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Variation in roots and mycorrhizae in tropical rainforests in relation with fertility

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1 Abstract

1.1 English abstract

The nutrient balances of nitrogen (N), carbon (C) and phosphorous (P) in ecosystems are changing while its effects on the Earth's system are still a research enigma. Roots and mycorrhizae in the tropics are important in global C storage and nutrient cycling, which makes it necessary to understand how these belowground structures react to different nutrient levels. We collected soil samples at two depths (0 -15 cm and 15 – 30 cm) along a precipitation gradient in French Guiana, as well as a topographic gradient within one site, to analyze how depth, bulk density, nutrient levels and pH affected root densities, root diameter, presence of ectomycorrhizal fungi (EMF), root colonization percentage of arbuscular mycorrhizal fungi (AMF), relative abundance of (AMF) and AMF species diversity. Along the precipitation gradient we found that the root densities correlated negatively with N in the deeper soil, the abundance and species richness of AMF were positively related with N and negatively with P (congruent with the "trade balance model"), while EMF did not significantly correlate with any nutrient. With increasing depth, root densities and EMF presence decreased, while AMF relative abundance increased and AMF species richness was stable. No relations with pH were found except for the root density in the upper soil layer. Root density was not affected by bulk density, nor did it differ with topography. The root density and diameter did however respond to P in the top plots while it correlated to N in the base and slope plots. We conclude that variation in AMF, particularly at greater depth, can be explained by varying nutrient concentrations while EMF is more depending on tree species, the amount of nutrients in the vertical soil layers in a way limit root density and the nutrient triggering root response can vary along a topographic gradient since the gradient affects N:P ratios.

1.2 Dutch abstract

De evenwichten van koolstof (C), stikstof (N) en fosfor (P) in ecosystemen veranderen als gevolg van CO_2 uitstoot en stikstofdepositie, maar de gevolgen hiervan op globaal niveau zijn nog onvoldoende onderzocht. Wortels en mycorrhiza in de tropen zijn belangrijk voor de globale koolstofopslag en nutriëntencycli, waardoor het noodzakelijk is om te begrijpen hoe deze ondergrondse structuren interageren met verschillende nutriëntconcentraties. We hebben bodemstalen verzameld in Frans Guyana op twee diepten (0 – 15 cm en 15 – 30 cm) langs een neerslaggradiënt en topografische gradiënt om te analyseren hoe de worteldichtheid, de worteldiameter, de aanwezigheid van ectomycorrhiza (EM), het kolonisatie-percentage van arbusculaire mycorrhiza (AM), de relatieve dichtheid van AM en de AM soortenrijkdom beïnvloed worden door de diepte, bulkdensiteit, pH en concentraties van N, C en P. Langs de neerslaggradiënt correleerde de worteldichtheden negatief met C in de diepere bodemlagen, volgde de relatieve AM dichtheid en soortenrijkdom het handelsbalansmodel en correleerde de aanwezigheid van EM niet met de onderzochte nutriëntconcentraties. Worteldichtheden en de aanwezigheid van EM verminderden met toenemende diepte terwijl de relatieve dichtheid van AM steeg; de AM soortenrijkdom bleef stabiel. Enkel bij de worteldichtheid in de bovenste bodemlaag werd een correlatie met pH aangetroffen. De worteldichtheid werd echter niet beïnvloed door de bulkdichtheid of topografie. Wel vonden we tussen de verschillende topografische niveaus een andere respons van worteldichtheid en worteldiameter op de aanwezigheid van N en P. De plots op de top correleerde met P terwijl de plots op de helling en basis correleerden met N. We concluderen dat variatie in AMF kan worden verklaard door variërende nutriëntenconcentraties, terwijl de variatie in EMF waarschijnlijk meer afhangt van aanwezige boomsoorten De nutriëntenconcentraties in de diepere bodemlagen een beperkende factor kunnen zijn op de worteldichtheid en dat het nutriënt dat een respons in worteldensiteit en worteldiameter veroorzaakt kan verschillen over een topografische gradiënt door verschillen in N:P ratio’s.

1.3 Layman abstract

The amount of nutrients available to organisms is globally changing due to the increase of CO_2 and deposition of nitrogen due to the use of inorganic fertilizer. Tropical forests are a great reservoir of CO_2 and hence very important in global climate regulation. CO_2 is captured by plants and incorporated in their trunks, leaves and branches but also, less obviously and also less studied, in their roots. Besides plants, also belowground fungi that live in close contact with plant roots, can store great amounts of carbon. These fungi and roots are also important in the cycling of nutrients from the soil back to living organisms. Since these belowground fungi and roots are so important in forest processes. In this research, we collected soil samples in French Guiana. The effect of nutrients, depth, soil density and acidity was analyzed on the presence of roots and belowground fungi living in close contact with these roots. With the variation in soil factors among different sites, we can try to predict how the roots and fungi of a certain soil would react if the amount of nutrients changed. We found that some fungi were indeed correlating with nutrients, while the presence of other fungi types was probably more influenced by the presence of certain tree species. In some cases, less fungi were present, due to competition with roots for the same nutrient. Also, we found that along the topographic gradient, the amount of different nutrients changed and as a result, the roots in the top would respond to a different nutrient than the roots in the base. Whit this kind of data, we can try to make predictions about the functioning of the earth and hence the implications for human life under these changing nutrient concentrations.

2 Introduction

Tropical forests are diverse and complex ecosystems that differ in important ways from ecosystems in temperate regions (Tripathi et al. 2016). Besides being major reservoirs of plant and animal biodiversity, tropical forests are also important in global climate regulation and biogeochemical cycling. However, tropical forests are under great threat, as the growing need for food, fiber and energy results in the conversion of tropical ecosystems to other uses (Fearnside 2000). Approximately 40 % of the global carbon (C) stock is stored in tropical ecosystems (Guerrero-Ramírez et al. 2016). As a result, it is crucial to understand how environmental factors and biogeographical patterns interact in the context of global change. The C:N:P balances in ecosystems are changing with no earlier equivalent in the history of the earth due to increasing availabilities of carbon (C) and nitrogen (N) in most areas of the globe (Peñuelas et al. 2012). These stoichiometric shifts will affect the structure, function and diversity of the Earth system whereas its impact still remains a research enigma. Data from field experiments of ecosystem nutrient limitations are necessary to predict these impacts using Earth system models (Ciais et al. n.d.). However, relatively few studies have examined belowground processes in the tropics due to methodological constraints of measuring root biomass (Guerrero-Ramírez et al. 2016) whereas significant amounts of C can be stored in roots (Ngo et al. 2013) and the rapid turnover of fine root systems are a major pathway of nutrient flow from plant to soils (Baddeley and Watson 2005). Also mycorrhizal fungi can promote below-ground storage of plant C (Rillig 2004) and are important in mobilization of N and phosphorus (P) from natural substrates (Read 2003). These properties make it valuable to research how nutrient balances affect both root density and mycorrhizal presence. The lowland tropical rainforests of French Guiana form a good location to do this investigation since long-term research has been conducted in the past and this created a well-established network of study sites.

In literature there has been a bias towards nitrogen (N) as the main limiting nutrient for plant productivity since most plant ecological research has been conducted in Western Europe and North America, which have relatively young postglacial landscapes with low amounts of N. On ancient soils like the soils of French Guiana, phosphorous (P) is often the limiting resource and this fact is now getting more recognition in literature (Lambers et al. 2007). The reason for the contrasting resource abundances in the world is the different source of N and P. The main reservoir of N is the atmosphere which composed out of 80% nitrogen gas (N_2). The key pathway for N to enter the ecosystem is via nitrogen fixation by bacteria that synthesize

NH_3. With addition of a proton from the soil, NH_3 forms NH_4^+ and this can get further converted to NO_3^- in the process of nitrification by nitrifying bacteria. Also precipitation, lightning and blowing dust can provide substantial inputs in ecosystems. No significant phosphorus-containing gases exist, so only small amounts of P move through the atmosphere as dust (Campbell et al. 2008). This contrasting atmospheric- versus rock-derived source distinguishes the availability of N and P over geologic time (Newman and Hart 2015). Young soils contain high levels of P but very low levels of N. Trough aging, the N levels will increase until a peak moment of nitrogen in the soil is reached due to microbial N_2 fixation and atmospheric deposition (Lambers et al. 2007). Meanwhile, P gradually disappears because of leaching and erosion, summarized as "weathering". For example in the chronosequence in Franz Josef (New Zealand), the total mineral N in the top 100 mm soil peaked at circa 9 g/kg and then declined to circa 3 g/kg with age. P declined from circa 800 to <100 mg/kg in the oldest studied stages (Richardson et al. 2004).

The rate at which soils develop is highly influenced by rainfall and temperature since this affects microbial activity, root metabolism and erosion, all of which contribute to soil weathering. Soils can however also be replenished trough processes such as glaciation, deposition of material from the ocean or from higher up in the landscape (Lambers et al. 2007). Soils in the tropics are infertile due to 1) their ancient status, 2) not having undergone glacial weathering or benefitted from post-glacial deposition, 3) rapid leaching due to high rainfall and temperature (Malhi 2011), or a combination of these factors. This holds true for French Guiana, which is composed out of soils from old granites and metamorphic rocks of Precambrian age (Damuth and Fairbridge 1970) and receives an annual rainfall of 2923 mm (Baraloto et al. 2011).

The infertile soils of tropical ecosystems are nonetheless known to harbor a great biodiversity and productivity (Malhi 2011). Terrestrial plants have adopted different mechanisms to overcome P limitations such as effective use of P for growth, resorption from falling leaves, internal recycling and P allocation from roots to shoots (Lambers et al. 2007; Horst et al. 2001). The uptake by roots can be improved by creating a steep gradient at the soil-root interface to benefit diffusion. However, in most soils it is likely that the transport of P from soil to the root is the main limiting factor for P acquisition rather than the root uptake (Horst et al. 2001) because roots can take up inorganic P much faster than that it moves in most soils (Lambers et al. 2007). Ways to improve this P acquisition can be achieved by increasing the range of chemical forms that can be absorbed (Read and Perez-Moreno 2003), increase the

supply rate of these compounds (Clarkson 1985) and an enlargement of the absorbing area (Lambers et al. 2007). The latter can be achieved by a large root system capable of exploring greater soil volume. Also changes in root architecture can occur in response to P limitations such as an enhanced lateral root formation, which increases the concentration of roots in the topsoil. The topsoil is nutrient rich compared to the deeper soil layers because the nutrient cycle occurs mostly in the upper soil layers (Poszwa et al. 2002). Research found that indeed significantly more root biomass can be found in the upper soil layers of tropical forests (Cavelier 1992; Sanford and Cuevas 1996). Cavelier (1992) argued that the vertical decrease of root biomass in a semi deciduous forest and lowland tropical forests in Panama is mainly controlled by the decreasing concentration of N and P. The author concluded that deeper roots mainly function for anchorage and water supply and that the root density in the deeper layers is thus constrained by nutrients.

Soil nutrients are a factor that can explain variances in root biomass between sites in tropical forests. Vitousek and Sanford (1986) predicted that trees allocate more energy to roots or otherwise maintain more extensive root systems on infertile sites, as the resulting increase in nutrient acquisition would increase growth and/or reproduction. Consequently, greater root biomass and/or root-shoot ratios might be expected on less fertile sites. Since most tropical forests are P-limited, tropical tree roots are expected to respond more to P than to N availability (Sanford and Cuevas 1996). Treseder and Vitousek (2001) indeed found greater root biomass and greater root length in P-limited Hawaiian islands than in fertile or N-limited islands. Also Gower (1987) found an inverse relation between root biomass and P but not within N in the non-flooded areas in the tropical forests of Costa Rica. The total root biomass in a P deficient soil might be larger, but root production rates are actually lower in P deficient soils due to the lack of P necessary for root growth. Higher biomasses can however be explained by the longer root lifetime experienced in these soils (Treseder and Vitousek 2001). Also a greater root-hair length and reduced root diameter can improve P acquisition in P deficient soils to enlarge the root surface area at minimal cost (Wissuwa et al. 2005; Lynch and Brown 2001; Horst et al. 2001). We might thus suspect higher root biomasses and lower root diameters in sites which are more P-deficient.

Apart from long-term processes such as mineral weathering, there can also be high local variation in nutrient availability for a number of reasons. A topographic gradient can for example alter the abiotic soil characteristics. Earlier research found higher N concentrations on the top (Ferry et al. 2010) but higher amounts of P in the base and slopes (Cox et al. 2002;

Chen et al. 2004; Ferry et al. 2010) along the topographic gradient. The high P in the base was caused by a fertility transfer from the upper parts of the topography through litter- and treefall and the dissolution of iron oxides by anoxic conditions due to the higher water table in the base (Ferry et al. 2010). These anoxic conditions would cause a reduction of Fe^{+3} to Fe^{+2}, which had a positive effect on the P availability in these soils since P is more strongly sorbed to Fe oxides than to the reduced forms of Fe (Silver et al. 1994). The lower nitrogen in the base was explained by the higher water table in the raining season causing a low soil oxygen availability and therefor decreasing N availability by inhibiting decomposition rates. Cuevas and Medina (1988) found that in non-flooded areas of tropical forests, root growth responded only to P availabilities while in flooded soils, roots would respond to N. This might also hold true for our topographic gradient i.e. a root response to N in the base but to P in the top.

Because of the higher P levels in the base, Ferry et al. (2010) concluded that fertility was higher in the bottomland than on the hilltop and slope. However, they also noted that the bottomlands are more susceptible to treefall, reducing the tree age in the bottomland. Cavalier (1992) argued that in older forests, the aboveground biomass increment is reduced while fine root production is incremented and Kozlowski (1997) concluded that a high water table suppresses root growth. The base regions might thus be more fertile, but less tree roots are expected due to higher p concentrations, a lower forest age and a higher water table suppressing root growth.

Besides enlarging the root area, also root excretions can also improve P supply rates. Acid secretions (H^+) for example improve the solution of P from the soil and excreted phosphatases can hydrolyze the ester-phosphate bonds in soil organic P and therefor release the phosphate into soil solution for uptake (Clarkson 1985; Treseder and Vitousek 2001). The conventional view states that plants take up N and P from soluble inorganic sources (nitrate and ammonium for N, phosphate for P) available in the soil solution (Lambers et al. 2007). To achieve an increased range and mobility of compounds, plants can establish symbiotic relations with mycorrhizal fungi (Shane and Lambers 2004; Lambers et al. 2007). Here, the fungus acquires nutrients from the soil and organic substrates in exchange for photo-assimilates from the plant (Madigan et al. 2012; Read and Perez-Moreno 2003).

