Responses of arbuscular mycorrhizal fungi to nutrient additions in a tropical montane forest of Southern Ecuador.

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Foreword

This dissertation is a cumulative work of the following publications:

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Summary

Tropical rainforests – characterized by an exceptional biodiversity, important for the regulation of climatic cycles and storing an estimate of 30% of terrestrial carbon – are severely endangered by human impacts. Increased nutrient depositions expected in the future by anthropogenic activities might affect these ecosystems primarily limited by nitrogen (N) and phosphorus (P). In Southern Ecuador consequences of these effects on a tropical montane forest are evaluated in the framework of a nutrient manipulation experiment. As part of this project we evaluated effects of moderate N and P additions on an important organism group involved in plant nutrient uptake – the arbuscular mycorrhizal fungi (AMF). Though AMF are known to represent the dominant mycorrhizal form in tropical forests, only few studies on AMF ecology were conducted in this diverse system. The main function of AMF represents increased nutrient uptake for the plant mainly of P, but also of N and other nutrients. Thus, since plants invest their carbon towards structures that are most limited (functional equilibrium model), theory predicts that increased nutrient availability will decrease AMF abundance. Based on this model and previous work conducted in temperate areas we tested our main hypotheses that (1) AMF abundance will be reduced following nutrient amendments and (2) AMF diversity will decrease and the AMF community shift towards lineages favored by fertilized conditions. In the case of AMF abundance we analyzed a time series from the beginning of the experiment up to five years of constant nutrient applications. Furthermore, effects were tested along an altitudinal gradient (1000 – 3000m above seal level) which is characterized by decreasing nutrient availability and a shift from N/P- towards N-limitation with elevation. For molecular analyses, after two years of nutrient applications the AMF community was analyzed in-depth by 454-pyrosequencing at 2000m. As part of this experiment, we aimed to characterize AMF in more detail in this tropical montane forest. Here, we focused on AMF distribution in the organic soil layer, since the dominance of AMF in such soils is contrary to common theories on AMF occurrence. Generally, we found some interesting patterns associated to this study site in AMF morphology and diversity, which we confirmed to be the dominant mycorrhizal form. Along the altitudinal gradient, as predicted by the functional equilibrium model, root and AMF abundance increased in parallel with decreasing nutrient availability. A more detailed molecular analysis at 2000m revealed a diverse community (74 operational taxonomic units) with a large proportion of Diversisporales. At this site, morphological analyses revealed a close association of AMF hyphae with barely decomposed plant material in the organic layer, though a great amount of hyphae was also detected on root surfaces.

Within the nutrient manipulation experiment and contrary to our first hypothesis, results show an overall positive effect of P additions on both, extra- and intraradical AMF biomass. However, N additions decreased AMF abundance. These effects were most pronounced at the intermediate site (2000m). This pattern is unusual and might be explained either by a predominant role of AMF in N uptake in this system or direct P-limitation of the fungus itself. Regarding AMF diversity, we observed a significant richness decrease in all treatments, with the strongest effect by concurrent application of N and P. Interestingly, Diversisporales and Glomerales were differentially affected by N and P, respectively, potentially pointing out group-specific traits. The findings presented here reveal insights in interesting patterns in AMF and their interrelations in this tropical montane forest, a biome where detailed knowledge on AMF is scarce. Direct conclusions on AMF functionality cannot be based solely on these observational results, but they allow the development of specific experimental designs for future studies. Nevertheless, the observed results indicate impacts of future nutrient depositions expected for the study area on the abundance and diversity of AMF, an important group influencing whole ecosystem processes.

Resumen

Los bosques tropicales - caracterizados por una biodiversidad excepcional, un papel importante en la regulación de ciclos climáticos y por mantener un estimado del 30% del carbono terrestre- se encuentran amenazados por actividades humanas. El incremento en la deposición de nutrientes debido a actividades antropogénicas podría afectar estos ecosistemas, limitados principalmente por nitrógeno (N) y fósforo (P). En el sur de Ecuador las consecuencias de estos efectos en bosques tropicales de montaña están siendo evaluadas en el marco de una manipulación experimental de nutrientes. Como parte de este proyecto, nosotros evaluamos los efectos de adiciones moderadas de N y P en un grupo de organismos importante en la absorción de nutrientes de las plantas: los hongos micorrícicos arbusculares (HMA). Aunque se sabe que los HMA representan la simbiosis micorrícica dominante en bosques tropicales, pocos estudios sobre la ecología de estos hongos se han realizado en estos ecosistemas. La principal función de los HMA es incrementar la capacidad de absorber nutrientes de las plantas, principalmente P pero también N y otros nutrientes. Por tanto, dado que las plantas invierten carbono a las estructuras que están más limitadas ("modelo de equilibrio funcional"), la teoría predice que un incremento en la disponibilidad de nutrientes disminuiría la abundancia de HMA. Basados en este modelo y en el trabajo preliminar conducido en sistemas templados, nosotros probamos las siguientes hipótesis: (1) la abundancia de HMA disminuirá al adicionar nutrientes y; (2) la diversidad de HMA disminuirá, resultando además en dominancia de linajes de hongos favorecidos por condiciones de alta fertilidad. En el caso de la abundancia de HMA, analizamos los resultados del registro de abundancia de HMA a lo largo de 5 años, desde el inicio de las adiciones constantes de nutrientes. Además, estos efectos fueron evaluados a lo largo de un gradiente altitudinal (1000-3000 msnm) que caracterizan una disminución en la disponibilidad de nutrientes y un cambio de una limitación de N/P hacia una limitación de N a mayor altura. Para la diversidad se realizó un análisis molecular: después de dos años de adiciones de nutrientes, la comunidad de HMA fue analizada por medio de la técnica de "pirosecuenciación 454 profunda" con muestras provenientes de sitios a una altura de 2000msnm. Como parte de este experimento, tuvimos como objetivo caracterizar los HMA en más detalle en este bosque tropical montañoso, especialmente en la capa orgánica de suelo ya que la presencia de HMA en este tipo de suelo contradice la teoría sobre el tipo de hábitat óptimo para los HMA.

En general, se encontraron patrones interesantes asociados a este sitio de estudio en cuanto a la morfología de HMA y su diversidad, confirmando que estos hongos representan el tipo micorrícico dominante. A lo largo del gradiente altitudinal, la abundancia de HMA incrementa a menor disponibilidad de nutrientes, tal como lo predice el modelo de equilibrio funcional. El detallado análisis molecular revela una comunidad de HMA diversa (74 unidades taxonómicas operacionales) con una larga proporción de hongos dentro del orden Diversisporales. En este sitio, observamos por medio de análisis morfológicos una asociación estrecha de hifas de HMA y material vegetal en descomposición en la capa orgánica de suelo, a pesar de que una gran cantidad de hifas fueron detectadas en la superficie de las raíces.

Dentro de la manipulación experimental de nutrientes, los resultados muestran un efecto positivo de la adición de P tanto en la biomasa extra- e intra- radical de HMA, contrario a nuestra primera hipótesis. Este efecto fue más pronunciado en sitio a intermedia altura (2000msnm). Sin embargo, a 2000msnm adiciones de N disminuyeron la abundancia de HMA. Este patrón es inusual y podría ser explicado ya sea por el papel predominante de los HMA en la absorción de N en este sistema o por una limitación de P del hongo en sí. En relación a la diversidad de HMA, observamos una disminución en la riqueza de especies en todos los tratamientos, siendo el efecto más pronunciado al adicionar N y P simultáneamente. Es interesante que hongos dentro de los órdenes Diversisporales y Glomerales fueron afectados diferencialmente por N y P, respectivamente, posiblemente indicando contrastantes características entre estos grupos.

En resumen, estos resultados revelan patrones interesantes en la asociación de HMA en bosques tropicales de montaña, un bioma sobre el cual hay muy poco conocimiento sobre los HMA. Conclusiones más directas sobre los efectos que estas manipulaciones tendrán en la función de los HMA no puede ser obtenida por medio de estudios observacionales como el presente, pero permitirán el diseño de experimentos con este propósito. En un contexto más amplio, cambios en la abundancia de HMA, su riqueza de especies y en la composición de la comunidad pueden ser esperados en respuesta al incremento de la deposición de nutrientes en este diverso ecosistema.

Zusammenfassung

Tropische Regenwälder – gekennzeichnet von außergewöhnlicher Biodiversität, wichtig für die Regulierung von Klimazyklen, Speicher von geschätzten 30 % des terrestrischen Kohlenstoffs – sind gefährdet durch menschliches Eingreifen. Zukünftige erhöhte Nährstoffdepositionen, verursacht durch anthropogene Aktivitäten könnten diese Ökosysteme, welche hauptsächlich durch Stickstoff (N) und auch Phosphor (P) limitiert werden nachhaltig verändern. Im Süden Ecuadors werden im Rahmen eines Nährstoff-Manipulationsexperimentes die Auswirkungen dieser Nährstoffdepositionen auf einen tropischen Bergregenwald getestet. Als Teil dieses Projektes haben wir den Einfluss von moderaten N und P Zugaben auf eine wichtige Organismengruppe untersucht, welche stark in die pflanzliche Nährstoffaufnahme involviert ist – die arbuskulären Mykorrhizapilze (AMF). Obwohl es bekannt ist, dass AMF die dominante Mykorrhizaform in tropischen Regenwäldern darstellen, gibt es nur wenige Studien zur Ökologie dieser Pilze in diesem diversen Ökosystem. Die Hauptfunktion von AMF für die Pflanze ist die verbesserte Nährstoffaufnahme, hauptsächlich von P, jedoch auch von N und anderen Nährstoffen. In Anbetracht der Grundlage, dass Pflanzen ihren Kohlenstoff an Strukturen verteilen welche am stärksten limitiert sind („Functional equilibrium model“), besagt die Theorie, dass erhöhte Nährstoffverfügbarkeit die AMF-Abundanz verringern wird. Basierend auf diesem Modell und vorherigen Ergebnissen aus temperaten Gebieten haben wir vorrangig die folgenden Hypothesen getestet: (1) AMF Abundanz wird in Folge von Nährstoffzugaben reduziert; (2) AMF-Diversität wird geringer und die Zusammensetzung der AMF Lebensgemeinschaft verschiebt sich zugunsten von Arten/Gruppen, welche durch die veränderten Bedingungen begünstigt werden. Basierend auf einer Zeitreihe von 5 Jahren ab Beginn des Experimentes wurden Untersuchungen zur AMF-Abundanz durchgeführt. Außerdem wurden Proben entlang eines Höhengradienten genommen (1000 – 3000 m ü. d. M.), welcher durch abnehmende Nährstoffverfügbarkeit parallel zu einer Verschiebung der Pflanzenlimitierung von P- bzw. N/P- hin zu N-Limitierung charakterisiert ist. Für die molekulare Analyse der AMF Lebensgemeinschaft auf 2000 m wurde nach zwei Jahren kontinuierlicher Nährstoffzugaben die detaillierte Methode der 454-Pyrosequenzierung angewandt. Weiterhin war es Teil dieses Projektes AMF im Allgemeinen in diesem besonderen System des tropischen Bergregenwaldes besser zu verstehen. Dafür lag der Fokus auf der AMF-Anpassung an die organische Auflage im Boden, da die Dominanz von AMF in solchen Böden früheren Theorien zum AMF-Vorkommen widerspricht.

Insgesamt konnten einige interessante Muster bezüglich der AMF-Morphologie und -Diversität im Zusammenhang mit dem Untersuchungsgebiet gefunden werden. Außerdem wurden AMF als vorherrschende Mykorrhizaform im Gebiet bestätigt. Im Einklang mit dem „functional equilibrium model", nimmt die Wurzellänge sowie die AMF-Abundanz parallel zur abnehmenden Nährstoffverfügbarkeit entlang des Höhengradienten zu. Eine detaillierte molekulare Analyse auf 2000 m offenbarte eine diverse Lebensgemeinschaft (74 „operational taxonomic units") mit einem großen Anteil an Diversisporales. Auf der gleichen Höhenstufe zeigte die morphologische Untersuchung des extraradikalen AMF-Mycels eine enge Assoziation der Hyphen mit im Abbauprozess befindlichem Pflanzenmaterial in der organischen Auflage, obwohl ein sehr großer Anteil an Hyphen auch auf der Wurzeloberfläche lokalisiert ist.

