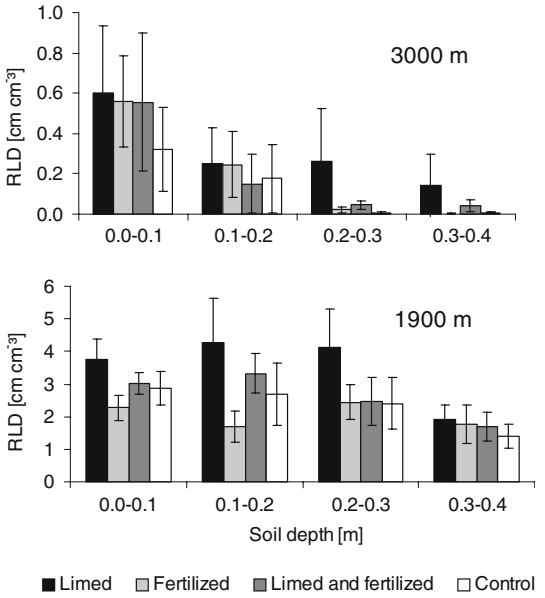

Fig. 6 Root length densities in ingrowth cores with different soil treatments at 1,900 and 3,000 m 1 year after installation. Error bars represent standard errors (n = 10). Note the difference in scale for 1,900 and 3,000 m

labelled fertilizer to different soil depths is a measure that integrates spatial availability of nutrients in soil to plant roots and the physiologically related ability of roots for nutrient absorption. As neither total plant biomass nor mean ^{15}N enrichment in the total biomass were determined, our data can only be used to assess the relative distribution of nutrient acquisition ability in different soil depths. The close correspondence of the vertical pattern of N uptake potential and RLD suggests that the physiologically based ability for nutrient uptake was not hampered in deeper soil layers.

At all three altitudes the organic layer was the horizon with the highest RLD (Fig. 1). This has also been reported for an old tropical montane forest (Hertel et al. 2003) and a lower montane forest in Costa Rica (Vance and Nadkarni 1992) and is in accordance with Ostertag (2001) who found highest root lengths in the upper soil layers of a Hawaiian tropical montane forest. RLD in the organic layer in this study were two times higher than in several tropical lowland sites (Powers et al. 2005).

In our study, there was no consistent effect of increasing altitude on RLD in the organic layer. In mineral soil, RLD below 0.1 m soil depth was substantially lower at the upper study sites in comparison to 1900 m. Where root growth is restricted, soil nutrients may be easily leached beyond the reach of plants (Rowe et al. 1999). The reduced access of roots to nutrients in mineral soil at high altitudes may imply that the potential risk of nutrient leaching is increased. Nitrate in soils with negligible anion exchange capacity is to the largest extent present in soil solution (Jungk 2002) and is therefore predisposed to leaching. However, at all altitudes RLD in all soil layers was high enough to assume effective nitrate retention by roots (Claassen and Steingrobe 1999). Sufficiently high RLD for effective nitrate retention and generally low soil N_{min} concentrations (Fig. 4) suggest that nitrate leaching in soil is negligible at all altitudes.

Many ecosystems show clear species differences in the vertical stratification of root systems (Van Noordwijk et al. 1996). For example, in a South African savannah grasses had shallower root systems than trees (Knoop and Walker 1985). In a seasonal tropical forest in the eastern Amazon herbaceous species had deeper root systems than trees (Sternberg et al. 1998). At our study sites, the vertical pattern of N uptake potential was very similar for trees, herbs and shrubs (Fig. 2). As an exception, N uptake from the deeper mineral soil at 1900 m was much higher from shrubs than from other plant groups. This might reflect a high abundance of young trees that had been classified as ''shrubs'', since root systems of young trees usually have less superficial root systems than adult trees (Polomski and Kuhn 2001). However, the high variability in N uptake from 0.4 m soil depth within plant groups did not allow a clear separation between the vertical rooting pattern of shrubs, trees and the understorey.

Possible mechanisms for the observed pattern of nutrient uptake ability

There are several possible reasons for the sharp decrease in RLD and N uptake potential in mineral soil at higher altitudes. As reported for other tropical montane forests (Cavalier 1992; Santiago 2000), mineral soils at 3,000 m were

often waterlogged, which may have caused oxygen deficiency in deep soil layers. Other impacts include shallower mineral soils at 2,400 and 3,000 m than at 1,900 m, less favourable soil chemical properties at higher altitude and differences in species composition between sites.