In this study, we will focus on ectomycorrhizae (EM) and arbuscular mycorrhiza (AM). Ectomycorrhizal fungi (EMF) typically form a mantle of hyphae around the root surface. These hyphae extend from the mantle into the soil and also grow in the extracellular spaces of

the root cortex to form a Hartig net that facilitates nutrient exchange with the plant (Campbell 2008). Arbuscular mycorrhizal fungi (AMF) do not form a mantle around the root but their hyphae penetrate the epidermal and cortical cells intracellularly. When the hyphae reach the inner cortex cells, they form oval structures called vesicles and dichotomously branched or coiled hyphal structures to improve nutrient transfer with the plant host (Madigan et al. 2012; Taiz and Zeiger 2010). Plants can produce Arum-type mycorrhizal colonization that form intercellular hyphae and tree-shaped arbuscules inside the cell or they can form the Paris-type which contains intracellular hyphae, coils and arbusculate coils (Dickson 2004). The chemical forms of nutrients that can be accessed depend on the type of mycorrhizal symbioses (Read and Perez-Moreno 2002). AMF take up predominantly inorganic P from the soil solution, whereas EMF also have access to insoluble organic forms through the exudation of phosphatases. EMF also play a key role in mobilizing N from soluble inorganic and soluble or insoluble organic sources. AMF deliver soluble inorganic N forms to its host (Lambers et al. 2007) but in contrast to the other types, they use organic N for their own nutrition (Dickie 2013). According to the different nutrient availabilities and limitations around the globe, patterns of mycorrhizal dominance can be expected (Read 1991). EMF are associated with relatively few plant species, but include widespread and dominant plant species of temperate, boreal and some tropical forests (Dickie 2013) while AMF colonize more than 85% of all terrestrial plants (Madigan et al. 2012) and dominate in tropical forests like French Guiana, together with some EMF (Read 1991; Read and Perez-Moreno 2002; de Grandcourt et al. 2003).

Environmental characteristics such as nutrient availability may determine optimal combinations of nutrient acquisition methods in a system. Plants will use resources that are available in excess to capture those that are not. In addition, an increase in availability of one nutrient may increase the demand and investment in procurement of another nutrient (Treseder and Vitousek 2001). The abundance of EMF (Wallander 1995) and AMF (Johnson 2009) are thus depending on the amount of both bioavailable N and P. According to the trade balance model (Johnson 2009), a soil deficient in both N and P will support beneficial mycorrhizal symbiosis but the C-for-P trade will be limited due to C limitation. Benefits would however be eliminated in soils high in P but low in N because plants and fungi may then both be competing for N and C. In tropical forests, high N and low P levels are expected. The model predicts in these conditions greatest benefits because the high N levels increase the photosynthetic rate of the host and thus also the need for P.

Soil properties can also affect root growth and mycorrhizal associations through their effect on nutrient availability, pH and bulk density. Bulk density (BD) can be defined as the mass of soil divided by its volume. The bulk density is strongly affected by the texture of a soil and the amount of organic matter (McKenzie 2004). Horizons high in organic matter cause lower densities (McKenzie 2004) while clayey and silty soils have lower BD compared to more sandy textures(Schaetzl and Thompson 2015) In these more sandy soils, smaller particles like silt and clay are able to fill the voids between sand grains, optimizing the packing of the soil matrix. Meanwhile, clayey soils have many micropores but these are more difficult to eliminate. (Schaetzl and Thompson 2015). Within the soil, some fraction of organic matter can be complexed with clays or physically protected within clay micro-aggregates. Consequently, clayey soils have higher organic matter contents than sandy soils and thus even lower BD (Anderson and Spencer 1991). Roots grow in the soil through large soil pores or will move soil particles aside when pores are smaller than the root tips. High bulk densities can therefore inhibit root growth because the roots cannot exert enough pressure to overcome the resistance to move soil particles.

The soil in tropical rainforests is often acidic due to the rapid litter decomposition rate and high levels of rainfall (Anderson and Spencer 1991). Carbon dioxide is produced as a result of decomposition of organic matter and it reacts with soil water. This carbon dioxide reacts further into hydrogen ions and bicarbonate, acidifying the environment. Also ammonia and hydrogen sulfide are produced in microbial decomposition processes that can form strong acids when oxidized (Taiz and Zeiger 2010). When pH decreases, Al^{3+} solubility increases which has the potential to induce toxic effects on plant tissues (Cavelier, 1992) and can bind with P. Both Al^{3+} and $Fe^{2+/3+}$ can bind phosphates and precipitate, lowering the direct P availability. A low pH can also increase the weathering of rocks and increase solubility of phosphate. Roots are thus affected by pH through its effect on P availabilities and by direct Al toxicity . Roots may therefore have to find balance between potential soil toxicity and the need to absorb nutrients that are low in supply.

In this thesis we are interested in how different abiotic factors affect total root density, root diameter and mycorrhizal presence of AM and EM. Multiple sites in the tropical forests of French Guiana were sampled to compare differences. Also, one site was studied into more detail according to an elevation gradient. Our hypothesis were:

1) Higher root densities in the upper soil layers, compared to the lower soil layers, are driven by the higher nutrient concentrations present in the upper zone; 2) root diameters will be smaller and root densities will be higher in sites with low soil P-levels; 3) high BD might reduce root densities and diameter since it can increase the root resistance to penetrate the soil; 4) root density and diameter might be differently affected by nutrients along a topographic gradient where root responses might be driven by P in the top but by N in the base; 5) AMF relative abundance, AMF species diversity and ectomycorrhizal presence can be explained by the trade balance model (Johnson 2009) in which bioavailable N and P explain benefits of the symbiotic relationship.

This thesis was part of the 'Imbalance-P' project, a project sponsored by the European Research Council, to investigate future shifts of stoichiometric C:N:P balances and their effects on natural ecosystems, the earth system and human society (Ciais et al., n.d.).

3 Methods & materials

3.1 Field description and sample collection

The field work was conducted in multiple sites within a lowland tropical rainforest spread over French Guiana (South America). The mean annual precipitation of the study sites varied along a precipitation gradient ranging from 2250 - 4000 mm (Figure 1). The climate is affected by the north/south movements of the inter-tropical convergence zone, which causes seasonal variations in rainfall. Samples were taken at the end of the wet season between mid-June and mid-July. In the period from May to June, 500-800 mm of precipitation falls while during the long dry season, from mid-August until the end of November, the region receives less than 100 mm per month (Wagner et al. 2014).

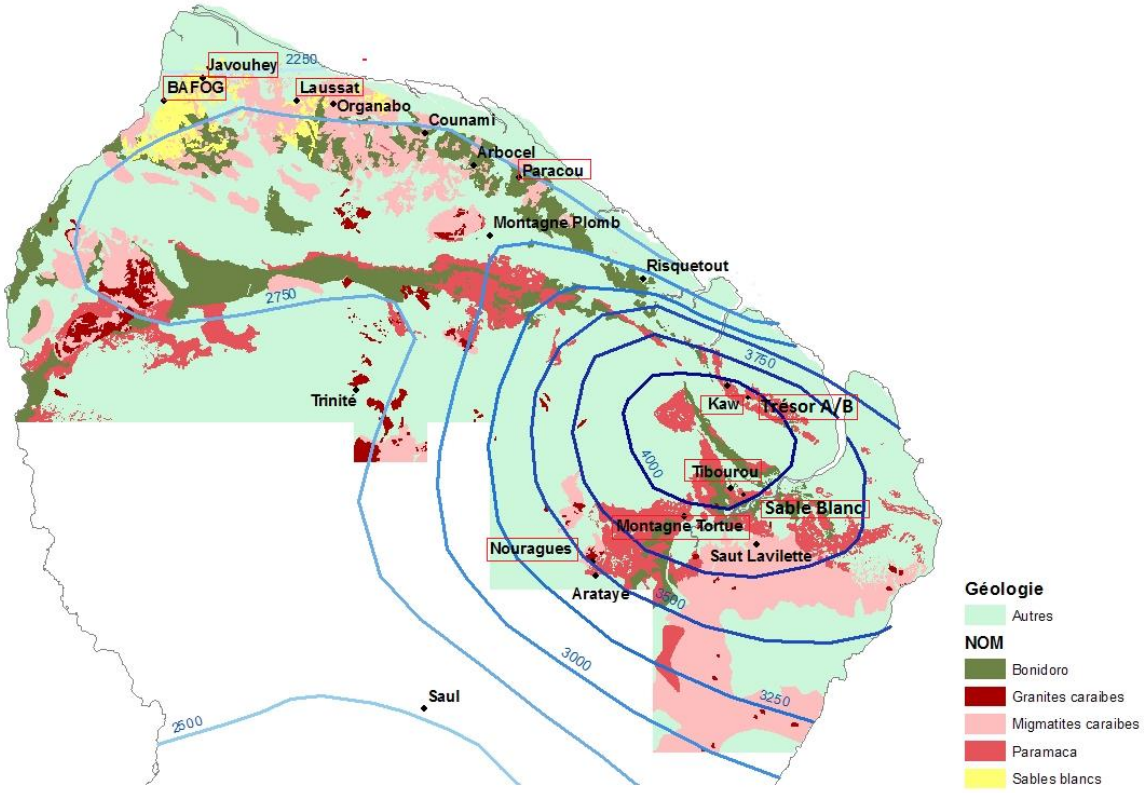

Figure 1. Map with overview of the different sample sites. Sites with red boxes were investigated in this paper. These include: Laussat (Lau), Bafog-A (BAA), Bafog-B (BAB), Javouhey (JAH), Kaw (Kaw), Tibourou (Tib), Sable blanc (Sal), Montagne tortue (Mon), Tresor site A (Tra), Tresor site B (Trb), Nouragues (Nou) and Paracou (Par). Blue lines represent equal estimates of annual rainfall. (Photo courtesy of Clement Stahl, INRA)

At every site, one plot of 20 by 20 m was studied with exception of Nouragues where at the top, slope and base each 4 plots were investigated. Every plot was subdivided in 5 zones according to the dice design (Fig. 2). Soil samples without litter were taken with a core of 15 cm height and 8 cm width to calculate bulk density and to collect roots. The core was inserted in the soil twice to obtain a separate sample from the 0-15 cm and 15-30 cm soil depth zones, taken in the middle of every zone of the plot. Additional cores of 30 cm height with a diameter of 3.3 cm were collected for the analysis of nutrients and mycorrhizal presence. Here three samples were taken per zone, a meter from where the bulk density sample was collected, and split into a 0-15 and a 15-30 section. The 3 cores of the same zone in the plot and the same depth were mixed to obtain a representative sample. Immediately after soil collection, samples were placed in Ziploc bags and the samples for nutrient and mycorrhizal composition were transported in a coolbox to avoid further decomposition of material by micro-organisms and hence changes nutrient composition. The same day, these samples were frozen using either dry ice or a freezer and were never thawed before freeze-drying.

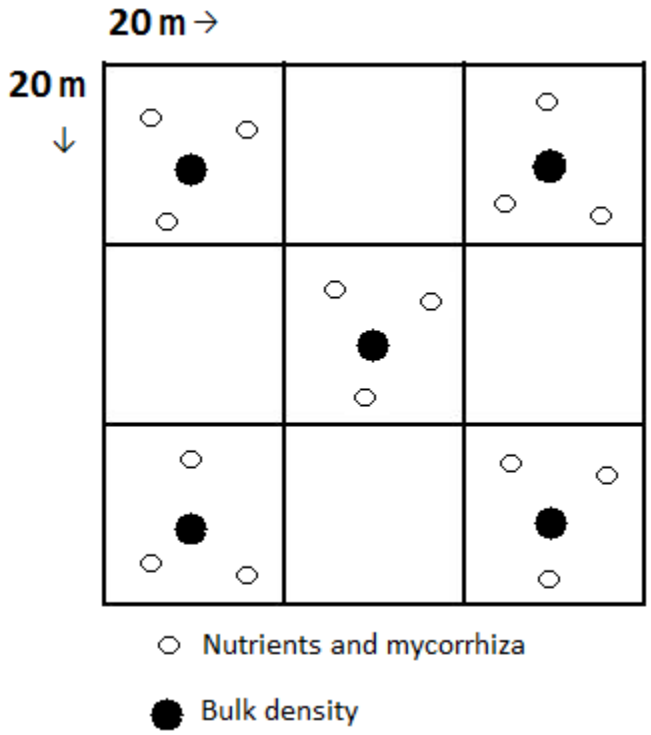

Figure 2. Systematic overview of the dice design. Per zone, one bulk density sample and three samples for nutrients and AMF analyses were collected at two different depths (0-15 cm and 15-30 cm).

3.2 Bulk density

Soil bulk density was defined as the ratio of the dry mass to the volume of the solid material smaller than 4 mm plus the volume of the pores. It varies with the structural condition of the soil, especially that related to packing (Blake 1965). Bulk densities can be defined in multiple ways. The formula we used is in accordance with Blake (1965) and Schaetzl and Thompson (2015) (Eq. 1)

$$(1) \quad Bulk\ density = \frac{Mass\ soil_{<4\ mm}}{\mathrm{Volume}_{\mathrm{core}} - \mathrm{Volume}_{\mathrm{soil>4mm}} - \mathrm{Volume}_{\mathrm{roots}}} = \frac{Mass\ soil_{<4\ mm}}{\mathrm{Volume\ soil}_{<4\mathrm{mm}}}$$

The different components of the formula were obtained by first sieving the soil through a 4 mm sieve. This soil was later dried at 110 °C for at least 24 h and weighted. Stones larger than 4 mm and roots laying on the sieve were collected and washed. Also, roots were collected from the sieved soil during a set time (± 5 min) and washed together with the other roots. The volume of the stones and roots was measured in a water-filled graduated cylinder with the water displacement method (Pang *et al.* 2011)**.** Stones were weighted after 24 h at 110 °C and roots were weighted after freeze-dried.

3.3 Soil analyses

Soil texture, pH, NO_3^-, NH_4^+ and Bray P analyses were carried out by Leandro Van Langenhove for the Nouragues sites while analyses of soil texture, pH, total N%, total C% and Bray P of all other sites were conducted by Dr. Jennifer Soong.