Im Gegensatz zu unserer ersten Hypothese, zeigten die Ergebnisse des Nährstoff-Manipulationsexperiments einen insgesamt positiven Effekt von P-Zugaben sowohl auf extra- als auch auf intraradikale AMF-Abundanz. Im Gegensatz dazu verringerten N-Zugaben die AMF-Abundanz eher. Die gefundenen Effekte waren auf der mittleren Höhenstufe (2000 m) am stärksten ausgeprägt. Dieses Muster ist ungewöhnlich und könnte entweder durch eine vorherrschende Rolle von AMF in der N-Aufnahme in diesem System, oder durch direkte P-Limitierung der Pilze selbst begründet werden. In Bezug auf die AMF-Diversität wurde in Folge aller Nährstoffbehandlungen ein signifikantes Absinken der Artenzahl beobachtet, mit dem stärksten Effekt bei gleichzeitiger Applikation von N und P. Interessanterweise wurden Diversisporales und Glomerales jeweils von N und P unterschiedlich beeinflusst, was auf bestimmte gruppenspezifische Eigenschaften hindeutet.

Zusammengenommen geben diese Ergebnisse Einblick in interessante Eigenschaften von AMF und ihren Wechselbeziehungen in diesem tropischen Bergregenwald, einem Biom für welches es an Detailwissen zu AMF noch mangelt. Direkte Schlussfolgerungen zur AMF-Funktionalität können alleinig basierend auf diesen Ergebnissen nicht getroffen werden, jedoch erlauben sie die Entwicklung von spezifischen experimentellen Designs für zukünftige Studien. Nichtsdestotrotz deuten die beobachteten Effekte daraufhin, dass zukünftige Nährstoffdepositionen, welche im Untersuchungsgebiet erwartet werden, Einfluss auf die Abundanz und Diversität von AMF haben werden, einer wichtigen Organismengruppe mit Einfluss auf ganze Ökosystemprozesse.

Chapter 1

General Introduction

Tropical rainforests represent ecosystems of high biodiversity which are important for the regulation of hydrological and climatic cycles and hold an estimated 30 % of terrestrial carbon (Dixon *et al.*, 1994; Brummitt & Lughadha, 2003). Especially in the light of global change scenarios the effects on tropical forests must be included in future predictions, since these systems are severely endangered by human impacts (Avissar & Werth, 2005; Gullison *et al.*, 2007). Most studies, particularly on biodiversity, have focused on plants and macroorganisms, demonstrating that many tropical forests represent hotspots of biodiversity (Olson *et al.*, 2001; Brummitt & Lughadha, 2003; Brehm *et al.*, 2008). In contrast, knowledge on the diversity and functionality of soil microorganisms is scarce (Borneman & Triplett, 1997; Alexander & Selosse, 2009). Beside the sampling bias, the great biogeochemical heterogeneity of tropical soils caused by high biological diversity but also great variation in soil age, chemistry and landscape dynamics might contribute to this knowledge gap in soil ecology (Townsend *et al.*, 2008).

This work focuses on a soil-inhabiting group of microorganisms associated with nutrient cycling, plant water uptake, carbon sequestration and soil structure – the arbuscular mycorrhizal fungi (AMF) (Rillig, 2004). This ancient group of obligate biotrophic plant symbionts is associated with 90% of all plant taxa worldwide (Smith & Read, 2008) and well known to represent the dominant mycorrhizal form in tropical forests (Shi *et al.*, 2006; McGuire *et al.*, 2008; Averill & Finzi, 2011), though basic knowledge on this group largely originates from temperate systems (Alexander & Selosse, 2009). Here, in the context of increased nutrient depositions by anthropogenic activities (Galloway *et al.*, 2008; Mahowald *et al.*, 2008), responses to nutrient additions in AMF abundance and molecular diversity were analyzed in a tropical montane forest in Southern Ecuador. As part of this study fundamental aspects of AMF morphology, functionality and community composition in this understudied ecosystem were elucidated.

The study area

The study area is located within the Cordillera Real in Southern Ecuador between the provinces Loja and Zamora-Chinchipe (Fig. 1.1). All study sites are within or adjacent to the Podocarpus National Park (PNP). Beside the core area at 2000m (Estación Scientifica San Francisco;

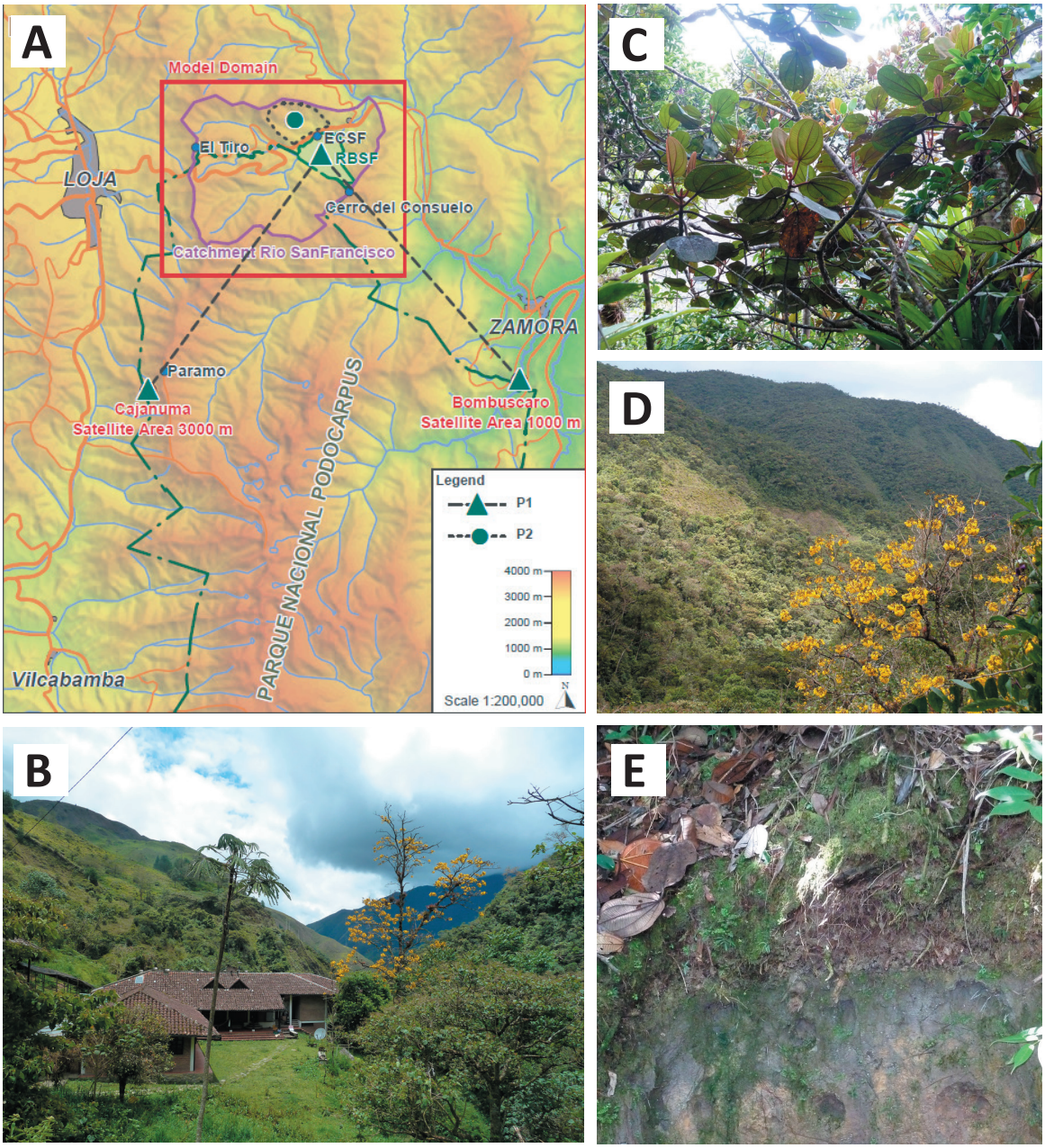

Fig. 1.1 Impressions of the study area including a map (A), which shows the different study sites within the Podocarpus National Park and the Reserva Biológica San Francisco (RBSF) (source: www.tropicalmountainforest.org). Pictures show the research station ECSF (Estación scientifica San Francisco) (B), a dominant tree of the study site at 2000m: *Graffenrieda emarginata* (C), a view on the pristine forest of the RBSF (D) and a soil profile at 2000m documenting the thick organic layer harboring the majority of tree roots (E).

3°98`S, 79°08`W), where most of the research was conducted, an altitudinal gradient comprising research sites at 1000m (Bombuscaro; 4°11`S, 78°96`W) and 3000m (Cajanuma; 3°98`S, 79°08`W) was analyzed (Fig. 1.1 A). Forest types span from premontane, lower montane towards upper montane forests, with a complete turnover of tree species (Homeier *et al.*, 2013). Interdisciplinary research conducted in this area since 1998 underscored the designation as a “hotspot” of biodiversity, regarding plant diversity - with more than 1200

species of spermatophytes recorded in PNP (Homeier & Werner, 2007; Homeier *et al.*, 2008) – and also other macroorganisms, especially moths (Brehm*, et al.*, 2008, Brehm *et al.*, 2005). The few studies investigating diversity of soil microorganisms (AMF: Haug *et al.*, 2010, oribatid mites: Illig *et al.*, 2010, testate amoebae: Krashevska *et al.*, 2013, Sebacinales: Setaro *et al.*, 2012), showed high but not exceptional species numbers, but several unknown taxa. The climate is characterized by high precipitation (2230, 1950 and 4500 mm $year^{-1}$ at the three elevational sites, respectively) without a pronounced dry season and mean annual air temperatures of 19.4, 15.7 and 9.4°C (Moser *et al.*, 2007). Soil types differ among altitudinal levels (Martinson *et al.*, 2013) and at 2000 and 3000m a thick organic layer is formed on top of mineral soil (Fig. 1.1 E), as often found in tropical montane forests (Edwards & Grubb, 1977; Unger *et al.*, 2010). Potential reasons are low temperatures, water-saturated soils and high amounts of phenolic compounds that slow down decomposition and mineralization processes (Kirschbaum, 1995; Wilcke *et al.*, 2002), which also decreases nutrient availability with elevation (Soethe *et al.*, 2008; Homeier *et al.*, 2010). Beside a general decrease in soil fertility, a shift from P- (or N/P co-limitation) towards N-limitation at higher altitudes was proposed (Graefe *et al.*, 2011; Moser *et al.*, 2011) as also predicted theoretically for Andean altitudinal gradients (Walker & Syers, 1976; Tanner *et al.*, 1998).

Arbuscular mycorrhizal fungi – morphology, physiology and diversity

Besides other important ecosystem functions AMF mainly improve plant nutrient uptake in exchange for carbon, since these fungi are obligate biotrophs gaining their carbon entirely from the plant (Smith & Read, 2008). Intraradical structures – namely coils and arbuscules – allow direct nutrient exchange with the plant, whereas a wide network of extraradical hyphae expanding the root depletion zone allows efficient nutrient uptake (Cavagnaro *et al.*, 2003; Parniske, 2008). Two root colonization types are differentiated: Paris- and Arum-type AMF (Smith & Smith, 1997). The Paris-type is characterized by the formation of intracellular coils without an intercellular phase, whereas in Arum-type AMF the root cortex is colonized by intercellular hyphae and subsequent intracellular arbuscules. However, the delineation of both types in natural systems is rather vague with several intermediate types occurring (Dickson, 2004). Extraradical hyphae may constitute 20 – 30 % of microbial soil biomass and exceed root length by several orders of magnitude, emphasizing their important role in ecosystems, but also the necessity to include this often neglected part of the symbiosis in analyses (Olsson *et al.*, 1999; Leake *et al.*, 2004).

Traditionally, AMF have been associated with plant P nutrition (Koide, 1991; Read, 1991), though in the last decade evidence accumulated that under certain conditions they also play a major role in the uptake of N (Hodge *et al.*, 2010; Veresoglou *et al.*, 2012; Hodge & Storer, 2015), as well as other nutrients, e.g. K, Zn and Cu (Marschner & Dell, 1994; Lehmann *et al.*, 2014). P uptake, transport and plant-fungus exchange has been investigated in detail (Parniske, 2008), whereas a role of AMF in N uptake has long been neglected due to the high mobility especially of nitrate in soil and high N demands of the fungus itself (Hodge *et al.*, 2010). However, studies using 15N tracers showed fungal transport of N to the plant, both from inorganic (Govindarajulu *et al.*, 2005; Tu *et al.*, 2006) as well as organic sources (Hodge *et al.*, 2001; Leigh *et al.*, 2009; Barrett *et al.*, 2011), and plant ammonium transporters involved in the symbiosis have been identified (Gomez *et al.*, 2009; Guether *et al.*, 2009). Still, opposing results on low or even a lack of N uptake by AMF inoculation (Marschner & Dell, 1994; Hodge, 2003; Reynolds *et al.*, 2005) indicate a potential dependency of this function on environmental conditions (Johansen & Jensen, 1996).