It is well known that rooting depth varies depending on plant species (Claus and George 2005; Coners et al. 1998; Silva and Rego 2003). The species composition of the forests in the present study changed significantly along the altitudinal gradient. However, plant diversity was extremely high (Homeier et al. 2002), suggesting a high genetic potential for complementary use of biotope space (Dimitrakopoulos and Schmid 2004) at all altitudes. It is therefore more likely that the vertical root distribution of the entire forest communities was governed by soil factors.

The soil pH was very low not only in the organic layer, but also in mineral soil, particularly at high altitudes (Fig. 5). Subsoil acidity has been shown to reduce vertical rooting intensity of *Picea abies* (Jentschke et al. 2001). The reduction of root growth in acid soil is usually related to high Al^{3+} and H^+ concentrations and low Ca/Al ratios in soil solution (De Graaf et al. 1997; Murach and Ulrich 1988). However, liming of the mineral soil did only marginally increase root growth at 1,900 and 3,000 m (Fig. 6). The lack of a clear effect of liming on RLD in the soil cores may have been caused by methodological problems. The initial difference in soil pH induced by liming was reduced during the 1 year period of the experiment from 1.0 to 0.3 pH units (decrease from pH 5 to pH 4.3) at 1,900 m and from 2.2 to 0.7 pH units (from pH 5 to pH 3.5) at 3,000 m. Alternatively, the lack of a significant rooting response may also indicate low susceptibility of root growth to low soil pH. Phytotoxic effects of low soil pH are usually reported for ecosystems exposed to anthropogenically induced soil acidification due to emissions of air pollutants (De Graaf et al. 1997; De Wit et al. 2001; Jentschke et al. 2001; Murach and Ulrich 1988). Native plant species growing on naturally acid soils are often adapted to low soil pH, e.g. by mycorrhizal symbiosis, rhizosphere alkalinization, Al chelation or by accumulating Al in extra-cytoplasmic compartments of root or shoot tissue (Cuenca et al. 1990; De Wit et al. 2001). In some cases plant growth of species adapted to acid soils is even increased by Al application (Watanabe and Osaki 2002). In the present study, the average leaf Al concentrations of woody plants were always higher than 1,000 mg kg^{-1} (data not shown), indicating that many woody species were Al accumulators (Cuenca et al. 1990). Accordingly, many Al accumulating species have also been observed in a tropical cloud forest in Venezuela (Cuenca et al. 1990). At least at 1,900 and 3,000 m, high organic carbon contents in mineral soil (data not shown) suggest high degrees of Al-complexation with organic compounds and thus low toxicity of Al for root growth (Jentschke et al. 2001). In summary, indirect evidence suggests that the differences in rooting depth at different altitudes are not caused by soil pH.

The N_{min} concentrations in mineral soil were very low, particularly at high elevations (Fig. 5). It is well documented, that external N_{min} concentrations have a strong impact on root growth (López-Bucio et al. 2003; Stitt and Scheible 1998). The variation of vertical fine root distribution in a montane rain forest in Panama was explained by differences in the concentration of N in soil (Cavalier 1992). However, fertilization did not significantly modify rooting into ingrowth cores (Fig. 6). This may be due to nutrient leaching during the 1 year of the study period or due to low rooting response of the forest plant community to fertilization.

Not only vertical fine root distribution in mineral soil but also in the organic layer showed different patterns between altitudes (Fig. 1). Whereas at 1,900 and 2,400 m RLD was similar in the O1 and O2 layer, at 3,000 m RLD was two times higher in the O1 layer than in the O2 layer. At 3,000 m, it was often observed that roots grew upwards along the trunk of their own or neighbouring trees, covered by thick moss layers. This was also observed in other tropical montane forests and is usually interpreted as a strategy to improve competition for nutrients that are leached from the canopy (Stewart 2000). The more superficial root distribution at higher altitudes supports the assumption by Cavalier (1992) that especially at higher altitudes of wet tropical regions more nutrients are taken up directly from litterfall, throughfall and stemflow. At a higher

altitude, nutrient input to soil is not only controlled by leaching from vegetation (Wilcke et al. 2001) but also by direct atmospheric nutrient input through fog and precipitation (R. Rollenbeck, pers. comm.).

Conclusions

In the studied tropical montane forest, the organic layer was always the preferred layer for nutrient acquisition. Root distribution and the ability for nutrient uptake were shallower at high altitudes. At 3,000 m, the marked concentration of fine roots to the surface of the organic layer was presumably related to high nutrient input from canopy by leaching. The specific constraints for root growth in the mineral soil of tropical montane forests warrant further investigations.

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