Soil textures were estimated per plot and depth by first sieving the soil (2 mm). H_2O_2 (30 %) was added in order to destroy organo-mineral bindings. After oven drying (110 °C, 72 h), sodium hexametaphosphate (Na-HMP, 5%) was added to remove remaining organo-mineral bindings and samples were shaken (18 h). The sample was sieved (63 µm) to separate the sand and this sand fraction was oven dried (110 °C, 24 h) and weighted. The filtrate composed out of clay and silt was diluted to a volume of 1 l with DI water. After 6 h, the silt had settled and the clay content could be measured which a hydrometer. This measurement was then corrected for solution viscosity with the blank measurement of only Na-HMP. The

proportion of sand (53-2000 µm) was calculated as the ratio of the oven-dried sand mass and the mass of total oven dried soil. The proportion of clay (< 2.0 µm) was the ratio of the corrected hydrometer measurement and the mass of total oven dried soil. Ultimately, the remaining proportion would be the silt fraction (2.0-53 µm) (American Society for Testing and Materials1, 1972; Gee and Bauder, 1986).

pH was measured from a solution with a volume ratio of 1:5 of soil and a KCl solution (1 M KCl) (Sikora and Moore KP 2014). For Nouragues, inorganic NH_4 and NO_3 concentrations were estimated with the 1.0 M KCl extraction method (Carter and Gregorich 2016). After addition of the KCl solution, the soil was shaken (30 min, 160 strokes/min) and afterwards filtered (2.5 µm) to remove the soil. For NO_3, the Cd-Reduction method was applied that gave the solution a red-purple color and for NH_4, phenol was added to create a blue color. Color intensity is proportional to the NH_4 and NO_3 present in the solution and therefor concentrations were obtained using a spectrophotometer. The NH_4 and NO_3 concentrations in dry (C_{dry}) soil was calculated using the formulas below. First the concentration in the moist soil (C_{moist}) was measured using the mass of the moist soil (m_{moist}), the concentration ($C_{extract}$) and the volume of the extract ($V_{extract}$) (Eq. 2). The concentration in dry soil (C_{dry}) was then calculated with the mass of dry soil (m_{dry}), concentration (C_{moist}) and mass of the moist soil (m_{moist}) (Eq. 3)

(2) $$C_{moist} = \frac{C_{extract} \; x \; V_{extract}}{m_{moist}}$$

(3) $$C_{dry} = \frac{C_{moist} \; x \; m_{moist}}{m_{dry}}$$

On other sites, the weight percent of total carbon and nitrogen content was determined after grounding and oven drying (100 °C, 24 h) soil using a combustion method. Here, samples were placed in a high temperature reactor, followed by analyses of the gases using a thermal conductivity detector (Flash 2000 series NC-Soils Analyzer). This detector sensed changes in thermal conductivity and compared it with a flow of Argon carrier gas.

To obtain the available soil P content, the Bray P method was chosen (Bray and Kurtz 1945). Soils in tropical rainforests are often acidic (Anderson and Spencer 1991) and this method performs best as an indicator of P available to plants in acid soils. The soils were extracted with HCl (0.025M) and NH4F (0.03M) for one minute and filtered over a 2.5 µm filter Fluoride (F^-) was added to enhance the release of P from aluminum phosphates in the soil by decreasing the Al activity trough formation of Al-F complexes and F^- was also effectively suppressing re-adsorption of solubilized P by soil colloids. The acidic nature of the extractant

(pH 2.6) also contributed to the dissolution of available P from calcium and iron-bound forms. The extractable P (mg P/kg soil) was then calculated as the concentration P in the extract multiplied by the volume of added extract divided by the soil mass (Eq. 4).

$$(4) \quad C_{extracted\ P} = \frac{C_{extract\ P}\ x\ V_{extract}}{m_{soil}}$$

3.4 Root traits and mycorrhizae

The collected roots, which were used to calculate BD, were categorized per sample based on a diameter larger or smaller than 2 mm and weighted. Roots were freeze-dried in French Guiana, but were placed in the oven in Antwerp for 15 min at 70 °C before weighing to make sure that no water had reabsorbed during the time it was transported. The roots with diameters smaller than 2 mm will be addressed as fine roots later on in this paper. During the sorting process based on diameter, the abundance of ectomycorrhizae on the fine roots (<2mm) was noted and samples were categorized according to the estimated proportion of infected roots to the total amount of roots in 4 categories (0: no infected roots spotted, 0.10: few roots infected, ±0.33 roots infected, ±0.50 roots infected). Colonized roots were recognized by their thicker and more branched shape than uncolonized roots and these colonized roots tend to be colored as well (Habte 2000).Roots were not sorted by species, and thus represented the community of plants at each site. Occasionally, large root pieces (>3ml) were found, these were not included in further analyses since they created large variances among samples.

The AM richness and abundance analyses were carried out by Prof. Dr. Verbruggen on isolated DNA from freeze-dried soil using the PowerSoil DNA Isolation Kit. A PCR was conducted using 1µl of the DNA solution, 1 Phusion High-Fidelity Mastermix and 200nM of the fungal primers ITS1f and ITS2 that were augmented with multiplexing barcodes. The PCR conditions comprised initial denaturing at 98 °C for 30 s, followed by an additional round at 98 C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s. The latter three steps were repeated 30 times and an additional final extension step of 72 °C for 10 min was added at the end. PCR products were loaded on agarose gels (1.5 %) to confirm whether PCR amplification was successful. Successful amplification products were pooled and cleaned with the Agencourt AMPure XP system and quantified with a Qubit fluorometer. This pool was then placed again on an agarose gel (1 %) to remove remaining primers, cleaned

using the QIAquick Gel Extraction Kit and again quantified. With an Illumina MiSeq (300 cycles), this product was sequenced.

Sequences were clustered into OTUs by first trimming sequences to 250 bp and undergoing a quality filtering (max. error of 0.5 %). Singletons were removed and others clustered to 97 % similarity. Chimera's were filtered using the UNITE database of ITS1 sequences as implemented in UCHIME. A representative sequence for each OTU was aligned with sequences of fungal representative species of the UNITE database using the BLAST algoritm. Non-fungal sequences were removed and the number of reads per sample was rarefied to 4000 reads. For the OTUs which were classified as belonging to the phylum Glomeromycota was the relative abundance within sample of total fungi (% AMF) and the number of unique OTUs per sample (species richness AMF) calculated.

To investigate the percentage of roots being colonized by AMF, microscopic slides with stained roots were made for the 0-15 cm depth zone and arbuscules, AM hypha and vesicles were scored. AM hyphae can be distinguished in the soil from other fungi by the absence of septa and by the dichotomic branching of hyphae. In roots it is characterized by its configuration in the cortex with hyphae, vesicles and arbuscules. Camenzind and Rillig (2013) used Tryphan blue as coloring agent, but other researchers such as Vierheilig et al. (1998) presented normal ink to be a less toxic alternative for root staining. In this study, both coloring agents were compared. We used a modified staining protocol of Camenzind and Rillig (2013) with reduced incubation time in KOH. To compare the two coloring agents, the same steps were followed with ink as coloring agent.

The root pieces were put overnight in 10 % KOH at 60 °C to perforate cell walls, washed with tap water, bleached from their natural dark pigments in 20 % H_2O_2 for 20 min. at RT and afterwards washed again. Next, they were re-acidified for 10 min. in 10 % acidic acid and then stained for 1.5 h in Trypan Blue at 60 °C or respectively with ink (Waterman Paris, intense black) at 90 °C for 10 min. and destained in lactoglycerlol.

3.5 Statistical analyses

All statistical analyses were performed with the R software (R Development core Team 2010). Mixed models were conducted with the R-package 'lme4' and 'lmerTest'. To compare estimates, factors were standardized by subtracting the mean and dividing the standard deviation to obtain a variable with mean 0 and standard deviation 1 to allow direct comparison of estimates and to make sure that the effect of a factor with large values would have the same 'power' on the independent variable as a smaller factor. Separate analyses were conducted on the dataset containing data from the topographic gradient in Nouragues and on the dataset with data along the precipitation gradient including only one random plot of Nouragues.

Before being standardized, concentrations of P, NH_4, NO_3 total C and total N were multiplied with their bulk densities. This was important since samples with a higher BD will contain more nutrients per volume element compared to samples with a lower BD while nutrient concentrations can be the same. It also enables the upscaling from point measurements towards estimates of nutrient and C stocks in the ecosystem. As a result, measurement comparisons with other studies can be made. Multiplying the nutrient concentrations solely with the soil mass (instead of actual BD) could result in similar amounts of nutrients in the core while soil and pore volumes can differ. However, because we are interested in the relationship between nutrients and roots, AMF and ectomycorrhizae we want to account for differences in soil and soil pore volume as these can affect these organisms differently. For instance, when the total amount of nutrients is similar, organisms in a low soil and pore volume (when there are many stones or a different soil texture present) need to explore a smaller area at small spatial scales to acquire the necessary nutrients compared to soils with higher volumes. To assess the differences in root diameter, the fine root mass was divided by the total root mass to create a ratio that could be used to compare along study sites. This ratio will be referred to as the fine root ratio.

A principal component analyses was carried out to assess interrelatedness of the various variables that had been measured: total N, total C, Bray P, pH, root density, fine root ratio, AMF abundance, AMF species richness and the proportion of infected roots with EMF. Means of the standardized variables per location and depth zone were used to avoid a lack of independence in the PCA.

Correlation analyses were carried out per depth zone for texture, pH, total N, total C, bray P and rainfall. Since this function is unable to deal with random terms, the mean value per location was used. When we were interested in how certain factors affected each other, we created mixed models per depth zone with location added as a random effect to avoid a lack of independence between the samples of the same location. This simple mixed model was used to test which factors influenced BD and root density. A separate model per variable was made for simplicity. When the relation between BD and texture was tested, the mean BD per location and depth zone was used since only 1 texture measurement per location and depth zone was conducted on a pooled sample. As a consequence, a simple linear model was used. When the effect of topography was tested in the Nouragues data on the BD, nutrients, root density and the fine root ratio, a similar model was built but with topography as fixed term and plot as random factor. Since every plot had a distinct number, it was not necessary to nest plot in topography level. A TukeyHSD test was performed to see how each topography level differed from each other using the package 'lsmeans'.

A generalized mixed model with a binomial distribution was made to assess the effect of the relative AMF abundance on nutrients, pH and the root density. The weight of every sample was equal. The same parameters were tested on the amount of AMF species but were carried out with a generalized mixed model with a poisson distribution. The proportion of infected roots with ectomycorrhizae was intended to be analyzed with a mixed model and binomial distribution but since this was not functioning properly, we decided to use the natural logarithm of the product of the proportions of infected roots multiplied with the fine roots mass. This was carried out to give samples with higher root masses more weight in the model because when more roots are found, the proportion of infected roots have more chance to reflect reality. To correct for the fine root biomass in the response variable, this term was also added in the models as a fixed term. Again, location was added as random factor and the effect of total N, total C, P and pH were analyzed as fixed variables. The fine root ratio was similarly analyzed transforming it with the natural logarithm.

When the effect of depth was analyzed on BD, nutrients, AMF relative abundance, AMF species richness, EMF presence and root density, depth was added in the above mentioned model as a fixed effect and replicate was nested in location. In other analyses, models were made per depth zone separately so that replicate did not need to be nested with location anymore. Since mixed models calculate the degrees of freedom, the degrees of freedom in the 'results' section were rounded to integers.

4 Results

4.1 Analyses along a precipitation gradient

In this section, the results are presented of the analyses that were conducted on all studied locations plus one random plot of the Nouragues station. Samples of the deeper zone in the Sal site were not taken due to extreme high water tables and are thus missing in our analyses.

4.1.1 The effect of depth

We found that depth had a major effect on the nutrient concentrations. The amount of P, total N and total C found in the core was significantly higher in the upper 15 cm of the soil compared to the 15-30 cm zone. Also pH showed a significant difference between depth zones, with a higher acidity in the upper soil layer (Table 1).

Table 1. Estimates, F- and p-values from the analyses of depth between 0-15 cm and 15-30 cm of the soil on separate models with pH, total C, total N and P per soil volume (g/m³) as fixed terms. Random terms included replicate that was nested in location. Estimates are from standardized variables to compare the magnitude of factors.* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$

Variable	Estimate	F	P
pH	0.90	$F_{1,50} = 134.7$	<0.001***
P	-0.59	$F_{1,43} = 43.98$	<0.001***
N	-0.65	$F_{1,40} = 26.61$	<0.001***
C	-0.75	$F_{1,40} = 34.94$	<0.001***

Besides the effect of depth on nutrients, we also found that roots and mycorrhizae were affected along the vertical gradient. Higher root densities were found in the upper soil layer ($F_{1,31}$= 63.09; $p < 0.001$) with a calculated mean decrease of 4.99 kg/m³ or 0.75 kg/m² in the deeper soil layer compared to the first 15 cm of the soil. Also ectomycorrhizal presence, measured as the proportion of infected roots, higher in the upper soil layers. The decrease in the deeper zone was roughly estimated (given the fact that we used classes) to be around 8% lower compared to upper 15 cm ($p = 0.003$, $F_{1,35} = 9.88$). The fine root ratio did not differ between depth zones ($F_{1,77} = 0.60$, $p = 0.44$), indicating that both fine and coarse roots decreased with depth equally. The relative abundance of AMF showed a significant increase ($p<0.001$, $\chi^2_1 = 1708.3$) of 163% in the deeper zone compared to the upper 15 cm. This is an indication that the amount of AMF increases, or decreases in a slower rate than other fungi do, with depth. The amount of AMF species was however stable ($p = 0.37$, $\chi^2_1 = 0.81$).

4.1.2 Cause and effect of bulk density

Figure 3 visualises the BD per site and shows that, with the exception of the Mon site, the BD was significantly higher in the deeper zone ($F_{1,100}$ = 26.16, p <0.001). Statistical analyses calculated an increase of 0.13 g/cm³ in the deeper zone compared to the first 15 cm. We investigated which variables were able to explain variations in BD between sites. We found that soil texture and the amount of organic material (Table 2). Table 2 shows that sites with a higher proportion of sand had higher BD whereas more silt and clay resulted in a lower BD. An overview of the textures per site and depth can be found in Appendix I. Table 2 also shows that sites with lower BD were characterized with higher amounts of organic material (total C) in the upper soil layer.