The degree of symbiotic association can be actively regulated by the plant via complex internal signaling depending on its nutritional status (reviewed in Gutjahr & Parniske, 2013; Carbonnel & Gutjahr, 2014). Thereby, complex trading of carbon for nutrients plays an important role in the interaction of plants and AMF, which might also affect the AMF community found in host roots (Bever *et al.*, 2009; Kiers *et al.*, 2011). Indeed, recent studies showed host-specific AMF community compositions, but no specialization of single host-AMF interactions (e.g. Vandenkoornhuyse *et al.*, 2003; Hausmann & Hawkes, 2009; Martinez-Garcia & Pugnaire, 2011). Other important factors which have been shown to affect AMF community composition are spatial effects (Lekberg *et al.*, 2007; Davison *et al.*, 2011), soil type, pH and soil fertility (Fitzsimons *et al.*, 2008; Dumbrell *et al.*, 2010b; Schechter & Bruns, 2013) as well as soil heterogeneity (Mummey & Rillig, 2008; de Carvalho *et al.*, 2012), though the relevant impact of each factor varies among systems. While AMF morphology is roughly equal among species except for differences in spore morphology, these alterations in species composition suggest differential traits among AMF species: in fact experiments confirmed to a certain degree differences in environmental demands as well as in colonization strategies (of roots versus soil) among AMF species (Lekberg *et al.*, 2007; Powell *et al.*, 2009; Ijdo *et al.*, 2010; Maherali & Klironomos, 2012). Global assessments of AMF diversity likewise revealed restricted occurrence of certain taxa in respect to plant type, habitat as well as climatic zones and continents, though some broad generalists exist (Öpik *et al.*, 2010; Kivlin *et al.*, 2011).

Taxonomic diversity of AMF – a monophyletic group within the Glomeromycota (Schüßler *et al.*, 2001) – is low: currently 330 morphospecies have been described (A. Schüßler's Glomeromycota phylogeny, http://schuessler.userweb.mwn.de/amphylo/; 10 January 2015) compared to more than a million Eumycotan species estimated (Blackwell, 2011). True AMF diversity though might be higher as indicated by molecular studies (Öpik *et al.*, 2014), especially since newly developed methods of high-throughput sequencing allow in-depth analyses of AMF communities (Öpik *et al.*, 2009; Dumbrell *et al.*, 2011). Still, molecular species delineation in AMF is problematic because of its asexual life form with multicore spores and hyphae (Kuhn *et al.*, 2001; Croll & Sanders, 2009), leading to high intraspecific variation (Ehinger *et al.*, 2012; Thiery *et al.*, 2012). Differences of intraspecific variability among AMF genera additionally complicates the clear determination of a universal sequence similarity cut-off for the classification of operational taxonomic units (OTUs) – the molecular synonym of species (Stockinger *et al.*, 2010). However, the traditional approach in AMF community analyses via spore sampling is also constrained by the influence of environmental conditions and species-dependent sporulation patterns (Clapp *et al.*, 1995; Sanders, 2004).

AMF in tropical rainforests

The original theory on AMF distribution proposed by Read (1991) restricted AMF occurrence broadly to areas characterized by P-deficient mineral soils of lower latitude and altitude. He already described AMF as the dominant mycorrhizal form in (lowland) tropical forests, as has been confirmed by numerous studies on the mycorrhizal status of tropical trees (e.g. Louis & Lim, 1987; Torti *et al.*, 1997; Husband *et al.*, 2002; Tawaraya *et al.*, 2003; Gehring & Connell, 2006; Shi *et al.*, 2006; McGuire *et al.*, 2008), also reviewed in Janos (1980b) and Averill *et al.* (2014). Since then, Read´s theory has been expanded by findings of high AMF abundances up to high latitudes (Väre *et al.*, 1997; Olsson *et al.*, 2004; Newsham *et al.*, 2009) and altitudes (Schmidt *et al.*, 2008; Liu *et al.*, 2011; Soteras *et al.*, 2015) and even in organic soils (Béreau *et al.*, 1997; Moyersoen *et al.*, 2001; Kottke *et al.*, 2004; Kessler *et al.*, 2014). Likewise, in tropical montane forests AMF have been repeatedly found to be the dominant mycorrhizal form up to the Páramo vegetation of the High Andes (Barnola & Montilla, 1997; Schmidt *et al.*, 2008; Urcelay *et al.*, 2011; Senes-Guerrero *et al.*, 2014). In the montane forest investigated here, Kottke *et al.* (2008) examined roots of 115 tree species: nearly all of them formed AMF, with only very few ectomycorrhizal species.

Despite this clear dominance of AMF in tropical systems, studies on AMF functionality are scarce in comparison to temperate areas (Alexander & Selosse, 2009). The majority of studies investigated the mycorrhizal status (as mentioned above) and several authors experimentally demonstrated a positive growth effect of AMF inoculum on tropical tree species (Janos, 1980b; Guadarrama *et al.*, 2004a; Urgiles *et al.*, 2014), often dependent on soil P levels (Moyersoen *et al.*, 1998a; Siqueira *et al.*, 1998; Guadarrama *et al.*, 2004b). A great number of studies focused on applied questions: effects of forest conversion on AMF abundance and diversity (Johnson & Wedin, 1997; Gavito *et al.*, 2008), impact of slash-and-burn practices (Guadarrama *et al.*, 2008; Aguilar-Fernandez *et al.*, 2009), comparisons of different land use systems (Fischer *et al.*, 1994; Leal *et al.*, 2009) as well as AMF potentials in afforestation (Urgiles *et al.*, 2014). Furthermore, certain aspects of tropical ecosystems, such as seasonal patterns (Lovelock *et al.*, 2003; Zangaro *et al.*, 2014), successional development (Zangaro *et al.*, 2000; Allen *et al.*, 2003) and influences of disturbance/gaps (Allen *et al.*, 1998; Cuenca *et al.*, 1998) have been investigated in more detail. Interestingly, few studies indicate potential particularities of AMF in certain tropical areas: Moyersoen *et al.* (1998b; 2001) showed unexpected overlaps of niches of ectomycorrhizal and arbuscular mycorrhizal fungi and Beck *et al.* (2007) described so far unknown AMF morphotypes on tree roots of montane forests, whose function remains unclear so far. Nevertheless, the small number of tropical studies to date does not allow for generalizations on this biome.

AMF community composition and diversity has been examined predominantly by spore analyses (Lovelock *et al.*, 2003; e.g. Leal *et al.*, 2013; Soteras *et al.*, 2015), but also by Sanger sequencing (Husband *et al.*, 2002; Aldrich-Wolfe, 2007; Haug *et al.*, 2010). Regarding richness, species numbers reported were generally high, though only slightly exceeding numbers found in temperate regions for both, spore counts and molecular OTU delineation (Gai *et al.*, 2009; e.g. Davison *et al.*, 2011; Schnoor *et al.*, 2011). However, variability among studies is high, probably depending on site characteristics as well as the study design applied and sampling depth. Indeed, some studies showed quite high richness estimates based on spore counts: Stürmer and Siqueira (2011) found 61 morphologically delineated species in the Amazon basin; Cuenca and Lovera (2010) found 50 in tropical shrubland, the same number as mentioned by da Silva *et al.* (2014) from a humidity gradient in Brazil. Nevertheless, the slight richness increase towards the tropics does not reflect the steep latitudinal gradient in plant diversity (Myers *et al.*, 2000), which is in line with findings reported for other soil fungi (Setaro *et al.*, 2012; Tedersoo *et al.*, 2014). However, especially the frequent lack of congruence of spore morphological types to known species (Cuenca & Lovera, 2010; Stürmer & Siqueira, 2011) - as well as OTUs to known sequences (Husband

et al., 2002; Haug *et al.*, 2010) - indicates a potential of several unknown AMF species remaining to be discovered in tropical forests.

AMF in response to nutrient additions

The functional equilibrium hypothesis states that plants invest their carbon towards structures that are involved in the acquisition of resources which are most limiting (Bloom *et al.*, 1985): if nutrients represent the limiting factor the belowground:aboveground ratio will increase, with stronger investment towards roots and mycorrhizae (Ericsson, 1995; Johnson, 2010). Indeed, along natural soil fertility gradients a decrease of AMF and root abundance with rising nutrient availability was shown (Bohrer *et al.*, 2001; Powers *et al.*, 2005). Likewise, pot experiments investigating AMF abundance in response to soil fertility gradients of P approved negative responses to high nutrient levels, however, at very low P values there was a primary increase towards a certain threshold (Abbott *et al.*, 1984; Bolan *et al.*, 1984; Breuillin *et al.*, 2010). In this context, Treseder and Allen (2002) proposed and tested a model of direct fungal nutrient limitation, which explains frequent observations of increases in AMF abundance following nutrient additions in low fertility soils (Treseder *et al.*, 2007; Alguacil *et al.*, 2010; Nijjer *et al.*, 2010). Nevertheless, in accordance with the functional equilibrium model the majority of fertilization experiments reported negative effects on AMF abundance, as summarized by a meta-analysis of Treseder (2004). In her analysis, the negative effects of P additions (on average -32%) were much more consistent than the effects of N additions (-15%). Positive effects of N additions are mainly discussed in the context of primary soil nutrient deficiency and a predominant role of AMF in P uptake: enhancing N availability for the plant results in growing P demands, thus, stronger investment in AMF (Eom *et al.*, 1999; Johnson *et al.*, 2003; Blanke *et al.*, 2012). This emphasizes that outcomes of nutrient manipulation experiments must be interpreted thoroughly including site characteristics but also all AMF structures relevant for the symbiosis, namely the intra- and extraradical phase.

Effects of nutrient additions on AMF diversity have also been investigated, quite consistently showing decreases in AMF richness following P (e.g. Alguacil *et al.*, 2010; Lin *et al.*, 2012; Gosling *et al.*, 2013), but also N additions (e.g. Johnson, 1993; Egerton-Warburton *et al.*, 2007; Antoninka *et al.*, 2011). Again, in the case of N results are more divergent (Eom *et al.*, 1999; Porras-Alfaro *et al.*, 2007). Generally, decreasing richness might be related to a parallel decrease in AMF abundance resulting in stronger interspecific competition. On the other

hand, changes in environmental conditions might favor specific AMF lineages promoting the dominance of certain groups: several studies found a shift in community composition, often towards lineages within the Glomerales (Johnson, 1993; Eom *et al.*, 1999; Egerton-Warburton *et al.*, 2007), which are known to be strong competitors (Helgason *et al.*, 2002; Maherali & Klironomos, 2012). Additionally, treatment related shifts in plant communities might affect the AMF community and vice versa, also influencing AMF abundance.

The majority of the above-mentioned studies on the effects of nutrient additions on AMF have been conducted in temperate systems. However, increased depositions of N by anthropogenic activities (Galloway *et al.*, 2008; Wilcke *et al.*, 2013a) and potentially also P (Mahowald *et al.*, 2005; Mahowald *et al.*, 2008) raise the question of future impacts on tropical forests, which are primary limited by these nutrients (Tanner *et al.*, 1998; Fisher *et al.*, 2013a). Impacts on AMF, which represent the interface of nutrient transfer between plants and soil, are important for the general understanding and should be considered in future scenarios.

Thesis outline

The central part of this thesis represents the evaluation of changes in AMF abundance and diversity in response to N and P additions, in order to understand potential consequences of future nutrient depositions but also to gain insights into the basic functionality of AMF in this tropical montane forest. Thus, we addressed the two main hypotheses that (1) AMF abundance will be reduced following nutrient amendments and (2) AMF diversity will decrease and the AMF community shift towards lineages favored by fertilized conditions. The results directly allow conclusions about the impact of nutrient additions on AMF, but also give general insights on AMF morphology and ecology in this special ecosystem. Analyses were conducted in the framework of a multidisciplinary nutrient manipulation experiment (NUMEX): at three altitudinal levels N and P were added in moderate amounts in a factorial design, starting in January 2008 (http://www.tropicalmountainforest.org/).

Chapter 2 examines the short-term effects (after one year) in NUMEX of N and P additions on several parameters of the whole ecosystem: nutrient fluxes and contents; microbial biomass and respiration; fine root biomass and intraradical AMF abundance; stem growth, litterfall and differential responses of the dominant tree species. This summary of datasets was used to determine (1) if added nutrients remain in the system, (2) in which way trees react to nutrient amendments, also regarding investment towards roots and mycorrhizae

and (3) if soil microbial activity is affected.

Chapter 3 focused on responses in AMF molecular diversity and community composition to nutrient additions. After two years of nutrient applications the intraradical AMF community was analyzed in-depth by 454-pyrosequencing. Here, the hypotheses were tested that (1) nutrient additions decrease AMF abundance, (2) AMF diversity, which is generally characterized by high richness values as expected by the application of high throughput-sequencing in this tropical biome, is reduced and (3) that a community shift towards lineages favored by fertilized conditions occurs.

In **Chapter 4**, the largest dataset covering responses in AMF abundance in detail was analyzed. Data collected after five years of nutrient addition along the altitudinal gradient were examined, aiming at testing the following hypotheses: (1) AMF as well as fine root abundance increases with elevation in parallel to decreasing nutrient availability, (2) N and P additions will decrease AMF abundance and (3) responses of AMF abundance will shift along the elevational gradient analyzed. Analyses included root length measurement, root colonization of AMF as well as of differential intraradical AMF structures. Importantly, also extraradical AMF abundance was included, measured by neutral lipid fatty analyses (NLFA) and partly also by spore counts and hyphal length measurements.

Chapter 5 represents a more general characterization of AMF in the highly organic soil typical of tropical montane forests. With a special focus on the extraradical mycelium, the general distribution of AMF in this soil - mainly consisting of barely decomposed plant material - was analyzed. Beside root colonization rates, hyphae were quantified separately on decomposing leaves, root surfaces and within the remaining particulate organic material. Thereby the hypothesis was tested that in such soil types AM hyphae grow in close contact with organic material.

A general discussion summarizing results and representing them in a broader context is given in **Chapter 6**.

Chapter 2

Tropical Andean forests are highly susceptible to nutrient inputs – Rapid effects of experimental N and P addition to an Ecuadorian montane forest

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Abstract

Tropical regions are facing increasing atmospheric inputs of nutrients, which will have unknown consequences for the structure and functioning of these systems. Here we show that Neotropical montane rainforests respond rapidly to moderate additions of N (50 kg ha^{-1} yr^{-1}) and P (10 kg ha^{-1} yr^{-1}). Monitoring of nutrient fluxes demonstrated that the majority of added nutrients remained in the system, in either soil or vegetation. N and P additions not only led to an increase in foliar N and P concentrations, but also altered soil microbial biomass, standing fine root biomass, stem growth, and litterfall. The different effects suggest that trees are primarily limited by P, whereas some processes - notably aboveground productivity – are limited by both N and P. Highly variable and partly contrasting responses of different tree species suggest marked changes in species composition and diversity of these forests by nutrient inputs in the long term. The unexpectedly fast response of the ecosystem to moderate nutrient additions suggests high vulnerability of tropical montane forests to the expected increase in nutrient inputs.

Introduction

Since the 1950/60s, anthropogenic changes to the cycling of the key nutrients nitrogen (N) and phosphorus (P) have dramatically altered the structure and functioning of many ecosystems in the world's industrialized regions (Tilman *et al.*, 2001; Mahowald *et al.*, 2005; Galloway *et al.*, 2008; Gruber & Galloway, 2008; Schlesinger, 2009; Janssens *et al.*, 2010). Elevated N and P inputs affect virtually all components and processes of terrestrial and aquatic ecosystems, including plant growth, plant longevity and stress tolerance, plant community composition and diversity, biotic interactions (plant-plant, plant-fungus, plant-animal), the composition and activity of heterotrophic communities, and the storage and cycling of carbon, nutrients and water (Elser *et al.*, 2007; Reay *et al.*, 2008; Bobbink *et al.*, 2010). This is because primary production is limited by N or P, or both in the vast majority

of ecosystems around the globe (Elser *et al.*, 2007; LeBauer & Treseder, 2008; Xia & Wan, 2008; Hedin *et al.*, 2009; Vitousek *et al.*, 2010; Harpole *et al.*, 2011).

In the past 30 years, research has focused on the structural and functional responses of temperate and boreal forests to atmospheric N inputs (Wright & Rasmussen, 1998; Stevens *et al.*, 2004; Högberg *et al.*, 2006; Bedison & McNeil, 2009) because the bulk of fertilizer use worldwide was in the industrialized nations of the northern hemisphere. Furthermore, these regions had particularly pronounced gaseous NO_x emissions originating from the combustion of fossil fuels and NH_3 emissions from animal production (Galloway *et al.*, 2008). However, this situation is changing rapidly. With the expansion of industrial agriculture into many tropical and southern hemispheric regions, the spread of N and P compounds to adjacent and more distant non-agricultural ecosystems in these regions has been greatly increased (Phoenix *et al.*, 2006; Galloway *et al.*, 2008; Bouwman *et al.*, 2013). In the future, tropical forests will be increasingly exposed to airborne N and P inputs. Higher P inputs are mostly due to the deposition of dust (Okin *et al.*, 2004; Fabian *et al.*, 2005; Mahowald *et al.*, 2005; Tamatamah *et al.*, 2005; Pett-Ridge, 2009), but sources of N can be varied, including oxidised and reduced N compounds emitted with farming, livestock breeding and the combustion of fossil fuels, and N released through biomass burning with the conversion of tropical forests (Matson *et al.*, 1999; Boy *et al.*, 2008; Galloway *et al.*, 2008).

Although tropical forests are likely to be sensitive to these changes, the size and direction of their responses are unclear (Hall & Matson, 2003; Lewis *et al.*, 2004; Wright, 2005; Li *et al.*, 2006; Phoenix *et al.*, 2006; Bobbink *et al.*, 2010; Corre *et al.*, 2010). A number of fertilization experiments in tropical forests have investigated responses to experimental high-dose treatments with N, or N and P (100-300 kg N ha^{-1} yr^{-1} and/or 50-100 kg P ha^{-1} yr^{-1}) (Tanner *et al.*, 1990; Vitousek & Farrington, 1997; Mirmanto *et al.*, 1999; Cavelier *et al.*, 2000; Nomura & Kikuzawa, 2003; Li *et al.*, 2006; Adamek *et al.*, 2009; Ostertag, 2010; Wright *et al.*, 2011). These experiments typically focused on selected ecosystem properties, such as changes in tree growth, fine litter production or soil carbon pools, but did not provide comprehensive insight into ecosystem responses to elevated N and P loads. Here, we report data from a nutrient manipulation experiment (NUMEX) investigating the response of an old-growth montane forest ecosystem in the Andes of southern Ecuador to moderate N (50 kg ha^{-1} yr^{-1}) and/or P (10 kg ha^{-1} yr^{-1}) additions considering a multitude of response variables. The study allows comprehensive insight into how highly diverse tropical montane forest ecosystems respond to moderate nutrient additions such as those predicted by climate change scenarios.

Results and Discussion

A large number of ecosystem properties and functions exhibited marked responses to the experimental nutrient additions after only one year.

Soil nutrient pools and soil biological activity

Nutrient addition did not result in a significant increase of the organic layer N pool while the P pool increased after combined addition of N and P (Fig. 2.1). The reason is likely the relatively high N stock in the thick organic layer. An adjacent micro-catchment between 1850 and 2150 m a.s.l. had 0.9- 21 Mg ha^{-1} N in the organic layer while the P stock ranged from 30-700 kg ha^{-1} (Wilcke *et al.*, 2002). Soil microbial biomass decreased after adding N (Fig. 2a), whereas P addition had no effect. A decrease in microbial biomass after N addition suggests detrimental effects of nitrogen on soil microorganisms and has been seen in other studies (Treseder, 2008; Janssens *et al.*, 2010), in particular with lignin decomposing fungi (Fog, 1988; Lu *et al.*, 2011). Conforming to the assumption of detrimental effects on microorganisms using complex organic compounds the metabolic activity of microorganisms significantly increased after N addition (Fig. 2.2b), indicating a shift towards microorganisms predominantly using more easily available carbon resources.

All treatments resulted in slightly higher net N mineralization rates, the increase after N addition was marginally significant ($p = 0.08$, Fig. 2.2c). N_2O emissions tended to increase after N+P addition ($p=0.05$) but not after addition of N or P only (Fig. 2.2d). This suggests that N is needed as substrate for denitrification while P is limiting the respective organisms.

The assumption that P addition stimulated microorganisms which are responsible for N transformations is further supported by the finding that the combined addition of N and P had a significant effect on the NH_4-N/NO_3-N ratio in mineral soil solution (Fig. 2.2e-f). Although we added urea, which is primarily a source of NH_4^+, NO_3^- concentrations increased after combined N and P addition while NH_4^+ concentrations did not. This was probably because of the combination of stimulated nitrification and microbial NH_4^+ retention. Our finding that only N and P addition together stimulated N mineralization (and possibly also nitrification) is different than reports from other tropical sites where N addition alone stimulated nitrification and triggered NO_3^- losses to the subsoil. This difference may be the result of no - or less pronounced - P limitation of the involved microorganisms (Lohse & Matson, 2005; Corre *et al.*, 2010). In contrast to many temperate forests where NO_3^- is the most abundant N form (Matzner *et al.*, 2004), NO_3^- only accounted for 3-5% of total N in the

soil solutions from our study site. Instead, the soil N pool was predominantly made up of dissolved organic nitrogen (DON) and NH_4^+ (contributing 50-70% and 27-43% respectively) (Goller *et al.*, 2006). This implies that small changes in N mineralization and nitrification rates can have a large impact on NO_3^- concentrations and fluxes. Our results indicate that the addition of different nutrients may stimulate or inhibit different processes in the ecosystem resulting in a complex system response to nutrient deposition.

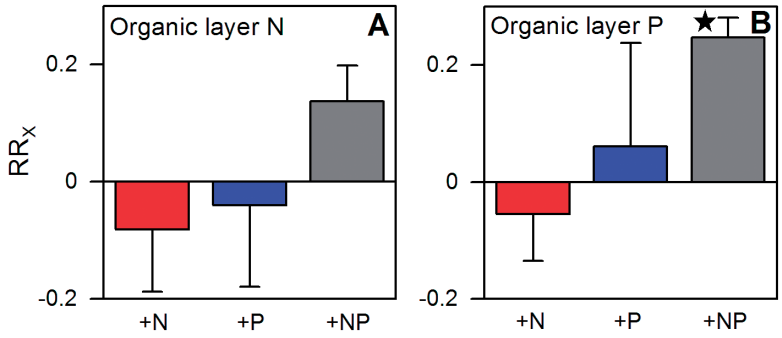

Figure 2.1. Effects of one year of experimental nutrient addition on various soil nutrient pools of a montane forest in Ecuador. Effects are presented as natural-log transformed response ratios (RR_x) in which the parameter in the enriched treatment is divided by its value in the control treatment and then ln-transformed. Hence, a value of 0.2 indicates a value in the manipulated treatment that is c. 23% higher than in the control, while a value of 0.5 indicates a 65% increase. Error bars indicate plus or minus one standard error. Data of the control treatment (mean ± 1 SE) are given in parentheses below. Asterisks indicate significant differences to the control ($P \leq 0.05$). **a.** Organic layer nitrogen pool (3.79 ± 0.31 Mg N ha^{-1}). **b.** Organic layer phosphorus pool (98.6 ± 6.8 kg P ha^{-1}).

Nutrient cycling

Increased N and P contents in litterfall and throughfall (Figs 2.3a-f) indicate that a large proportion of the added nutrients was taken up by trees and subsequently accelerated nutrient cycling through higher N and P return with litterfall and leachate (Wullaert *et al.*, 2010) and through stimulated litter decomposition by higher N and P concentrations (Wieder *et al.*, 2009). The annual increase in N and P fluxes in litterfall and throughfall after fertilization was equivalent to 25.4% and 26.7% of the applied N, after N addition and after N and P addition, respectively, and 3.8% and 6.1% of the applied P, after P addition and after N and P addition, respectively. Neither organic layer N and P pools nor N and P losses to the atmosphere or to the subsoil were significantly increased by N or P addition (Figs 2.2d and 2.3i, j), suggesting that the bulk of N and P added was retained in the ecosystem (Wullaert *et al.*, 2010).