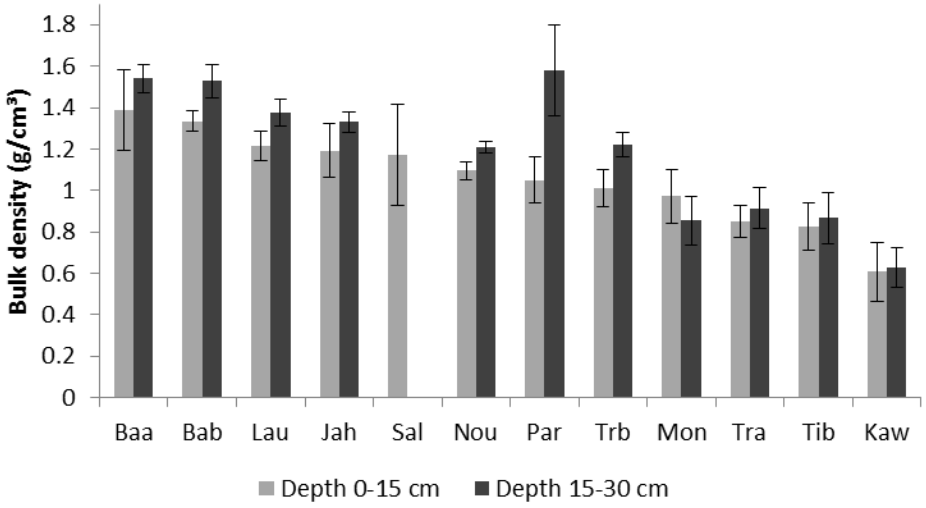

Figure 3. Bulk densities per site and depth zone. Error bars represent standard deviations. Sites were ordered according to values at depth 0-15 cm for visual comparison.

Table 2: Response estimates, p-values and F-values of the mixed models, including only one variable per model, with BD as a function of the percentage of C and P as fixed terms and location as random effect. To estimate the effects of sand, silt and slay, a linear model of mean BD per site was fitted for each soil fraction separately (see M&M for explanation). Estimates are from standardized variables to compare the magnitude of factors. * p < 0.05, ** p < 0.01 and *** p < 0.001.

	0-15 cm			15-30 cm		
	Estimate	**F**	**P**	**Estimate**	**F**	**P**
Sand	0.63	$F_{1,10}$ = 21.23	<0.001***	0.63	$F_{1,9}$ = 6.66	0.029*
Silt	-0.58	$F_{1,10}$ = 14.36	0.004**	-0.52	$F_{1,9}$ = 2.68	0.14
Clay	-0.64	$F_{1,10}$ = 15.83	0.003**	-0.66	$F_{1,9}$ = 7.32	0.024*
C %	-0.22	$F_{1,16}$ = 54.80	<0.001***	0.14	$F_{1,34}$ = 1.10	0.30
P	-0.044	$F_{1,56}$ = 0.17	0.68	0.12	$F_{1,42}$ = -0.26	0.61

Even though we observed differences in BD, no significant pattern was found in the analyses of total root density and the fine root ratio with BD (Table 3). We found however, a non-significant trend towards more fine roots when bulk densities are high in the upper soil layer.

Table 3. Response estimates, p-values and F-values of the mixed models with separately the total root density and the fine root ratio as a function of BD as fixed terms and location as a random effect. . Estimates are from standardized variables to compare the magnitude of factors.* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

	0-15 cm			15-30 cm		
	Estimate	F	P	Estimate	F	P
Total root density	-0.30	$F_{1,50} = 2.48$	0.12	0.08	$F_{1,10} = 2.49$	0.15
Fine root ratio	0.22	$F_{1,40} = 4.08$	0.06	-0.04	$F_{1,35} = 0.05$	0.82

4.1.3 Cause and effects of nutrients and pH

Soil nutrient contents and pH varied between sites (Figure 4). We noticed that soils are very acidic but were quite homogenous between sites with exception of Lau and Sal that were even more acidic (Figure 4a). While the N and C levels among sites are strongly positively correlated (Figure 4b, 4c) there is a negative correlation between the presence of these nutrients and P (compare Figure 4d with 4c and 4b) (p-values: See Appendix II).

These differences in nutrient contents between sites were affected by a varying soil texture, pH and the annual precipitation. A high sand content was correlated with a low pH and higher P levels, while a more clayey substrate was less acid but contained high N and C levels but a low amount of P(p-values: Appendix II). The rainfall correlated positively with N and C, but negatively with P in the deeper depth zone (p-values: Appendix II).

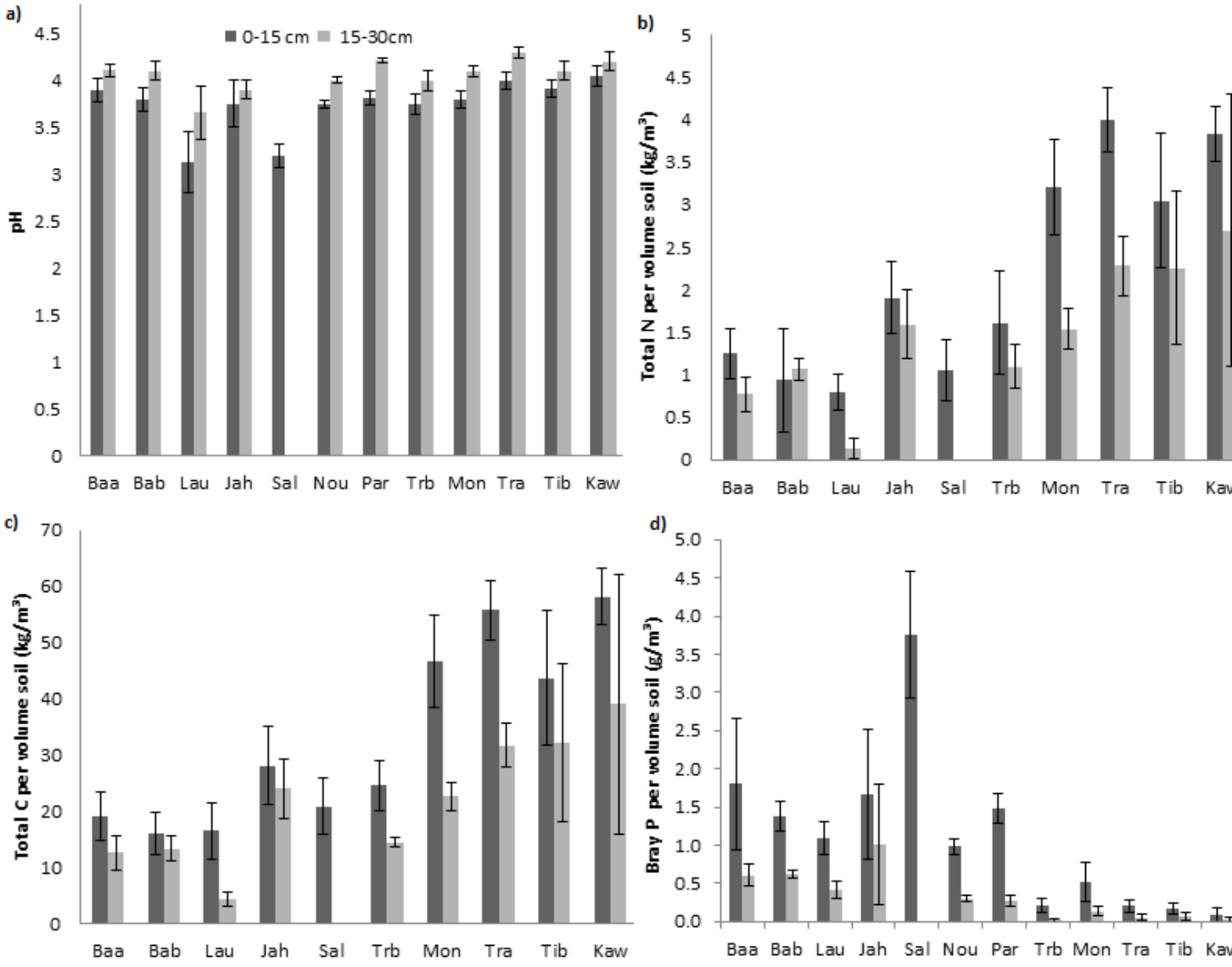

Figure 4. pH (a), total N in kg/m³ (b), Bray P in g/m³ (c) and total C in kg/m³ (d) per site and depth zone. Black and grey bars show the 0-15 cm and 15-30 cm zone respectively. Sites are ordered from high to low BD (BD of 0-15 cm zone). Error bars represent standard deviations.

In figure 5, the biplot shows how total N, total C, P and pH interact with the root density (Root), the fine root ratio (Ratio), AMF species richness (AMRich), AMF abundance (AMabun) and the proportion of roots with ectomycorrhizae (Ecto) in the two depth zones. The position of AMF abundance and species richness in the biplot is closely assembled to N, C and pH but on the opposite end of P. The root density, fine root ratio and EMF are more distant from other factors.

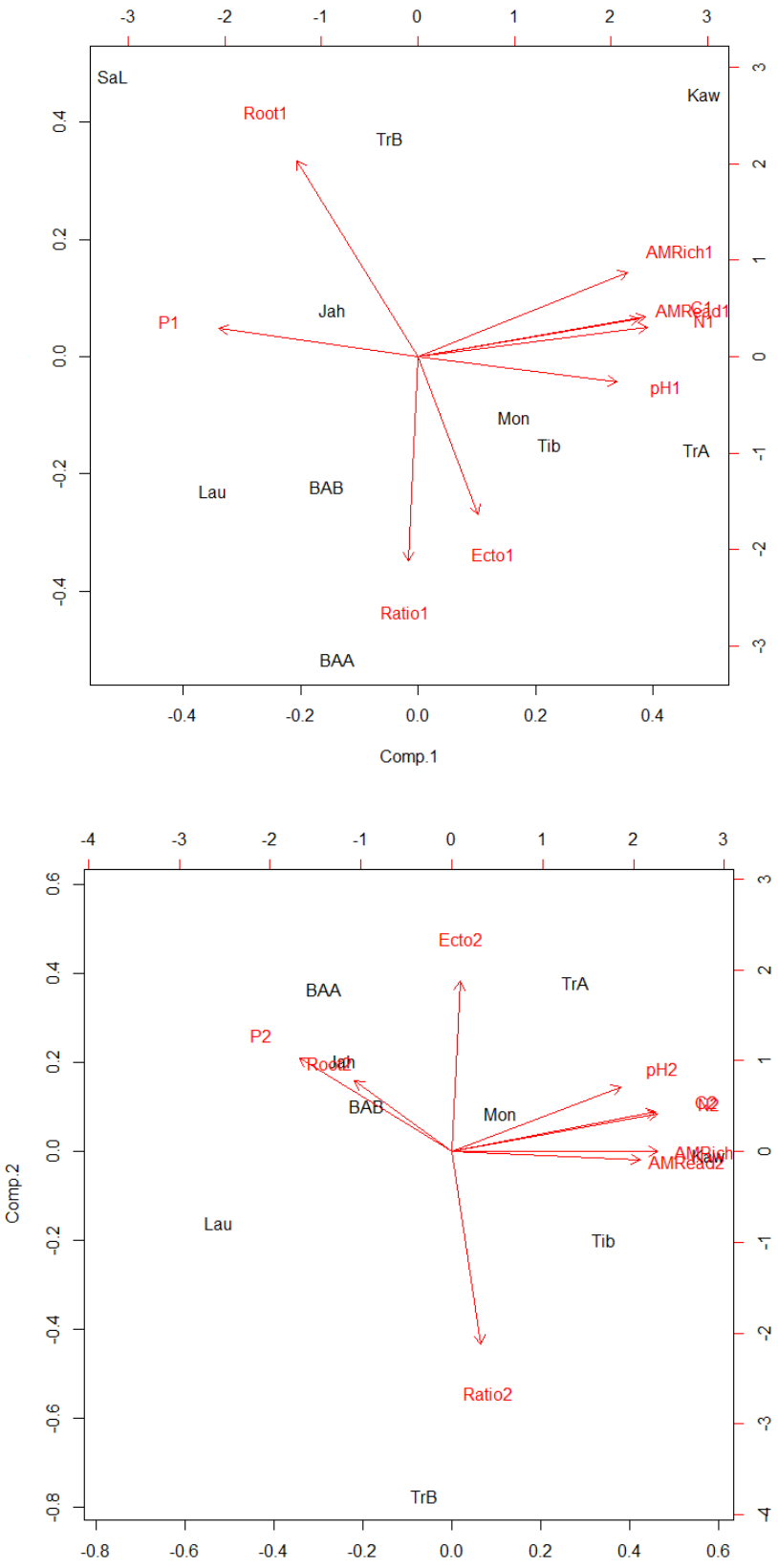

Figure 5. Biplot of the two most important components for A) the 0-15cm zone and B) the 15-30 cm zone which together contain respectively 76% and 73% of the variation. The factors included are: total C (C), total N (N) and Bray P (P), the pH, root density (Root), the proportion of infected roots with ectomycorrhizae (Ecto), the relative abundance of AMF (AMRead) and the AMF species richness (AMRich) .

An overview of the root densities per site are shown in Figure 6. It's relation with nutrients and pH was analyzed but only the pH showed a significant relationship in the upper soil layer, whereas N correlated significantly with the root densities in the deeper soil layer (Table 4). The relation with C was not significant, but showed a tendency towards significance in the deeper zone.

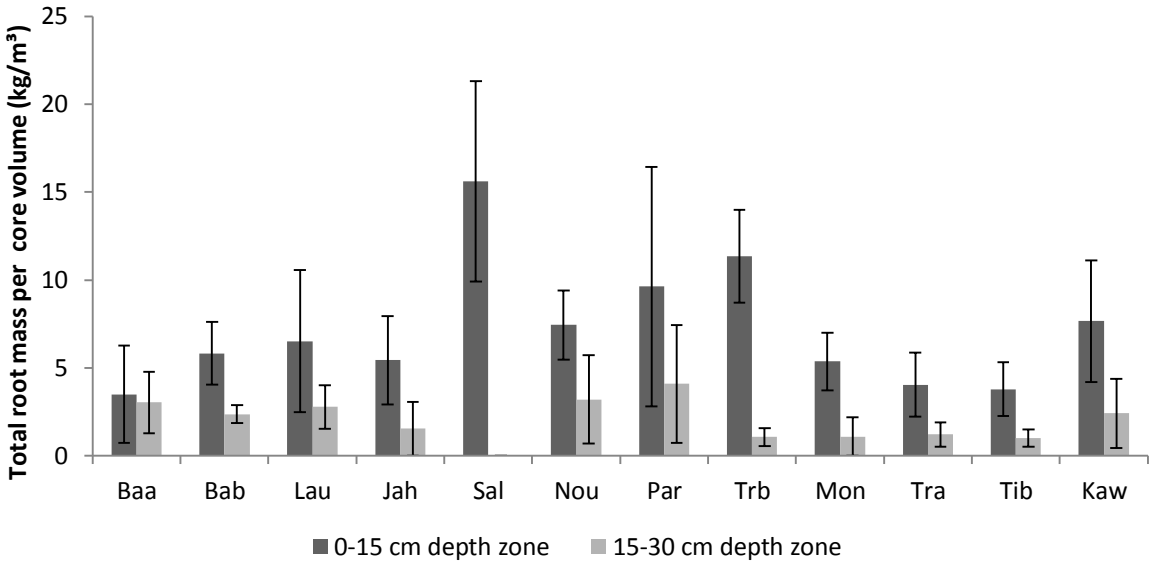

Figure 6. The total root densities per site and depth zone. Sites are sorted from high to low 0-15 cm bulk density. The figure of total root density based on 57 and 52 observations in respectively the 0-15 cm and 15-30 cm zone. Error bars represent standard deviations.

Table 4. Response estimates, p-values and F-values of the mixed models, including only one variable per model, with total root density as a function of pH, P (g/m³), total C and total N (g/m³) as fixed terms and location as random effect. Estimates are from standardized variables to compare the magnitude of factors.* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$

	0-15 cm			15-30 cm		
	Estimate	F	P	Estimate	F	P
pH	-0.55	$F_{1,42} = 13.14$	< 0.001***	-0.04	$F_{1,23} = 0.14$	0.70
P	0.18	$F_{1,30} = 1.50$	0.23	0.06	$F_{1,23} = 0.40$	0.53
N	-0.14	$F_{1,25} = 0.50$	0.48	-0.14	$F_{1,14} = 4.83$	0.04*
C	-0.10	$F_{1,26} = 0.25$	0.61	-0.13	$F_{1,15} = 4.03$	0.06

An overview of the fine root ratio in shown in Figure 8. The figure shows that there is relatively little variance across sites. When the relation between the fine root ratio and nutrients and pH was analyzed, we noticed that it didn't correlate with any variable.

Figure 7. Fine root ratio per site and depth zone. Sites are sorted from high to low 0-15cm bulk density. The figure was based on 42 and 37 observations in the respectively 0-15 cm and 15-30 cm zone. Error bars represent standard deviations.

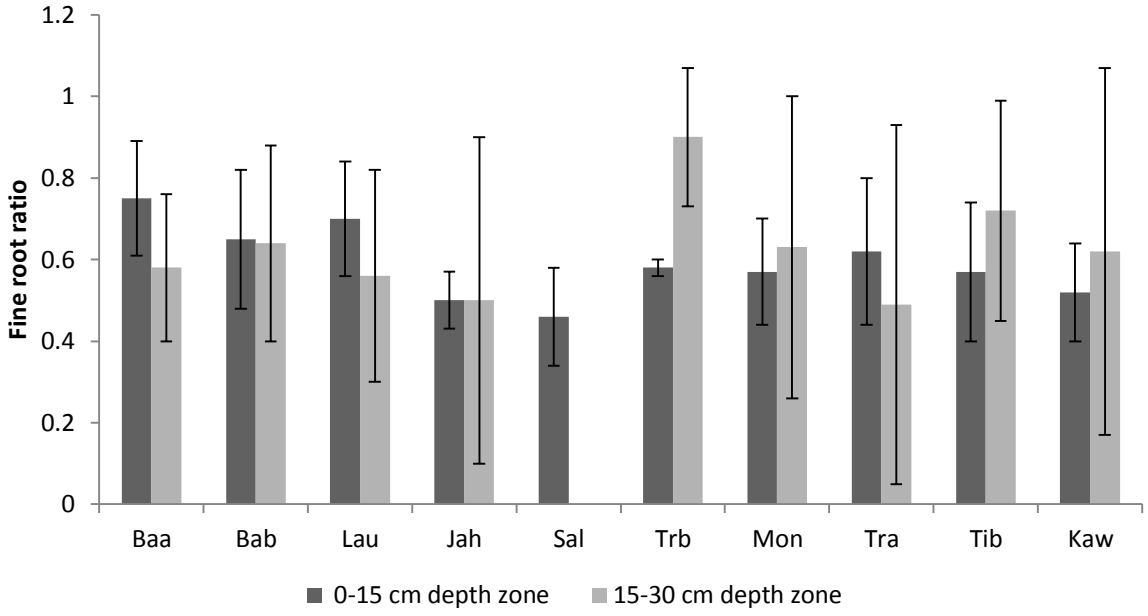

Table 5. Response estimates, p-values and F-values of the mixed models, including only one variable per model, with the fine root ratio as a function of pH, P (g/m³), total C and total N (g/m³) as fixed terms and location as random effect. Estimates are from standardized variables to compare the magnitude of factors.* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$

	0-15 cm			15-30 cm		
	Estimate	**F**	**P**	**Estimate**	**F**	**P**
pH	0.04	$F_{1,14}= 0.17$	0.68	-0.17	$F_{1,35}= 0.31$	0.58
P	-0.003	$F_{1,10}= 0.001$	0.96	0.23	$F_{1,35}= 0.21$	0.64
N	0.009	$F_{1,10} = 0.01$	0.92	0.03	$F_{1,30}= 0.01$	0.89
C	0.03	$F_{1,10}= 0.18$	0.77	-0.01	$F_{1,27} = 0.002$	0.96

Figure 8 visualises the variability in the number of AMF species (Figure 8a) and the relative abundance of AMF (Figure 8b). The relative AMF abundance and species richness is clearly the highest in the Kaw site. Notably, the Sal site, which had extreme P levels, shows the lowest AM abundance. Indeed, statistical analyses showed that the abundance of AMF was negatively influenced by the presence of P in the deeper layers (Table 6). Meanwhile, N and C had a positive correlation with the relative AMF abundance in both depth layers. Higher densities of total roots in the deeper zone were negatively correlated with AMF relative abundance while pH did not show any correlation.

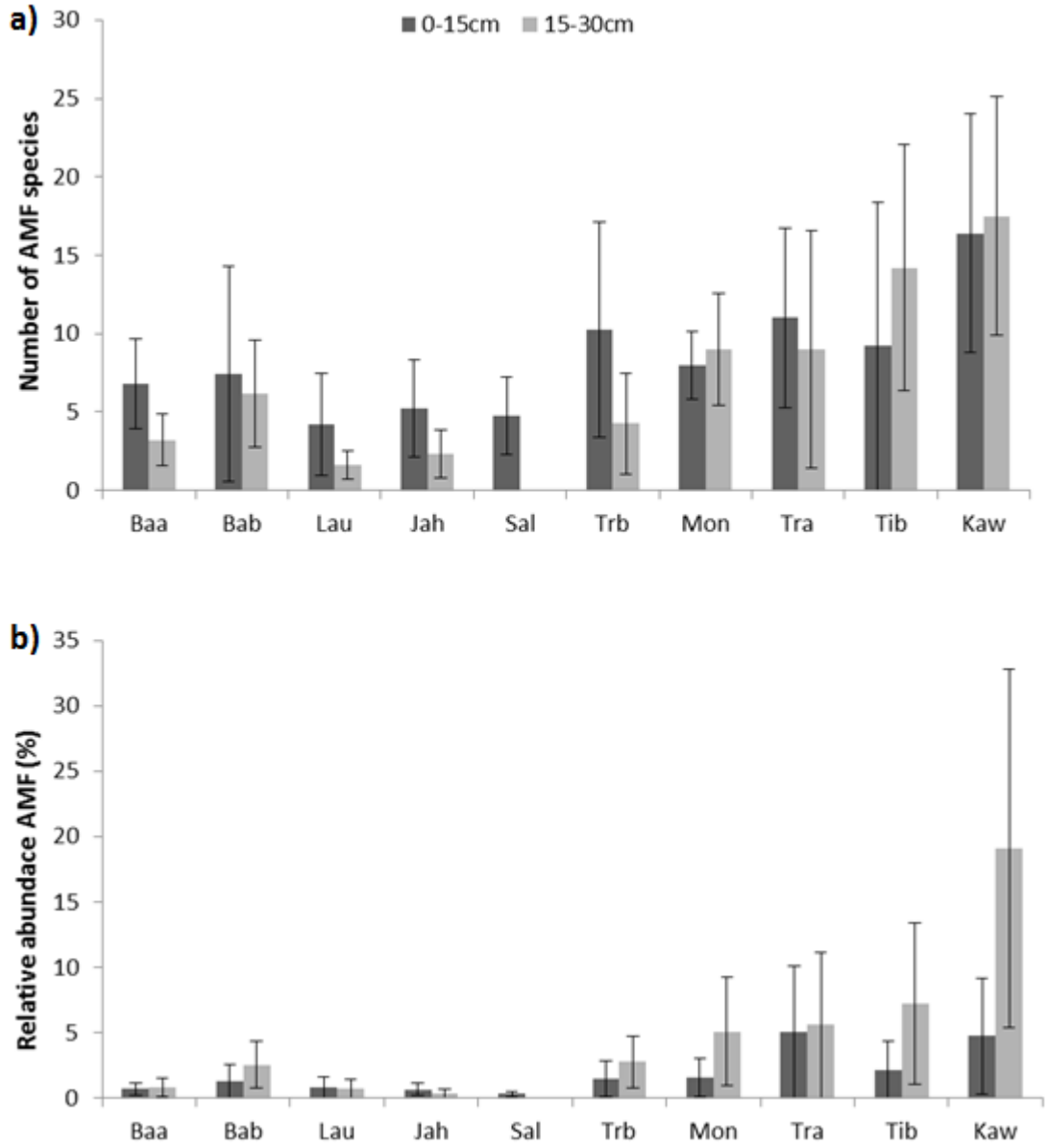

Figure 8. The amount of AMF species (a) and AMF relative abundance (b) per site and depth zone. Sites are sorted from high to low 0-15 cm bulk density. The figure of AMF species and relative abundance is based on 50 and 40 observations in the respectively 0-15 cm and 15-30 cm zone. Error bars represent standard deviations.

Table 6. Response estimates, p-values and χ^2-values of the generalized mixed models, including only one variable per model, with a binomial distribution of relative AMF abundance as a function of pH, P (g/m³), total of C and total N (g/m³) as fixed terms and location as random effect. Estimates are from standardized variables to compare the magnitude of factors.* p < 0.05, ** p < 0.01 and *** p < 0.001

	0-15 cm			15-30 cm		
	Estimate	χ^2_1	P	Estimate	χ^2_1	P
pH	-0.037	0.07	0.80	-0.10	0.20	0.65
P	0.11	3.30	0.07	-1.34	8.46	<0.01**
N	0.37	16.36	<0.001***	0.47	21.93	<0.001***
C	0.34	12.49	<0.001***	0.46	24.0	<0.001***

Table 7. Response estimates, p-values and χ^2-values of the generalized mixed models with a poisson distribution, including only one variable per model, of AMF species richness as a function of pH, P (g/m³), total C and total N (g/m³) as fixed terms and location as random effect. All variables were standardized except for AMF richness because a poisson distribution cannot handle negative values for the dependent factor.* p < 0.05, ** p < 0.01 and *** p < 0.001

	0-15 cm			15-30 cm		
	Estimate	χ^2_1	P	Estimate	χ^2_1	P
pH	0.02	0.03	0.85	-0.09	0.20	0.65
P	-0.12	1.95	0.16	-1.25	8.46	0.004**
N	1.93	19.88	<0.001***	3.41	20.25	<0.001***
C	0.34	12.50	<0.001***	0.46	24.01	<0.001***

Besides the relative AMF abundance, we intended to calculate the AMF colonization percentage of roots. No results can however be shown because we failed to make proper AMF counts because the destaining of the cortex cells did not occur efficiently for most roots of the different locations. Some plots which contained very fine roots (probably understory instead of adult tree roots) destained well. This was the case for the wet soils of TRB and SAL. The roots from other plots were thicker and had thicker cell walls that could be as thick as the AM hyphae themselves. This resulted in an accumulation of many cell layers which was not transparent enough to spot hyphae. The comparison of roots stained with Tryphan blue and ink had similar results. Of the AMF that were noted, none were having arbuscules, suggesting that these mycorrhizal fungi belonged to the Paris-type which is known for not having arbuscules.

Figure 9 shows an overview of the proportion of roots infected by EMF. The large error bars show the high standard deviations within site. In the Trb site, no infected roots were found. When we statistically analyzed the relation between EMF and nutrient and pH we could not related EMF to any of our studied factors (Table 8).

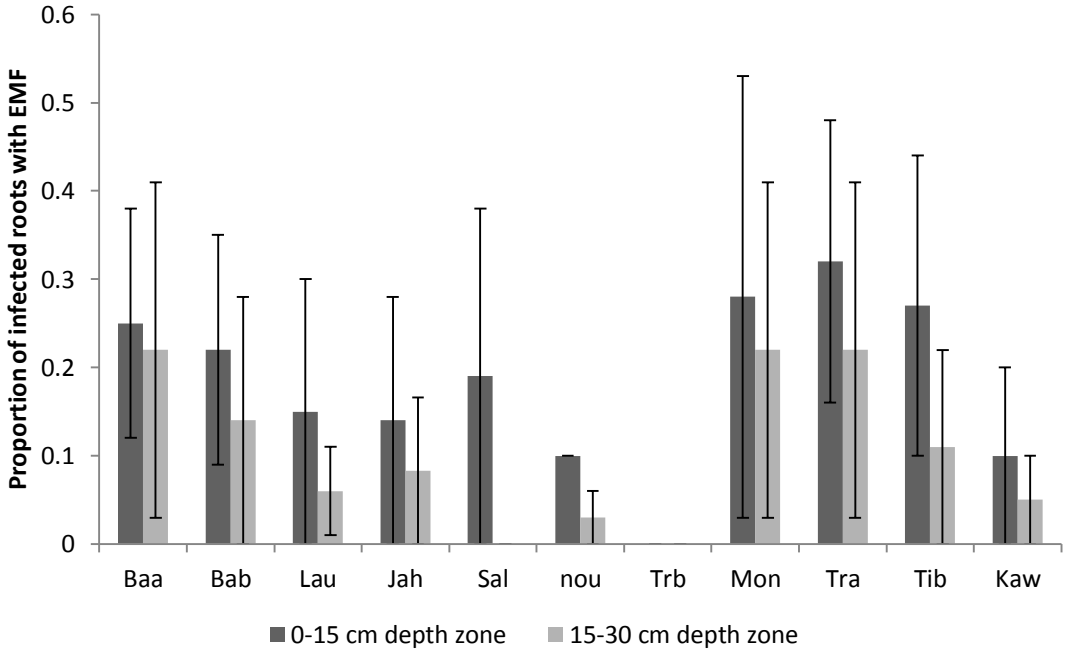

Figure 9. The proportion of roots infected with ectomycorrhizae per site and depth. Sites are sorted from high to low 0-15 cm bulk density. This figure is based on 41 and 30 observations in the respectively 0-15 cm and 15-30 cm zone. Error bars represent standard deviations.