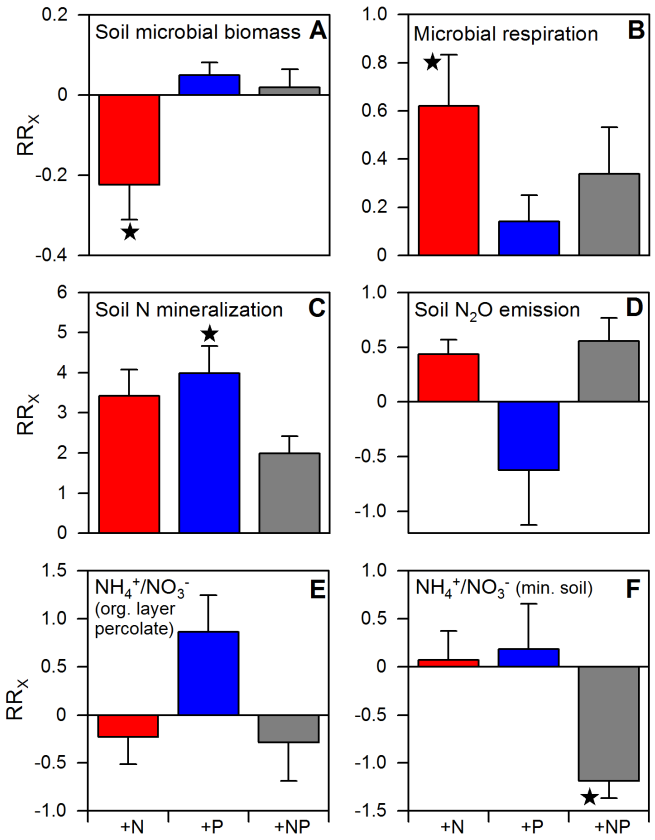

Figure 2.2. Effects of one year of experimental nutrient addition on biological soil activity of a montane forest in Ecuador. **a.** Soil microbial biomass in May 2009 (5881 ± 25 µg C_{mic} g^{-1} soil dry mass). **b.** Respiration of soil microorganisms in May 2009 (5.066 ± 0.36 µl O_2 mg C_{mic}^{-1} h^{-1}). **c.** Net N mineralization in September 2008 (23.4 ± 10.5 ng N cm^2 h^{-1}). **d.** Annual emission of N_2O (0.25 ± 0.03 kg N ha^{-1} yr^{-1}). **e.** Mean NH_4^+/NO_3^- ratio of organic layer percolate from February 2008 to January 2009 (15.7 ± 6.7) and **f.** mean NH_4^+/NO_3^- ratio of mineral soil solution from February 2008 to January 2009 (8.9 ± 2.0). Error bars indicate plus or minus one standard error. Data of the control treatment (mean ± 1 SE) are given in parentheses in the legend. Asterisks indicate significant differences to the control ($P \leq 0.05$). For interpretation of graph see legend of figure 2.1.

The N and P effects on nutrient cycling were interrelated. Phosphorus increased the retention of N in the system since aboveground N losses were slightly reduced (Fig. 2.2d) and losses by leaching were negligible (Fig. 2.3i-j). We attribute this mainly to the stimulation of the N-mineralizing microbial community by P addition as reflected by the significantly increased net N mineralization rates (Cleveland & Townsend, 2006). Furthermore, N application increased the P return with litterfall and throughfall when added in combination with P (N+P treatment: Figs 2.3d,f). The positive effects of N addition on litter P concentration and P return with litterfall and throughfall might be a result of better soil P availability to plants by increased extracellular phosphatase activity after N addition. This has been observed in several temperate and tropical forest studies (Olander & Vitousek, 2000; Treseder & Vitousek, 2001; Gress *et al.*, 2007).

Tree biomass and forest productivity

Stand leaf area index (LAI) tended to increase after addition of N and P alone (although not significantly), while leaf litter production increased only after N+P addition (Figs 2.4a,c). Since N fertilization tended to increase the specific leaf areas and foliar N concentrations of the four most common tree species (Table 2.1; difference significant only for *Myrcia* sp.), two foliage attributes generally associated with a shorter leaf lifespan (Poorter & Bongers, 2006), we infer that N addition has stimulated leaf production. The LAI increase after P addition probably resulted from an extension of mean leaf lifespan as indicated by the observed slight reduction in litter production. This is supported by results from Hawaiian montane forests, where leaf production and leaf longevity were increased after P addition (Vitousek *et al.*, 1993).

Stand basal area increment as a proxy of aboveground productivity tended to increase in all fertilization treatments (Fig. 2.4b), as reported from other Neotropical montane forests after addition of N (Tanner *et al.*, 1990; Vitousek *et al.*, 1993; Vitousek & Farrington, 1997), P (Tanner *et al.*, 1990; Vitousek & Farrington, 1997) or N and P (Tanner *et al.*, 1992).

All fertilizer treatments resulted in a marked reduction in standing tree fine root biomass (by 15-28%) with the effect being strongest after P addition (Fig 2.4d). This treatment also led to a strong increase in standing fine root necromass, while the N and N+P treatments had no effect on necromass (Fig. 2.4e). Presumably, the accumulation of fine root necromass in the P treatment resulted from reduced root litter decay due to an unfavorable litter N: P ratio for decomposers (Güsewell & Gessner, 2009). A decline of fine root biomass and a concurrent increase of dead roots after nutrient addition have also been shown in other montane forests after N addition (Cusack *et al.*, 2011: Puerto Rico) or after N, P and N+P addition (Gower & Vitousek, 1989: Hawaii).

The observed decrease of fine root biomass does not necessarily indicate a reduced fine root production. More likely is a stimulation of fine root production by nutrient addition and a concurrent increase of fine root turnover (Nadelhoffer, 2000; Yuan & Chen, 2012), the combination of both effects could result in a reduced standing fine root biomass.

The root colonization by arbuscular mycorrhizal fungi (AMF) was not significantly affected by nutrient addition, with values remaining more or less constant at about 50% (Fig. 2.4f). This result contrasts with the rapid response of fine root biomass and does not fit to the predictions of the functional equilibrium model of AMF (Johnson *et al.*, 2008).

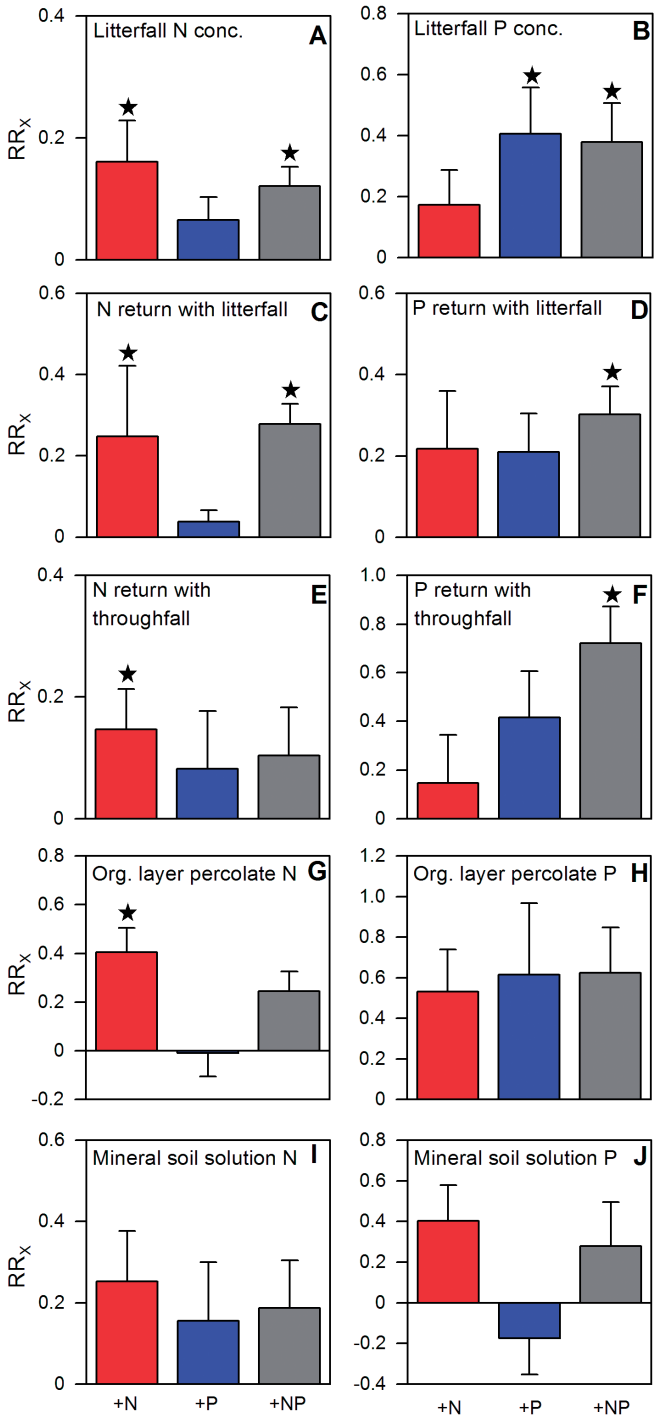

Figure 2.3. Effects of one year of experimental nutrient addition on nutrient cycling of a montane forest in Ecuador. **a**. Nitrogen and **b**. phosphorus concentrations of the litterfall in January 2009 after one year of nutrient addition (0.87 ± 0.03 % N and 0.025 ± 0.006 % P). Total annual return of **c.** nitrogen and **d.** phosphorus with litterfall (41.7 ± 5.0 kg N ha^{-1} yr^{-1} and 1.49 ± 0.24 kg P ha^{-1} yr^{-1}). Total annual return of **e.** nitrogen and **f.** phosphorus with throughfall (10.1 ± 1.0 kg N ha^{-1} yr^{-1} and 0.15 ± 0.03 kg P ha^{-1} yr^{-1}). **g**. Annual flux of nitrogen and **h**. phosphorus found in the organic layer percolate (14.65 ± 1.21 kg N ha^{-1} yr^{-1} and 0.13 ± 0.02 kg P ha^{-1} yr^{-1}). **i**. Annual flux of nitrogen and **j**. phosphorus found in the soil solution at 0.3m soil depth (3.26 ± 0.59 kg N ha^{-1} yr^{-1} and 0.03 ± 0.004 kg P ha^{-1} yr^{-1}). Error bars indicate plus or minus one standard error. Data of the control treatment (mean ± 1 SE) are given in parentheses in the legend. Asterisks indicate significant differences to the control ($P \leq 0.05$). For interpretation of graph see legend of figure 2.1.

Empirical data show root colonization by AMF to be reduced when N and particularly P availability is increased (Treseder, 2004). These results indicate that in our study area the addition of P (and to a lesser extent of N) relaxed the growth limitation imposed by P (and N) scarcity and prompted the trees to allocate more carbon into aboveground structures and productivity. Our finding that fine root biomass was significantly reduced upon N and P addition, while the mycorrhizal infection of the trees remained unchanged, points at the importance of AMF functions other than nutrient acquisition in controlling the plant-fungus interaction (Powell *et al.*, 2009), or resource uptake rates that are independent of infection rate.

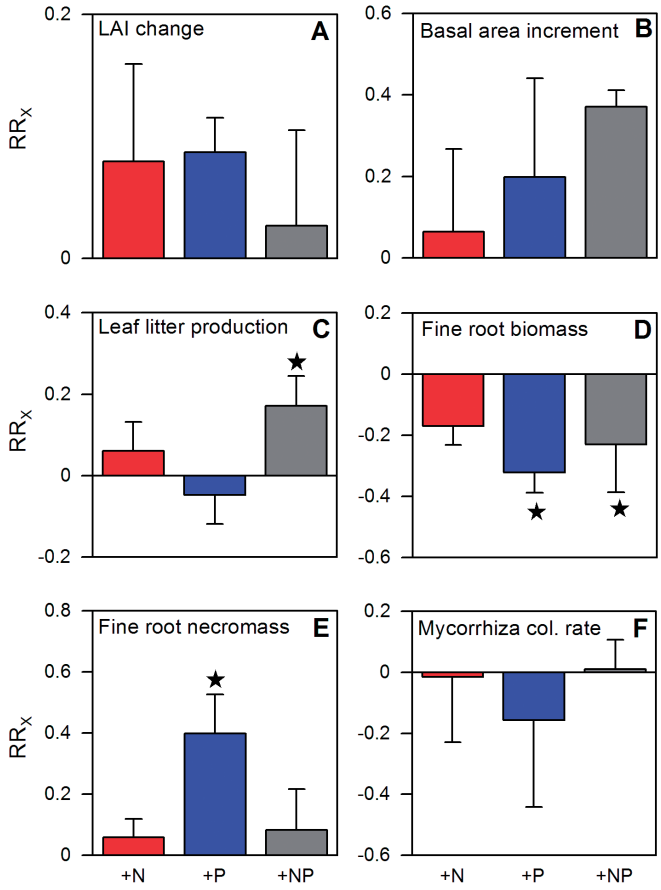

Figure 2.4. Effects of one year of experimental nutrient addition on vegetation related parameters of a montane forest in Ecuador. **a**. Relative change of leaf area index (LAI) after one year of nutrient addition (measurements from January 2009 were compared to measurements prior to nutrient addition in January 2008; control mean changed from 4.6 ± 0.2 in 2008 to 4.7 ± 0.4 in 2009). **b**. Plot basal area increment from February 2008 to January 2009 (0.111 ± 0.018 m^2 ha^{-1}). **c**. Annual leaf litter production from February 2008 to January 2009 (3.46 ± 0.46 Mg ha^{-1}). **d**. Fine root biomass in January 2009 (443 ± 28 g m^{-2}), **e**. fine root necromass in January 2009 (426 ± 29 g m^{-2}), and **f**. rate of fine root colonization by arbuscular mycorrhizal fungi in January 2009 (53.3 ± 6.2%). Error bars indicate plus or minus one standard error. Data of the control treatment (mean ± 1 SE) are given in parentheses in the legend. Asterisks indicate significant differences to the control ($P \leq 0.05$). For interpretation of graph see legend of figure 2.1.