Table 8. Response estimates, p-values and F-values of the mixed models, including only one variable per model, of the logarithm of the mass of fine roots infected with ectomycorrhizae as a function of pH, P (g/m^3), total C and total N (g/m^3) as a fixed term and location as random effect. Estimates are from standardized variables to compare the magnitude of factors.* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

	0-15 cm			15-30 cm		
	Estimate	**F**	**P**	**Estimate**	**F**	**P**
pH	-0.32	$F_{1,8} = 0.62$	0.45	-1.47	$F_{1,26} = 0.002$	0.96
P	-0.28	$F_{1,14} = 0.84$	0.37	1.37	$F_{1,13} = 1.91$	0.18
N	0.09	$F_{1,13} = 0.12$	0.74	-0.56	$F_{1,3} = 0.34$	0.59

4.1.4 Relations between belowground biota

When we looked at how total root mass affected the presence of mycorrhizal fungi, we found that root density correlated negatively with the relative AMF abundance and the amount of AMF species (Table 9). The proportion of ectomycorrhizae was however not affected by the root density.

Table 9. Results of the mixed models used to test the effect of total root density as a fixed term on the relative AMF abundance, AMF species richness and the proportion of roots infected with EMF and location as random effect. The relative AMF abundance and AMF species richness followed respectively a binomial and Poisson distribution, while the proportion of roots with EMF had undergone a natural logtransformation..* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

	0-15 cm			15-30 cm		
	Estimate	χ^2_1	**P**	**Estimate**	χ^2_1	**P**
Rel. AMF abundance	0.078	1.10	0.29	-0.57	4.55	0.033*
AMF Species richness	0.09	1.94	0.16	-0.50	3.86	0.049*
	Estimate	**F**	**P**	**Estimate**	**F**	**P**
Prop EMF	-0.22	$F_{1,32} = 0.55$	0.55	0.41	$F_{1,26} = 0.17$	0.67

4.2 Analyses along a topographic gradient

In this section, the results are presented of the analyses that were conducted on data from the Nouragues field station along a topographic gradient.

Along the topographic gradient, we noticed that that BD decreased with increasing height (Table 10). The BD of the top differed significantly from the other topographic levels while the base and slope did not differ significantly from each other. The soil in the top was mainly composed out of the less coarse particles silt and clay whereas the base and slope are mainly consisting out of sand. Texture data per topographic level can be retrieved in Appendix III and the statistical results of how these textures differed along the topographic gradient are shown in Appendix IV.

Table 10. Estimated differences and p-values in BD between base (B), slope (S) and top (T) plots for both depth zones. Estimates are unstandardized to show real BD differences represented in g/cm³. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

	0-15cm		**15-30cm**	
	Estimate	p-value	Estimate	p-value
B-S	0.06	0.57	0.08	0.50
B-T	0.34	<0.001***	0.43	<0.001***
S-T	0.28	<0.01**	0.35	<0.001***

Besides a changing BD, also nutrient levels were affected by the topography. Table 11 shows that he top plots had most N but less P whereas the base plots contained more P but less N. The soil on the slopes had a lower P concentration than the base but was similar to the P levels in the top. N did not differ significantly with the base but was lower than in the top. Additionally, moisture levels were compared along the gradient. The top was significantly dryer, but base and slope had equal water content. Boxplots of the nutrients and pH per topography level can be found in the appendices (Appendix V).

Table 11. The p-values and estimated differences of a Tukey test to compare pH, inorganic N and P between topography levels pet depth zone. Variables were not standardized but are represented in kg/m³.* p < 0.05, ** p < 0.01 and *** p < 0.001.

	Depth(cm)		Base-Slope	Base-Top	Slope-Top
pH	**0-15**	Difference	0.02	-0.07	-0.11
		P-value	0.84	0.36	0.17
	15-30	Difference	0.03	-0.14	-0.18
		P-value	0.73	0.02*	<0.01**
N	**0-15**	Difference	2.29	-3.01	-5.30
		P-value	0.26	0.13	<0.01**
	15-30	Difference	6.17	-1.80	-2.41
		P-value	0.83	0.25	0.10
P	**0-15**	Difference	1.04	1.26	0.22
		P-value	0.03*	<0.01**	0.78
	15-30	Difference	0.5	0.61	0.11
		P-value	0.06	0.02*	0.83

Although differences in nutrient composition and bulk density along the different topographic regions were found, the total root density and the fine root ratio did not appear to be changing along the elevation gradient (Table 12).

Table 12. Estimated differences in total root density and fine root ratio between topography levels for both depth zones. Given the high p-values, no pattern along the topography gradient could be found. Estimates are unstandardized to show real BD differences represented in kg/m³. * p < 0.05, ** p < 0.01 and *** p < 0.001.

	Depth(cm)		Base-Slope	Base-Top	Slope-Top
Total Root density	**0-15**	Difference	-2.17	-0.878.	1.299
		P-value	0.42	0.86	0.71
	15-30	Difference	-0.284	-0.194	0.090
		P-value	0.90	0.95	0.99
Fine root Ratio	**0-15**	Difference	0.10	-0.03	-0.13
		P-value	0.35	0.91	0.20
	15-30	Difference	-0.09	0.02	0.11
		P-value	0.67	0.98	0.56

Figure 10 shows that in the same topographic region, the amount of roots and fine root ratio still contained large variations.

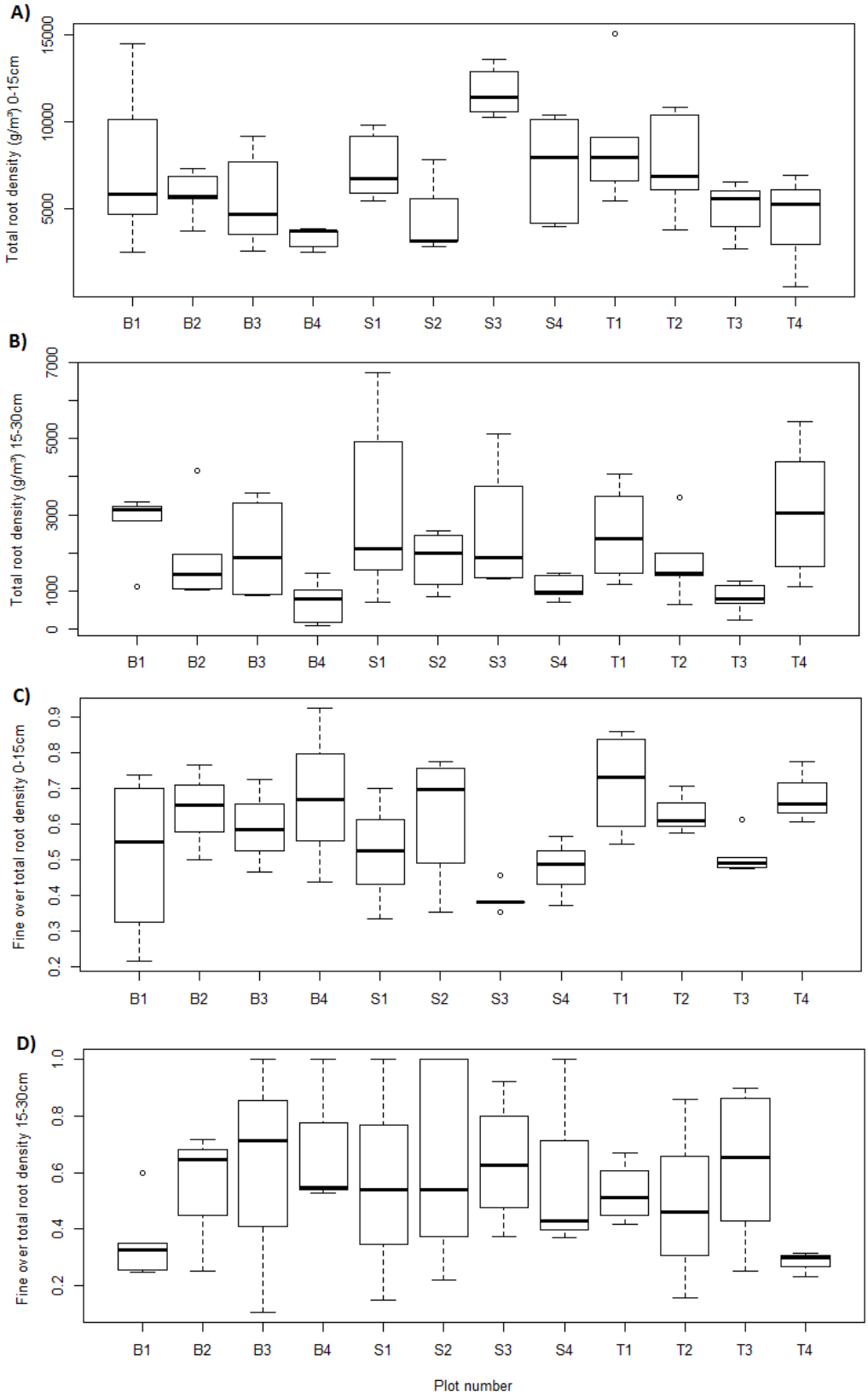

Figure 10. Boxplots of total root density (g/m³) in the 0-15 cm zone (A) and 15-30 cm zone (B) and boxplots of the ratio of fine over total root density in the 0-15 cm (C) and 15-30 cm (D) depth zone per plot along the elevation gradient in Nouragues.

When investigating the effect of P and N on the root density per topographic level, we noticed that root density on the top plots was positively affected by P in the deeper zone (Table 13). This suggests that when more P is present in the deeper soil layers, roots will grow deeper to scavenge for this resource. In the slope plots, the relation between the root density and N is approaching significance, suggesting that N might be the limiting resource here. The estimate is negative, so when N levels are low more roots might be necessary to acquire N. In the base plots, p values for N and P are similar but estimates are larger for P suggesting that even though P is more prevalent in the base, it still might explain variation in root density. The fine root ratio was subjected to the same analyses. Table 14 shows that there is a significant relation between the fine root ratio in the base with N in both depth zones. In the upper zone, it correlates negatively suggesting that less root surface is needed to collect N when N levels are higher. In the deeper zone, the correlation is positive, suggesting that when N levels are high in the deeper soil layers, the roots will be thinner to explore more soil surface thus increase the uptake capacity for this resource.

Table 13. Response estimates, p-values and F- values of the mixed models, including only one variable per model, with root densities within a topography level as a function of N and P levels per depth zone. Estimates are from standardized variables to compare the magnitude of factors. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Factor	Depth (cm)	Top	Slope	Base
N	**0-15**	Est = 0.03 p-val = 0.92 $F_{1,17} = 0.01$	Est = -0.43 p-val = 0.07 $F_{1,17} = 3.8$	Est = -0.33 p-val = 0.20 $F_{1,8} = 1.9$
	15-30	Est = -0.03 p-val = 0.82 $F_{1,17} = 0.05$	Est = -0.03 p-val = 0.91 $F_{1,8} = 0.01$	Est = -0.22 p-val = 0.20 $F_{1,16} = 2.5$
P	**0-15**	Est = -0.52 p-val = 0.40 $F_{1,4} = 0.85$	Est = -0.54 p-val = 0.42 $F_{1,9} = 0.69$	Est = -0.62 p-val = 0.21 $F_{1,3} = 0.21$
	15-30	Est = 0.87 p-val = 0.03* $F_{1,17} = 5.79$	Est = -0.09 p-val = 0.88 $F_{1,8} = 0.022$	Est = -0.32 p-val = 0.48 $F_{1,11} = 0.52$

Table 14. Responds estimates, p-values and F- values of the mixed models, including only one variable per model, of the log transformed fine root ratio within a topography level as a function of N and P levels per depth zone. Estimates are from standardized variables to compare the magnitude of factors .* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Nutrient	Depth (cm)	Top	Slope	Base
N	**0-15**	Est = -0.03 p-val = 0.84 $F_{1,12} = 0.0$	Est = 0.29 p-val = 0.17 $F_{1,14} = 0.17$	Est = -0.46 p-val = 0.04* $F_{1,11} = 5.57$
	15-30	Est = 0.09 p-val = 0.86 $F_{1,12} = 0.03$	Est = 0.13 p-val = 0.87 $F_{1,12} = 0.03$	Est = 1.26 p-val = 0.04* $F_{1,11} = 5.43$
P	**0-15**	Est = 0.21 p-val = 0.66 $F_{1,5} = 0.21$	Est = 0.22 p-val = 0.66 $F_{1,5} = 0.21$	Est = 0.67 p-val = 0.17 $F_{1,11} = 2.15$
	15-30	Est = -1.68 p-val = 0.21 $F_{1,12} = 1.74$	Est = -2.15 p-val = 0.17 $F_{1,12} = 2.18$	Est = -0.85 p-val = 0.69 $F_{1,11} = 0.16$

5 Discussion

5.1 Analyses along a precipitation gradient

5.1.1 The effect of depth

When we analyzed the difference in root densities between the two depth zones, we found significantly more roots in the upper 15 cm of the soil compared to the 15-30 cm soil layer. This was in accordance to our hypothesis and in line with previous research that found decreasing root masses with increasing depth (Sanford and Cuevas (1996), Cavelier (1992)). Cavelier (1992) explained this observation with the fact that most nutrients are also present in the upper soil layer. This was also confirmed in our research since significantly more N, C and P were found in the 0-15 cm zone compared to the subsequent 15 cm. Cavelier (1992) described these higher nutrient levels as a consequence of litter fall, stemflow and canopy leachate and the fact that this is the place where most litter is decomposed. Another reason proposed by Cavelier (1992) was an increasing Al toxicity with increasing depth. The fine root ratio however, did not differ between the two depth zones. This suggests that even though the highest root densities were observed in the upper soil layers, the fine root mass in the 15-30 cm zone decreased at the same rate as the total root mass. The roots in the upper layer have thus no higher ratio of fine roots to acquire nutrients.