The stand-level N and P use efficiencies in the control plots were in the upper range of values reported from other tropical forests (Vitousek, 1984b) (Fig. 2.5). Addition of nitrogen, phosphorus, or both, led to a significant decrease (10- 25%) in the N or P use efficiencies, respectively, within one year, suggesting rapid relaxation from growth limitation by P and N and most likely decreased nutrient resorption efficiencies of the vegetation. Lower nutrient resorption efficiencies with increasing green leaf nutrient status were also reported by Kobe et al. (2005) for a global data set of perennial plant species.

Divergent tree species growth responses

We found a highly variable response of stem diameter growth upon N and/or P addition among the tree species in the fertilized plots. Depending on species, growth rates were either higher or lower relative to the control plots (Table 2.2). However, two of the most common species (*Hieronyma fendleri* and *Alchornea lojaensis*) showed increases in stem diameter growth rates after addition of N, P or N+P, while two other species (*Graffenrieda emarginata* and *Myrcia* sp.) tended to reduce growth upon N or P fertilization. The divergent growth response of different tree species is in agreement with fertilization studies from other tropical montane forests (Tanner *et al.*, 1990; Cavelier *et al.*, 2000). It appears that the responsiveness to N or P addition of stem diameter growth is highly species-specific and that while some species will increase, others will reduce their competitive strength with continued nutrient addition, likely resulting in species composition changes and diversity reductions in this species-rich forest over time (Gusewell, 2004; Bobbink *et al.*, 2010; Lu *et al.*, 2010). Since the forest canopy will become denser in response to fertilization (increasing the LAI) and the trees will be relieved of growth constraints due to limited N and/or P availability, light competition will become more important with increasing input of nutrients. These changes may reduce the competitive ability of the seedlings and saplings of the currently abundant tree species, and will probably result in their eventual replacement by species adapted to more fertile soils. Changes in tree species composition (from slow-growing species adapted to nutrient-poor soils to faster growing species adapted to more fertile soils) will most likely accelerate the projected shifts in the C cycle by increasing the biomass turnover rate. The N:P ratio in leaf biomass of the unfertilized trees (means of 20-31 in the four most common species) provides support for the conclusion that tree growth in the studied forest is mainly limited by P (Townsend *et al.*, 2007). Consequently, P was accumulated to a larger extent in the foliage than N after addition of P or N, and the N:P ratio responded more to P addition (decrease) than to N addition (no uniform effect; Table 2.1). The increased foliar N concentrations in three of the four studied common tree species

after addition of N or N+P, respectively, should result in higher photosynthetic carbon gain, since photosynthetic capacity is closely related to foliar N (Wright *et al.*, 2004).

Table 2.1. Effects of nutrient addition on foliar nutrient concentrations and leaf morphology of the four most common tree species of a montane forest in Ecuador.

			Deviation from control (%)		
	Tree species	**control**	**+N**	**+P**	**+NP**
Foliar N (mg g^{-1})	*Graffenrieda*	12.2	+5	±0	+4
	Myrcia sp.	11.3	**+9***	-6	**+11***
	Hieronyma	13.8	+1	-4	**+11***
	Alchornea	13.8	+7	**+15***	**+14***
Foliar P (mg g^{-1})	*Graffenrieda*	0.43	-2	**+37***	+21
	Myrcia sp.	0.43	**+28***	-5	**+33***
	Hieronyma	0.54	+2	+11	**+28***
	Alchornea	0.71	-11	**+34***	**+42***
Foliar N/P ratio	*Graffenrieda*	31	+7	**-31***	-17
	Myrcia sp.	27	-17	-4	-18
	Hieronyma	26	-3	**-14***	**-14***
	Alchornea	20	**+16***	**-15***	**-21***
Leaf area (cm^2)	*Graffenrieda*	178.2	+28	-1	+15
	Myrcia sp.	17.9	+3	-15	+12
	Hieronyma	26.8	-13	-29	-23
	Alchornea	30.5	-8	+1	+2
Specific leaf area (cm^2 g^{-1})	*Graffenrieda*	38.4	+9	+7	+10
	Myrcia sp.	40.2	+2	-5	+2
	Hieronyma	69.3	+6	+1	-7
	Alchornea	40.9	+13	+5	+12

Given are the absolute values for the control treatment and the percental effects of the treatments. Asterisks indicate significant differences to control ($P<0.05$). The number of sampled trees was for *Graffenrieda emarginata*: 5 (control), 6 (+N), 6 (+P) and 6 (+NP), for *Myrcia sp.:* 6, 5, 5 and 6, for *Hieronyma fendleri* 2, 5, 5 and 3 and for *Alchornea lojaensis*: 5, 4, 5 and 4.

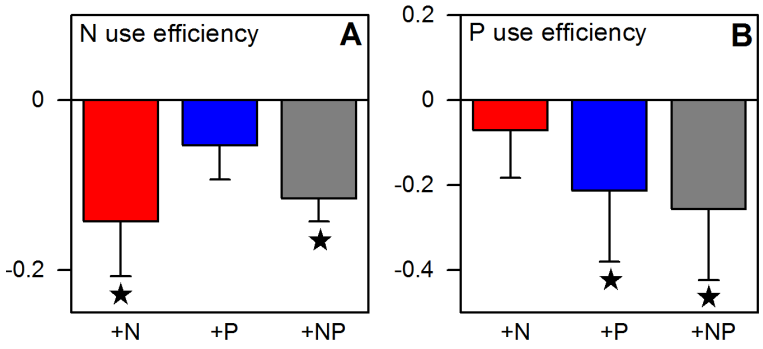

Figure 2.5. Nutrient use efficiencies and monthly nutrient return with litterfall. Nutrient use efficiencies (i.e. the ratio of total litterfall dry mass to nutrient content [64]) of the different treatments in the studied montane forest in Ecuador after one year of nutrient addition (samples from January 2009, means of N = 24 litter traps per treatment). **a**. N use efficiency (116.5 ± 3.8 g g^{-1}), **b**. P use efficiency (4751 ± 782 g g^{-1}). Error bars indicate plus or minus one standard error. Data of the control treatment (mean ± 1 SE) are given in parentheses in the legend. Asterisks indicate significant differences to the control (P ≤ 0.05). For interpretation of graph see legend of figure 2.1.

Table 2.2. Effects of nutrient addition on annual stem diameter growth of the four most common tree species of a montane forest in Ecuador.

		Deviation from control (%)		
Tree species	**control (mm)**	**+N**	**+P**	**+NP**
Graffenrieda emarginata	0.84 (49)	-12 (49)	-21 (47)	+16 (54)
Myrcia sp.	0.80 (17)	-39 (15)	**-50*** (16)	-5 (11)
Hieronyma fendleri	0.07 (6)	+157 (22)	+71 (13)	**+414*** (13)
Alchornea lojaensis	0.12 (20)	**+33*** (18)	+17 (16)	**+42*** (9)
all other species pooled	0.28 (78)	-11 (94)	+82 (77)	+25 (89)

Given are the absolute values for the control treatment (February 2008 – January 2009) and the percental effects of the treatments. Asterisks indicate significant differences to the control (P<0.05).

Conclusions

Overall, the strong and complex short-term response of the tropical montane forest ecosystem to moderate nutrient inputs suggests major consequences of expected future nutrient inputs into these ecosystems. This is particularly evident at our study site. The effects that we observed were larger than those reported from tropical lowland forests on more fertile soils, where only long-term nutrient addition resulted in significant effects (Wright *et al.*, 2011). Several of the responses to nutrient addition are similar to those

known from other tropical montane forests, where they occurred either after chronic nutrient addition or after fertilization with higher amounts of N.

Provided that these initial trends persist, continued addition of substantial amounts of N and P will probably result in taller forests with a higher above-ground biomass but smaller below-ground biomass (Yuan & Chen, 2012). However, the below-ground response of the system to nutrient addition is still poorly understood. Given the large stocks of carbon in the organic layer, stimulated mineralization and soil respiration rates and less belowground C sequestration may turn these ecosystems into a significant future source of CO_2 to the atmosphere.

Further studies have to show how nutrient cycles and key ecosystem services such as carbon storage will adjust to continuing input of moderate amounts of N and P and how community composition will change in the long run.

Cross-study comparisons of nutrient manipulation experiments could contribute to a better understanding of ecosystem responses to increasing nutrient deposition, but the currently published studies are hard to compare due to different levels of fertilizer addition and methodological differences among the various studies. A network of coordinated experiments adding low amounts of nutrients to tropical forests, that covers a wide range of environmental conditions (climate, soil), would be the method choice of obtaining general patterns of tropical forest ecosystem responses to increasing nutrient availability.

Materials and Methods

Study area

The study was conducted at about 2000 m elevation, in a tropical montane moist forest of the San Francisco Reserve in the Andes of southern Ecuador (3° 58’ S, 79° 04’W) (Fig. S2.1). This forest is in nearly pristine condition, and is one of the best-studied tropical montane forests worldwide, known for its extraordinary richness in tree species as well as other plant and animal groups (Beck *et al.*, 2008; Homeier *et al.*, 2010). The forest harbors more than 300 tree species with Lauraceae, Melastomataceae and Rubiaceae being the plant families with the highest species numbers. The study site has a mean annual precipitation of ~2200 mm and an annual mean temperature of ~15°C. The most abundant soil types at the study site are Cambisols that developed on paleozoic metamorphosed schists and sandstones. Soils are heterogeneous but usually nutrient-poor (thick organic layers can harbor locally

high nutrient stocks, but often these are only slowly bioavailable) (Wilcke *et al.*, 2002). Estimates of total annual nutrient depositions (based on the monitoring of bulk and dry deposition between 1998 and 2010) range from 14 – 45 kg N ha^{-1} and 0.4 – 4.9 kg P ha^{-1} for the study area.

All necessary permits were required for the described field studies.

Experimental design

A full-factorial nutrient manipulation experiment (NUMEX) was conducted in 16 plots of 400 m^2 (20m x 20m) consisting of four treatments (N, P, N+P, control) with four replicates in a stratified random design in four blocks at 2020 - 2120m a.s.l. (Fig. S1). Minimum distance between two plots was 10 m.

Nitrogen and phosphorus were added at an annual rate of 50 kg N ha^{-1} as urea and 10 kg P ha^{-1} as monosodium phosphate. The fertilizer was dispersed homogeneously over the plots with two application dates per year (January 26 and July 26) starting in 2008.

The dominant tree species in the NUMEX plots, making up about one quarter of all stems (dbh ‘ 10 cm), was *Graffenrieda emarginata* Ruiz & Pav. (Melastomataceae); other frequently found species were *Myrcia* sp. nov. (Myrtaceae), *Alchornea lojaensis* Secco and *Hieronyma fendleri* Briq. (both Euphorbiaceae). The mean number of trees, mean tree diameter and stem basal area per plot (pre-fertilization survey of trees ≥ 10 cm dbh) were 45.7, 15.0 cm and 0.91 m^2, respectively. Average stand height was 12 to 14 m.

Organic layer nutrient pools

In April 2009, the soil organic layer (including the Oi, Oe, and Oa horizons) was sampled with a 0.2 x 0.2 m frame to the depth of the organic layer/mineral soil boundary at five randomly selected points within each permanent plot. Samples were dried to constant mass at 40° C.

The organic horizons were separated from the underlying mineral soil at the point where bulk density abruptly increases from < 0.2 g cm^3 in the organic layers to > 1g cm^3 in the mineral soil (Wilcke *et al.*, 2002).

The N concentrations in the ground samples were determined with a CHNS-analyzer (Vario EL Cube, Elementar Analysensysteme GmbH). After microwave digestion with HNO_3 (Mars 5 Xpress, CEM Corporation, Matthews, NC), the total phosphate concentration was

detected photometrically (Continuous Flow Analyser; Bran+Luebbe GmbH, Norderstedt, Germany). Soil bulk density was determined with two additional samples per plot which were dried at 105°C for 24h. Detailed results are shown in Table S2.1.

Microbial biomass

In May 2009, three samples per plot were collected in the upper organic layer to a depth of 5 cm using a metal corer (5 cm diameter). The upper litter layer (1-2 cm) was removed and the three samples were pooled and stored at 5°C. Before measurements, roots >2 mm were removed from the soil and the remaining material was chopped to pieces of <25 mm², homogenized and pre-incubated at 20°C for five days.

Respiration of soil microorganisms was measured as O_2 consumption using an automated electrolytic O_2 microcompensation apparatus (Scheu, 1992). Respiration was measured at hourly intervals at 22°C for 24 h. Basal respiration (BR) of microorganisms was calculated as the mean oxygen consumption rates of hours 14-24 after the start of the measurements without addition of substrate. Microbial biomass carbon (C_{mic}) was calculated from substrate induced respiration measuring the respiratory response to D-glucose which activates the metabolism of living microorganisms in the soil (Anderson & Domsch, 1978). After adding Glucose (80 mg g^{-1} dry mass in 300 µl deionized water) the mean of the lowest three readings within the first 5-10 h was taken as maximum initial respiratory response (MIRR; µg O_2 h^{-1} g^{-1} soil dry mass). Microbial biomass (µg C g^{-1} soil dry mass) was calculated as 38 x MIRR (Beck *et al.*, 1997).

Net rates of nitrogen cycling in the soil

Net rates of N cycling in the soil were measured in October 2008 using the buried bag method (Hart *et al.*, 1994). In each plot a soil sample was taken from 0 to 5 cm depth. One subsample was extracted immediately in the field with 0.5 mol L^{-1} K_2SO_4 to determine initial NH_4^+ and NO_3^- levels (T_0). The other sample was put into a plastic bag, reburied in the soil, incubated for ten days and afterwards extracted with 0.5 mol L^{-1} K_2SO_4 (T_1). The plastic bag was closed with a rubber band to prevent rain coming in but not too tight to permit air exchange. Net N mineralization and nitrification rates were calculated as the difference between T_1- and T_0- NH_4^+ and NO_3^- concentration.

Trace gas measurements

Nitrous oxide was measured monthly using static vented chambers. Four permanent chamber bases made of polyvinyl chloride (area 0.04 m^2, height 0.25 m, ~0.02 m inserted into the soil) were randomly placed in four of six subplots per plot at least four weeks before the first measurement, resulting in 16 chamber bases per block and 48 in total. Four gas samples (100 ml each) were removed at 2, 14, 26 and 38 min after chamber closure with an acrylonitrile butadiene styrene (ABS) lid and stored in pre-evacuated glass containers (60 mL) with stopcocks (Koehler *et al.*, 2009). Gas samples were transported to the laboratory in Loja (Ecuador) within two days and analyzed using a gas chromatograph (Shimadzu GC-14B, Duisburg, Germany) equipped with an electron capture detector (ECD) and an autosampler (Loftfield *et al.*, 1997). Gas concentrations were determined by comparison of integrated peak areas of samples to standard gases (320, 501, 1001 and 3003 ppb N_2O; Deuste Steininger GmbH, Mühlhausen, Germany). Gas fluxes were calculated from the linear increase of gas concentration in the chamber vs. time, and were adjusted for air temperature and atmospheric pressure (Koehler *et al.*, 2009). Zero fluxes were included.

Litterfall

Six litter traps (each 0.36 m^2 in surface area positioned 1 m above ground) were randomly placed in each plot. The litterfall was collected every four weeks starting on November 6th, 2007. The collected samples were oven-dried at 60 °C before determining the dry weight.

Leaf morphology

Leaf samples from sun-exposed branches of each of 4–5 trees per treatment from four common species (*Alchornea lojaensis*, *Graffenrieda emarginata*, *Hieronyma fendleri* and *Myrcia* sp. nov.) were collected in January 2009 to quantify changes in leaf morphology and foliar nutrient concentrations one year after the onset of the experiment. For each sample 10-25 fresh leaves were scanned using a flat bed scanner (CanonScan LIDE 30, Canon). The images were analyzed subsequently with the WinFolia 2001a software (Regent Instruments Inc., Quebec, Canada) for calculation of leaf area. The leaves were then dried at 60 °C to constant mass. Specific leaf area (SLA) was calculated as the ratio of leaf area and leaf dry weight.

Foliar and litter nutrient contents

The concentrations of total C and N in leaf and litter mass were determined with a C/N elemental analyzer (Vario EL III, elementar, Hanau, Germany). The concentrations of total P were analyzed using an Inductively Coupled Plasma Analyzer (Optima 5300DV ICP-OES, Perkin Elmer) after digesting the samples with concentrated HNO_3.

Throughfall and soil solutions

Throughfall was collected with 20 randomly distributed, fixed-positioned funnel gauges in each plot. The volume of throughfall water was measured in the field with a graduated cylinder, and the samples were then bulked according to their relative volume to result in a single sample per plot per collecting date.

Litter leachate was collected using three zero-tension lysimeters per plot, which consisted of plastic boxes with a collecting surface area (polyethylene net) of 0.15 m x 0.15 m, installed below the organic layer. All collected litter leachate samples of a plot were bulked to yield a single sample per plot per collecting date.

In each plot, mineral soil solution was collected using three suction lysimeters (ceramic suction cups with 1 µm pore size) at 0.15 and 0.30 m depth, installed so that bulking of the soil solution per soil depth occurred *in situ*.

Throughfall, litter leachate and mineral soil solutions were sampled fortnightly. After collecting the mineral soil solution, a vacuum was applied to the suction lysimeters in order to collect sufficient sample for the next sampling period.

After field collection, throughfall, litter leachate, and soil solution samples were transported to our field laboratory where an aliquot was filtered (ashless filters with pore size 4-7µm, folded filter type 389; Munktell & Filtrak GmbH, Bärenstein, Germany) and frozen until transport to Germany for further analysis. Samples were analyzed for concentrations of NH_4^+, NO_3^-, total dissolved N, and total dissolved P using continuous flow analysis (CFA, Bran+Luebbe GmbH, Norderstedt, Germany). Dissolved organic nitrogen concentrations were calculated as the difference between total dissolved N and the sum of total inorganic nitrogen ($NH_4^+ + NO_3^-$), assuming that NO_2^- concentrations were negligible.

Leaf area index

The LAI was quantified in the plots with two LAI-2000 plant canopy analyzers (LI-COR Inc., Lincoln, NE, USA). The LAI measurements were conducted in the remote mode, i.e. by synchronous readings below the canopy at 2 m height above the forest floor and in a nearby open area ("above-canopy" reading) using two devices. One measurement was done above each litter trap and a second at the same time outside the forest. Measurements were done in January 2008 (before 1st fertilization) and in January 2009 (one year after the 1st fertilization).

Stem diameter growth and basal area growth per plot

The stem diameter growth of all trees present with a dbh ≥ 10 cm was monitored in the 16 plots every six weeks with permanent girth-increment tapes (D1 dendrometer, UMS, Munich; 713 stems in total). The cumulative increase in plot basal area per year was calculated as the sum of all tree basal area increments in a plot between February 15th, 2008 (after the first fertilization) and January 19th, 2009.

Fine root biomass

For measuring fine root biomass, we took six root samples per plot to a depth of 20 cm using a soil corer of 3.5 cm in diameter in January 2009. The soil samples were transferred to plastic bags and transported to the laboratory, where processing of the stored samples (4°C) took place within six weeks. In the lab, the samples were soaked in water and cleaned from soil residues using a sieve with a mesh size of 0.25 mm. Only fine roots (roots <2 mm in diameter) of trees were considered for analysis. Live fine roots (biomass) were separated from dead rootlets (necromass) under the stereomicroscope based on color, root elasticity, and the degree of cohesion of cortex, periderm and stele (Persson, 1978; Leuschner *et al.*, 2001). The fine root biomass of each sample was dried at 70°C for 48 h and weighed.

Mycorrhiza

Root colonization by arbuscular mycorrhizal fungi was measured at 200x magnification following clearing and staining with 0.05% Trypan Blue according to (Rillig *et al.*, 1998), additionally including fungal structures as defined in (Beck *et al.*, 2007).

Statistical analyses

The effects of N and/or P addition on the various investigated parameters were expressed by a response ratio metric (RR_x = *ln* (measured value in nutrient addition treatment / measured value in the control)) (Elser *et al.*, 2007) in order to compare the response of plant- and soil-related state variables or flux parameters in relative terms. Non-transformed data are shown in Table S2.2.

Effects of the addition of N and/or P on individual parameters were analyzed using linear mixed models (package lme4, R version 2.13.0) (R Development Core Team, 2011). We included the fertilization treatments as fixed effects and the factor "block" as a random factor in the models, since in most parameters, samples were nested within plots. P-values for the fixed effects were calculated with the "cftest" function of the package "multcomp" (R version 2.13.0) (R Development Core Team, 2011).

Acknowledgments

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Chapter 3

Nitrogen and phosphorus additions impact arbuscular mycorrhizal abundance and molecular diversity in a tropical montane forest

http://dx.doi.org/10.1111/gcb.12618

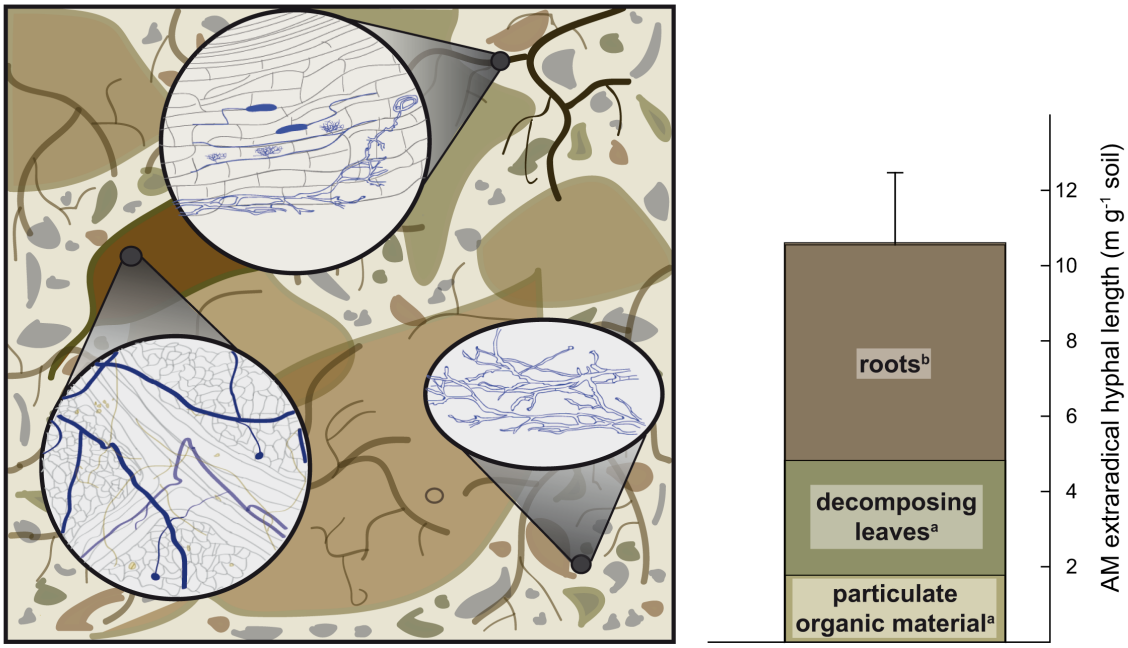
Chapter 4

Opposing effects of nitrogen versus phosphorus additions on mycorrhizal fungal abundance along an elevational gradient in tropical montane forests

http://dx.doi.org/10.1016/j.soilbio.2015.11.011

Chapter 5

Extraradical arbuscular mycorrhizal fungal hyphae in an organic tropical montane forest soil



Graphical abstract: Sketch of the organic layer in a tropical montane forest soil, consisting of roots, decomposing leaves and particulate organic material. The ramification of arbuscular mycorrhizal extraradical hyphae is illustrated. Bar represents the mean, error bar s.e.m. (linear-mixed effect model ($P < 0.05$) including the respective Sample ID as random effect).

http://dx.doi.org/10.1016/j.soilbio.2013.04.011

Abstract

Previous research from the tropics indicates that AMF may be well adapted to organic soils and even represent the dominant mycorrhizal form, though the extraradical part of the symbiosis was omitted as in most other tropical studies. Our study aims at characterizing the extraradical part of arbuscular mycorrhizal fungi (AMF) in a highly organic tropical montane forest soil in Southern Ecuador. Based on recent studies on the interaction of AM fungal hyphae and litter we hypothesized that within the organic layer AM hyphae grow in close contact with decomposing material. To test this idea, AM fungal hyphal distribution in the organic layer was determined by directly staining roots and decomposing leaves and extracting hyphae from the remaining particulate organic material. AM and non-AM fungal hyphae were analyzed, as well as root colonization patterns. Our results showed that AMF

indeed represented the dominant mycorrhizal form with an average root colonization of 43%. The extraradical AM hyphal length ranged from 2 – 34 m g^{-1} soil with a mean of 10.4 m g^{-1} soil (equals 3.1 m cm^{-3} soil), and therefore exceeded root length about 13-fold. As hypothesized, 29% of AM extraradical hyphae were closely attached to decomposing leaves. These hyphae were mainly located at the leaf surface, though in some parts leaf veins and inner leaf tissues were colonized. More than half of AM hyphal biomass was detected on the root surface, a pattern potentially driven by the predominant Paris-type AMF. Non-AM fungal hyphae colonized decomposing material to a signifcantly greater extent, though hyphal length attached to roots was equal. This study supports the adaptation of AMF to highly organic soils in the tropics and the existence of a widespread extraradical mycelium, which is not readily detectable by standard methods. The close association with decomposing leaves most likely improves direct nutrient uptake from decomposed material and points to a potential indirect contribution of AMF to the decomposition process.