The effect of depth on EMF and AMF was analyzed by Neville et al. (2002) on trees of *Populus tremuloide.* In the >10 cm soil depth they found significantly less amounts of EM root tips compared to the 0–5 and 5–10 cm depths while AM colonization was significantly lower in the top 5 cm of the soil compared to the 5–10 and >10 cm depths. They therefore suggested that EM and AM are partitioned at different soil depths. In our research, the proportion of roots infected with EMF was lower in the deeper zone while the species richness of AMF did not differ between depth zones. The relative abundance of AMF did however increase with increasing depth suggesting that either biomass of AMF increases with depth, as observed by Neville et al. (2002), or that other fungi (EMF included) decrease at a faster rate with depth than AMF do. Data on absolute AM abundance would shed more light on the exact relationship between AMF and depth.

5.1.2 Cause and effect of bulk density

Variations in BD were observed between the studied locations. There variations could be explained with the soil texture and carbon content as a proxy for organic matter. The greatest impact was observed for texture: a higher sand content increased BD whereas silt and clay had a decreasing effect. According to Schaetzl and Thompson (2015) clayey and silty soils have a lower BD compared to more sandy soils because they have many micropores which are more difficult to eliminate. The second greatest impact on BD was its negative correlation with the amount organic material. This is due to the fact that organic material is considerably lighter than mineral soil (Hagan et al. 2013) and because organic matter tends to attract soil fauna which also create pores (Schaetzl and Thompson 2015).

We hypothesized that a high BD could impede root growth and reduce the root diameter. While we found these differences in BD between sites, no relation was however discovered with the root density in the upper zone nor in the deeper zone which had higher bulk densities. This might be because BD did not reach values that could impede root growth or it might be a consequence of the extraction method of the roots that was not showing enough detail. Our method combined root collection and BD estimation and may as a result have been too coarse to give accurate results on root density and biomass. The method involved the sieving of soil in which fine roots break into even smaller fractions and get lost under the sieved soil. The soil structure might have created extra bias since clayey soils were harder to sieve resulting in even larger root damages. It would therefore be better to use more gentle techniques like soil washing. We noticed however a trend towards a higher fine root ratio with increasing BD in the upper soil layer. This suggests that BD, even in our observed range, might show an effect on root diameter when more precise root collection methods are applied and thus roots might be more fine at high BD in order to grow in the smaller micropores of the soil.

5.1.3 Cause and effects of nutrients and pH

Variations in pH, P, total N and total C were observed in the studied locations. Some correlations were found between these nutrients and the texture composition of the site. Sites high in sand contained more P but less organic material (N and C) whereas clayey soils contained more organic material but less P. This is in accordance with Anderson and Spencer (1991) who stated that some fraction of organic material can be complexed with clays or be physically protected within clay micro-aggregates. The sandy soils had a slightly lower pH, which could promote the release of Al and Fe that binds P and thus holds more P in the soil (McClellan, n.d.). PH did not correlate with precipitation. The precipitation did however

correlate positively with N and C levels but negatively with P in the deeper soil layers. We also found that sites high in N and C had lower P levels and vice-versa. This might be explained by the presence of organic anions can cause displacement of phosphate from soil anion exchange sites and increase phosphate bioavailability and hence lower the amount of P we extracted. Also humus can interfere by forming a coating around Al and Fe which improves P release to the soil solution and makes it more bioavailable. (McClellan, n.d.). These differences in nutrient concentrations, influenced by different soil factors and precipitation, can help explain variation in the belowground biotics in which we are interested.

We hypothesized that root diameter would decrease and total root biomasses would increase on the sites with lower soil P concentrations (Gower, 1987; Treseder and Vitousek 2001) . Treseder and Vitousek (2001), already found higher biomass in the P deficient soils and they explained this with a longer root lifetime. Also did we expect a lower root diameter on these P deficient sites since it increases the root surface area at a low cost (Horst et al. 2001; Wissuwa et al. 2005). Our analyses showed however that variation in fine root ratio between different sites, could not be explained with our analyzed nutrients or pH. Also, the total amount of roots did not correlate with P as we expected. It did however correlate negatively with N in the deeper zone. This is in contradiction of Cavelier (1992) who studied the relation between the vertical distribution of root biomass and chemical soil properties in a semi-deciduous and a lower montane rain forests in Panama. The author argued that the vertical decrease of root biomass in was mainly controlled by the decreasing concentration of N and to a smaller extent to P. He thus suggests that the root density in the deeper layers was constrained by nutrients while we find that if more N is present in the deeper zone, a lower root density can be found. It might thus be that the roots in sites with high N concentrations can acquire already enough N so that less roots in the deeper soil layers are necessary. In sites with low N concentrations, more effort is however done to acquire the N. We can therefore conclude that N is probably might also be a limiting nutrient in the forests that we studied.

More correlations could have been found if, like stated before, the root collection method was not damaging roots while the soil was sieved. Also, we inserted the core manually and could therefore not always collect a sample every time we inserted the core due to the presence of a thick soil mat or a root that was too large to be cut by the core edges. This probably created a bias towards the sampling of soils that were easy to penetrate. Including factors such as

species composition and the presence of bushes in the understory could also shed more light on the variation of root densities and nutrients.

Besides the relationship between root density and nutrients, we also analyzed the correlation between root density and pH. When pH in the upper soil layers was low, higher root densities were found. A lower pH might indicate higher decomposition rates of organic matter and thus also higher rates of nutrients becoming available again for roots. Therefor roots might have been attracted to these zones with high decomposition rates. However, it might also be an effect of the high root density itself though exudation of organic acids. These root exudates are excreted to solubilize unavailable soil Ca, Fe and Al phosphates and might as a result lower the pH (Mamo et al. 2003). This is interesting because one would expect that at a certain threshold, the pH would cause negative effects due to Al-toxicity (Cavelier 1992) but it appears that the roots in these lowland tropical forests are adapted to these acid conditions.

Besides studying the effects of nutrients on root responses, we were also interested in the response of mycorrhizae on different nutrient levels. We hypothesized that the presence of AMF and EMF could be explained by the trade balance model argued by Johnson (2009). The author suggests that the mycorrhizal symbiosis depends on the stoichiometry of both available N and P. Looking at the nutrient availabilities in our analyzed sites, we could categorize our sites in 3 categories: relatively high N and low P (Mon, Tra, Tib & Kaw); relatively lower N and higher P (Baa, Bab, Lau & Trb) and sites in very high P and low N (Sal). Johnson (2009) argues that 75% of N in leaves are invested in chloroplasts to produce carbon photo-assimilates. As a consequence, when N is in abundance, the photosynthetic capacity of the host plant is high and C-products can be traded for P. Johnson (2009) argues that in this situation mutualistic benefits are expected to be the greatest. Indeed, investigated sites with lower P and high N had the highest relative abundance of AMF and AMF species richness (Mon, Tra, Tib & Kaw). However, the benefits of C-for-P trade would be eliminated in conditions with high P and limiting N since both plants and fungi will compete for N and C and as a consequence AM growth will be limited. This is in our case visible in the Sal site with extreme P values but relatively low N levels. Here the observed relative AMF abundance and AMF species richness reached the mean minimum. In the other sites which had still high P levels, but lower than in Sal, and relatively high N (Baa, Bab, Lau & Trb), relative AMF abundances and AMF species richness were low but in mean still higher than in Sal. In the upper soil layer, the relation between AMF and P was however not significant suggesting that this competition between AMF and roots is less influence in the upper soil layers.

In contrast with AMF, the proportion of infected roots with ectomycorrhizae did not correlate with nutrient levels. This is in accordance with the results of Verbruggen (Unpublished) who analyzed DNA of EMF that were collected at the same sites as this research. In those analyses, no relation was found between nutrients or pH with the relative abundance and species richness of EMF. Ectomycorrhizae are associated with relatively few plant species of which only some occur tropical forests (Dickie 2013). Since they are more depending on the right tree species than AMF do, their distribution might be more irregular and hence it is harder to collect a sample that gives a good representation of the area. The collection of ectomycorrhizal data is thus more bound to chance making it harder to link with other environmental factors than for AMF.

Besides doing analyses on the relative AMF abundance, we also intended to study the percentage of AMF root colonization. No counts were however made because the roots were not destained enough. Roots from some locations of which the roots were finer destained well, suggesting that the collected roots were too large to do proper microscopic analyses. Both the Tryphan Blue and ink staining gave however the same results, suggesting that ink would be a better alternative because we should strive for less toxic compounds in our methods. Of the visible AMF, we did not spot any arbuscules, suggesting that AMF are of the Paris-type. This is in accordance with the research of Grandcourt et al. (2003) and Béreau et al. (2000) who also observed that most mycorrhizas in French Guiana were of the Paris-type. Smith and Smith (1997) found that certain plant families were more likely to be observed with the Arum or Paris type of AMF. Dickson (2004) found however that besides the effect of the plant family or species, there is also an effect of the fungus itself. He suggests also that environmental factors such as soil and nutrient conditions might contribute to morphological variability. More research is however needed to fully understand the mechanisms of morphological variance if we want to explain why more Paris type AMF in the tropical forests of French Guiana.

5.1.4 Relations between belowground biota

When the relation between root densities and mycorrhizae was studied, we didn't find a correlation between root density and EMF but we found a negative correlation between the root density and both relative AMF abundance and AMF species richness. In the previous section we found that more roots are present in the deeper soil layers when N concentrations are low. We also found, in accordance to the trade balance model, that in sites with low N the plants and fungi will compete for N and C. As a result can the cost of supporting mycorrhizae overshadow their benefits and hence it might be cheaper for the plant to produce roots that also acquire water and create support. Thus in case of low N levels, we can indeed expect high root densities and low AMF abundancies which explain the negative correlation between root density and AMF in the deeper zones.

5.2 Analyses along a topographic gradient

The effect of topography was tested on the BD, nutrients, pH and root density and diameter. The top plots had a significant lower BD compared to the slope and base plots. Again, this was probably due to the texture variation, with more sand in the lower plots and more clay and silt in the top. BD did however not explain variation of root density or fine root ratio suggesting that the BD range along the topographic gradient was not impeding root growth.

The amount of nutrients did also differ along the topographic gradient. The soil on the top plots contained less available P, while assimilable P in the base levels were significantly higher. These findings are in accordance with the results found by Ferry et al. (2010) who studied the topographic effects on a hillslope in Paracou (Par), French Guiana. They claimed that the high P levels in the base were due to the anoxic conditions because of a higher water table and a fertility transfer from the upper parts of the topography through litter- and treefall. The anoxic conditions would cause a reduction of Fe^{+3} to Fe^{+2} , which could have a positive effect on the P availability in these soils since P is more strongly sorbed to Fe oxides than to the reduced forms of Fe (Silver et al. 1994). Ferry et al. (2010) also noticed that the higher soil P availability in the base led to higher amounts of phosphorus in the tree litter, probably because P resorption is less proficient suggesting that P is relatively not the most limiting nutrient. Meanwhile lower amounts of inorganic N were retrieved in the slope and base plots compared to the top plots. Ferry et al. (2010) explained the lower N in base plots because of the higher water table, especially in the raining season, due to lower soil oxygen that inhibits decomposition rates.

Even though we observed these variations in nutrient levels along the gradient, the root densities and fine root ratios did not differ significantly. Because the base was expected to have more P than the other topography levels, higher treefall rates (Ferry et al. 2010) and a high water table that can suppress root growth (Kozlowski 1997), we expected lower root densities in the base plots. We observed however that the root density did not change along the topographic gradient. The base might thus be limited by a different nutrient than P that requires a higher root density.

When the effect of N and P on the root densities within the same topographic level was tested, we found indeed a different response along the gradient on N and P levels, like we hypothesized. In the top plots, the root density correlated significantly with the P levels in the deeper zone. This positive relation indicates that when the soil contains more P In the deeper soil layer, the roots are more likely to grow deeper to reach it. This was only noted in the top plots which were more P limited than the other topography levels. In the slope plots, which had on average the lowest N levels, the relation between N and root density was negative suggesting that when less N was present, more roots per m^3 were necessary to acquire it. When the effect of nutrients on the fine root ratio was analyzed, only the base plots responded significant to N. In the upper zone this relation was negative, suggesting that there was more need of a greater soil surface to be explored when N levels were low. In the deeper zone, the relation was positive suggesting that when N was still present in the deeper soil layers, energy is invested to create a greater root surface in the deeper zone. These analyses show that in the slope and base, where N is least present, the roots are more responsive to N while in the top plots the roots are more responsive to P.

6 Conclusion

In this study we found various relationships between roots, mycorrhizal fungi, and soil nutrients. Some of these supported our hypotheses while others did not, providing new and sometimes surprising insights into the workings of lowland tropical rainforests in French Guiana. The data that included all study sites along a precipitation gradient showed that more nutrients were found in the upper soil layers and as a result, more roots were also found in this layer of the soil profile. With regards to the response of root density and diameter to soil nutrients, we expected a higher root density and a lower root diameter in sites with low P-levels. However, we did not find any relationship with P in any layer whereas in the deeper soil layers, we found a negative significant correlation with N. This suggest that in sites with high N levels, plants require fewer deep roots to acquire enough N compared to sites with lower N levels. Along the topographic gradient, we did not observe differences in root densities and fine root ratios. However, when the relationship of root density and root diameter with nutrients was analyzed within one topographic level, we observed that roots in the top plots correlated with P, while in the base and slope the roots correlated with N. Root density in the top plots were higher in the deeper zone if there was more P present whereas root densities were tending to increase in the slope plots when N concentrations were low in the upper layers. The fine root ratio only responded significantly to N in the base. In the upper zone, the diameter decreased if N concentrations were low in the upper zones, while in the deeper zones the diameter decreased if N was high. This suggests that there is a different limiting nutrient along the topography. Along the precipitation gradient we only found a negative correlation with root density and N in the deeper zones, suggesting that N can also be limiting in the forests we studied.

The trade balance model (Johnson 2009) explained variation in AMF relative abundancies and AMF species richness but not of EMF probably because these symbionts are more bound to certain tree species compared to AMF. It was therefore harder to link EMF with other environmental factors such as nutrients. The presence of EMF would however decrease with depth while the relative AMF abundance would increase with depth and AMF species richness did correlate with depth. The amount of AMF species and the relative AMF abundance would however be lower in the deeper zone if more roots were present. This is due to their contrasting response to low N in the deeper soil layers. If N is low, the cost of supporting mycorrhizae might thus be greater than its benefits. In general, AMF colonization might however decrease at a slower rate with increasing depth than EMF do, but data of

absolute AMF abundance is needed to shed more light on the relation between AMF and depth.

Besides the effect of nutrients and depth also the effect of pH was included. PH did not appear to affect mycorrhiza but it was negatively correlated with root density which confirms that roots in these lowland tropical forests are probably adapted to acidic conditions and higher Al levels caused by low pH. Ultimately, BD did not explain variation in root densities along the precipitation gradient but we observed a non-significant tendency for a larger fine root ratio suggesting that at higher BD. This suggests that the observed BD might have an effect on root morphology but a more detailed collection method is needed to prove this statement. We would therefore advise future research on root biomass and densities to wash core samples and use an automated core.

7 Acknowledgements

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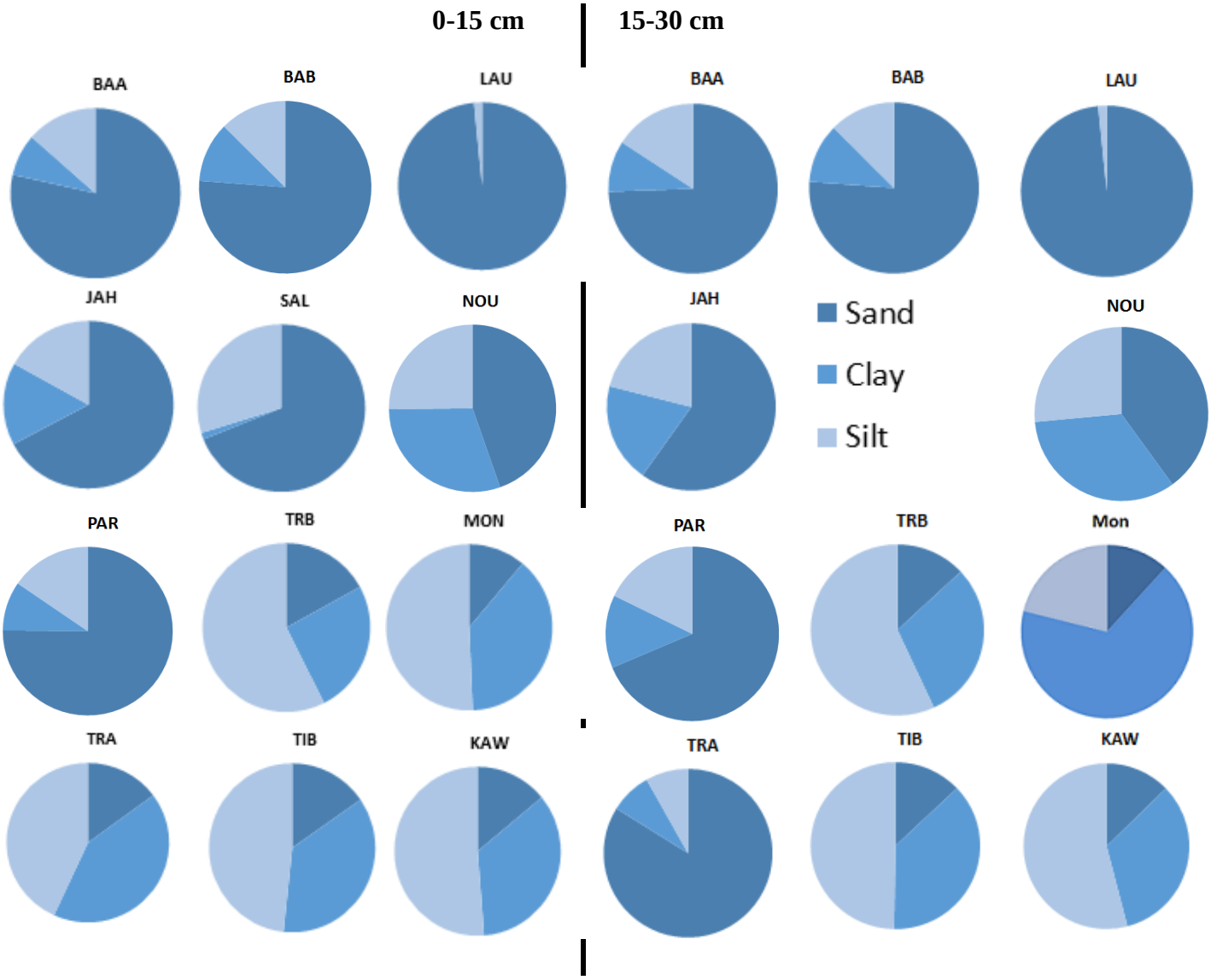
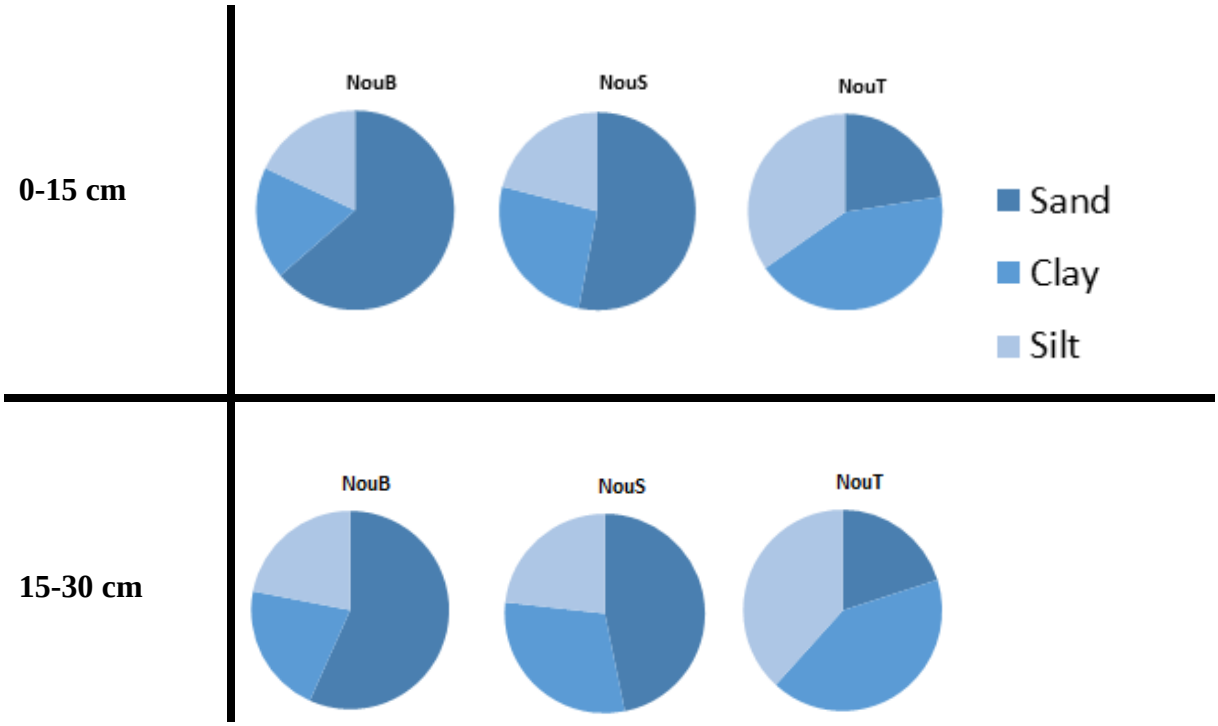
8 Appendices



Appendix I. Pie diagrams of soil textures from the different sample sites with on the left side the 0-15 cm zone and right the 15-30 cm zone. Sites are sorted from high to low BD (BD of 0-15 cm). Data based on one measurement per depth zone and site.

Appendix II. Correlation estimates, t-values and p-values between soil characteristics based on the factor means per site to account for independent values..* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$

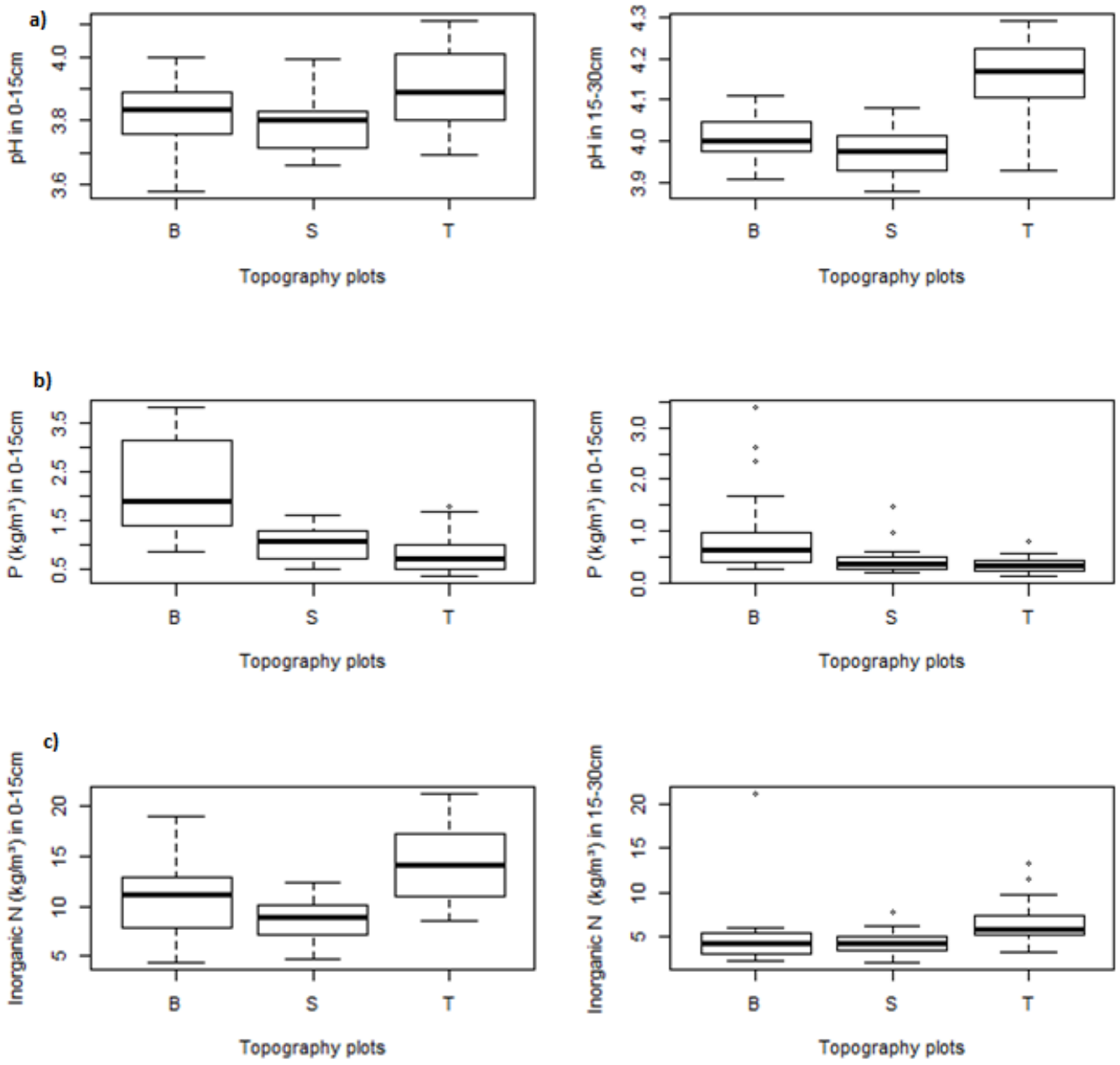
	0-15 cm		**15-30 cm**	
	Cor. Estimate	p-value	Cor. estimate	p-value
N-P	-0.67	0.036 *	-0.46	0.22
N-C	1	<0.001 ***	1	<0.001 ***
C-P	-0.66	0.039 *	-0.45	0.23
Sand-C	-0.84	0.0019 **	-0.53	0.14
Sand-N	-0.85	0.0015 **	-0.53	0.14
Sand-P	0.69	0.028 *	0.56	0.12
Clay-C	0.92	<0.001 ***	0.48	0.19
Clay-N	0.93	<0.001 ***	0.48	0.19
Clay-P	-0.77	0.008 *	-0.47	0.20
Silt-C	0.69	0.03*	0.45	0.22
Silt-N	0.69	0.03*	0.46	0.21
Silt-P	-0.50	0.09	-0.52	0.10
pH-P	-0.66	0.038 *	-0.47	0.20
pH-C	0.62	0.054	0.68	0.04*
pH-N	0.66	0.037 *	0.72	0.028*
pH-Clay	0.74	0.01 *	0.35	0.36
pH-Sand	-0.66	0.04 *	-0.35	0.35
Rainfall- N	0.64	0.05*	0.67	0.05*
Rainfall -C	0.65	0.04*	0.66	0.05*
Rainfall -P	-0.30	0.35	-0.88	<0.001***
Rainfall-pH	0.22	0.49	0.43	0.18



Appendix III. Pie diagrams of mean soil textures from the base, slope and top with on upper side the 0-15cm zone and lower the 15-30 cm zone, soarted from high to low BD (BD of 0-15cm).

Appendix IV. Results of a Tukey test to analyze differences in texture along the topographic gradient. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$

	Dept (cm)	S-B	T-B	T-S
Sand	**0-15**	Diff = -10.58 p-val = 0.30	Diff = -41.16 p-val<0.001***	Diff = -30.57 p-val<0.01**
	15-30	Diff = -9.33 p-val = 0.32	Diff = -35.83 p-val<0.001***	Diff = -26.5 p-val<0.01**
Silt	**0-15**	Diff = 2.94 p-val = 0.69	Diff = 16.60 p-val<0.01**	Diff = 13.66 p-val<0.01
	15-30	Diff = 1.10 p-val = 0.92	Diff = 15.99 p-val<0.01	Diff = 10.90 p-val<0.01**
Clay	**0-15**	Diff = 7.64 p-val = 0.11	Diff = 24.55 p-val = <0.001***	Diff = 16.91 p-val<0.01**
	15-30	Diff = 8.23 p-val = 0.09	Diff = 19.83 p-val<0.001***	Diff = 11.60 p-val = 0.02*



Appendix V. Boxplots of mean pH (a), Bray P (b) and inorganic N (c) per topography level with the upper boxplots and lower boxplots are respectively of the 0-15cm and 15-30 cm depth zones.

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