Introduction

Arbuscular mycorrhizal fungi (AMF) explore the soil with their extraradical mycelium, transferring nutrients to the plant via specialized intraradical structures (Smith & Read, 2008). Generally, beside other functions, such as pathogen protection and soil stabilization, the uptake of mineral nutrients as phosphorus (P), nitrogen (N) and various micro-nutrients is regarded the main benefit to the plant (Johnson, 2010).

AMF are associated with more than 80% of land plants and occur globally in most terrestrial ecosystems (Treseder & Cross, 2006). However, in contrast to the hypothesis proposed by Read (1991) - limiting the dominance of AMF to ecosystems characterized by “mineral soils with lower altitude” - several studies performed in nutrient-poor organic soils in the tropics have shown that AMF represent in fact the dominant mycorrhizal form (Béreau *et al.*, 1997; Moyersoen *et al.*, 2001; Kottke *et al.*, 2004), even up to high altitudes of the Páramo vegetation (Barnola & Montilla, 1997; Aristizabal *et al.*, 2004; Camenzind, unpublished data). Likewise, a direct competition with ectomycorrhiza for the same niche was reported (Moyersoen *et al.*, 1998b). Apart from the work of Aristizabal *et al.* (2004), these studies employed AMF root colonization as a measure of abundance, neglecting the extraradical phase and thus an important functional part of these fungi and the symbiosis (Leake *et al.*, 2004). This omission is likely related to methodological constraints. Only some early studies attempted a functional appreciation of mycorrhizal hyphae in tropical soils:

Went and Stark (1968) proposed “direct nutrient cycling” based on the simple observation that in tropical forests a dense network of hyphae covers the decomposing litter layer, which is in close contact to roots colonized by “endotrophic mycorrhiza”. They hypothesized that a hyphal connection between decomposing leaves and roots directly cycles mineralized nutrients back to the plant. Herrera *et al.* (1978) supported the idea by morphological analyses of root and litter samples collected in the Amazon basin. They described “hyphae bridges” as a direct connection between decomposing leaves and roots and even reported a direct transfer of labeled ^{32}P. However, the respective fungal group was simply specified as “numerous septate fungal hyphae bridges”, and thus not necessarily restricted to AMF. Only recently, this idea was picked up and decomposing leaves from the litter layer collected at different sites in Colombian tropical forests were stained and analyzed (Aristizabal *et al.*, 2004; Posada *et al.*, 2012). These authors reported extensive litter colonization by AM fungal hyphae comparable to the amount of saprobic fungi (specified as aseptate hyphae), especially in highly decomposed material with easily accessible nutrients, located in close contact to roots.

Based on these findings we here aimed at identifying and quantifying the extraradical mycorrhizal fungal phase in a tropical forest soil characterized by a thick organic layer, typical of tropical montane forests (Grieve *et al.*, 1990; Wilcke *et al.*, 2008). The study area was previously characterized to be dominated by arbuscular myorrhizae (Kottke *et al.*, 2004) and high rates of AM root colonization (Homeier *et al.*, 2012) suggesting a high contribution of AM hyphae to soil fungal biomass (Leake *et al.*, 2004). However, this hyphal biomass is only detectable by methods adapted to leaf litter samples, whereas standard hyphal extraction protocols designed for mineral soils are ineffective. These findings lead us to the hypothesis that within the thick organic soil layer the majority of extraradical AM hyphae is closely attached to decomposing leaves, similar to previous findings in litter of certain plant species (Aristizabal *et al.*, 2004; Posada *et al.*, 2012). To test this hypothesis, hyphal length was quantified across soil depths by a direct observation of hyphae attached to every single soil fraction (leaf litter, root surfaces, and remaining particulate organic material). Additionally, the distribution of non-AM fungal hyphae in the substrate was evaluated, which represent other fungal groups, such as saprobes.

Materials and Methods

Study area

The study site of the Reserva Biologica San Francisco, bordering the Podocarpus National Park, is located in the Cordillera Real, an eastern range of the South Ecuadorian Andes (Beck *et al.*, 2008). This area represents a hotspot of biodiversity, with more than 280 tree species described, with Lauraceae, Melastomaceae and Rubiaceae as the dominant plant families. *Graffenrieda emarginata* Triana (Melastomaceae) is the most abundant tree species at the site. The sampling site is located at 2030 – 2120 m a.s.l. (3°59`S, 79°05`W). The vegetation type is an evergreen lower montane forest (Homeier *et al.*, 2008). The climate is warm humid with an average annual temperature of 15.2°C at 1950 m and an annual precipitation of approx. 2600 mm. Precipitation is particularly high from April to September without a pronounced dry season. The soil is a Stagnic Cambisol (IUSS Working Group WRB 2007) with a thick organic layer up to 35 cm (Wullaert *et al.*, 2010). The organic layer is divided into three horizons: The Oi horizon consisting of fresh litter, the Oe horizon of fragmented litter and the Oa horizon of humified material of un-recognizable plant origin. The pH of the organic layer ranges from 3.8 – 5.0, C/N ratio from 23.6 ± 1.3 in the Oa horizon to 34.0 ± 3.2 in the Oi horizon (Wullaert *et al.*, 2010).

Sampling design

In October 2010, six soil samples were taken with a soil corer (3 cm diameter, 15 cm in depth). Three sampling sites were randomly chosen at 2050 m, the other half at 2100 m, at both altitudes within an area of approx. 800 m² in the undisturbed forest. Every soil core was divided into three soil samples from different depth layers (upper: 0-5 cm; middle: 5-10 cm; lower: 10-15 cm). These layers do not correspond to O-horizons of the organic layer. Soil samples were oven-dried at 40°C.

Determination of soil fractions

The proportion of leaf and root weight as well as the respective surface area in the soil profile was determined (see below). The remaining material of non-recognizable plant origin was hereafter referred to as “particulate organic material”. Using forceps we picked all recognizable pieces of leaves and roots (regardless of size) out of each 5 g soil sample. The dry weight of roots and leaves was determined and the respective surface area ($cm^2\ g^{-1}$ soil) as well as root length ($cm\ g^{-1}$ soil) analyzed with WinRhizo (version 2007, Regent Instrument Inc., Quebec,

Canada). Results were expressed on a soil weight basis. The (dry) bulk density of the organic layer at the study site was 0.31 ± 0.03 (mean ± standard error) g cm^{-3} soil.

Method optimization

In the course of optimizing morphological methods suitable for the quantification of extraradical hyphal biomass, two different protocols were tested. Results and detailed methodological descriptions are provided in the supplementary materials. AMF hyphae were identified by the following criteria: Non-regularly septate hyphae with characteristic unilateral angular projections, that are stained dark- to light-blue by Trypan Blue (Mosse, 1959). All other fungal hyphae were referred to as non-AMF, since there is no reliable further morphological differentiation of fungal groups.

The first protocol tested was an aqueous filtration extraction method slightly modified from Bardgett (1991). The second protocol was based on a method originally described for hyphal quantification in tropical leaf litter (Herrera *et al.*, 1986; Aristizabal *et al.*, 2004). Considerable modifications were applied in order to meet the requirements of an accepted morphological differentiation of AM and non-AM hyphae: Besides the prevention of severe hyphal losses through the 40 µm sieve, a staining step with Trypan Blue was inserted and hyphae were quantified at 200x magnification instead of 100x.

Hyphal quantification in single soil fractions

Testing the hypothesis that extraradical AMF hyphae are attached to decomposing leaves, the amount of hyphae attached to every soil fraction (leaf litter, root surfaces and particulate organic material) was determined separately.

Roots

Roots were stained with a modified staining protocol of Phillips & Hayman (1970). 1-2 cm root pieces were cleared in 10% KOH at 60°C for 1-2 days, bleached in 20% H_2O_2 for 20-30 min. at RT, acidified in 1M HCl and stained for 1.5 – 2.5 hours in 0.05% Trypan Blue at 60°C. The duration of staining depended upon the respective root diameter. Root colonization was counted at 200x magnification using the line-intersect method described by McGonigle et al. (1990). For the calculation of extraradical hyphae attached to the root surface, half of one line incorporated into the microscopic lens was projected upon the stained root, consistently extending from the vascular tissue into the root cortex. The number of hyphae crossing was

counted and incorporated into Newman´s (1966) formula, inserting "root surface (mm²)" as *A*:

$R = ((\pi NA) / (2H)) \times D \times$ efficiency factor x (weight of soil expressed per g) (1)

(*R* = hyphal length (mm (g^{-1} soil)); *N* = Number of hyphal intersects; *A* = Area of the filter paper (mm²); *H* = length of lines (mm); *D* = dilution factor).

Decomposing leaves

Leaves were stained with Trypan Blue using the same protocol as described for roots, with slightly modified staining times: 1day 10% KOH, 20 min. 20% H_2O_2 and 5 hrs. 0.05% Trypan Blue. Depending on the amount of available leaf material, up to four slides per sample were produced, resulting in an average of 167 ± 26 intersects per sample. Again, the number of hyphae crossing one line incorporated into the microscopic lens was counted and the total hyphal length calculated with Newman´s formula (equation 1), taking leaf surface area as the respective Area (*A*).

Particulate organic material

To quantify hyphal length in the remaining particulate organic material, the modified aqueous filtration extraction method described above was used. The efficiency factor was estimated for four samples, including hyphae lost during the sieving step (Miller *et al.*, 1995) as well as non-extractable hyphae still attached to the soil. Hyphal length on the latter fraction was estimated by adding Trypan Blue to decanted soil subsamples (about 1 g) and counting the number of hyphal fragments of approx. 0.5 mm length within 27 defined square plots (11 mm²). The quotient of AM to non-AM hyphae was adjusted according to its proportion determined on the filter paper.

Statistical analyses

For the calculation of differences in the response variables across the depth profile, linear mixed effect models (lmer) including "Sample" as a random effect were run in R (vers. 2.15.0, R Development Core Team, 2011) using the package "lme4" (Bates *et al.*, 2012). Corresponding p-values were calculated using the function "cftest" included in the package "multcomp" (Hothorn *et al.*, 2008; Everitt & Hothorn, 2010). All models were tested for the underlying assumptions of normality and homogeneity. In case these were not met, data were log or square root transformed.

In order to compare the amount of hyphae attached to leaves, roots and particulate organic material, mean values of every soil core were used (without separation into different depth

layers). Again, a linear mixed effect model was run with “Sample” as random effect. The same was done to compare the density of AM and non-AM hyphae attached to the leaf and root surface, respectively.

For linear correlations between response variables the impact of the respective depth layer as well as the sample affiliation was included in the model. Therefore, an ancova including depth as categorical independent variable was performed, additionally including “Sample” as random effect.

Results

Composition of the organic layer

The organic layer at the sampling site consisted on average of 12.1 ± 2 % by weight of roots and 8.6 ± 2 % by weight of leaf litter. The remaining soil refers to particulate organic material of non-recognizable plant origin. Root surface (12.31 ± 1.5 $cm^2\ g^{-1}$ soil) formed a larger proportion of “hyphal accessible soil surface” than leaves (9.91 ± 2.9 $cm^2\ g^{-1}$ soil).

However, leaf litter mainly accumulated in the upper layer, which includes the Oi horizon consisting of fresh litter: 76% of the observed leaf material was accumulated here (significant differences to middle and lower layer: $P < 0.001$). In contrast to leaves, root length was highest in the middle layer, though these differences were only significant compared to the lower layer ($P < 0.001$) (Table 5.1).

Intraradical AM root colonization

AM intraradical root colonization was on average 43%, ranging from 6 – 64%. The morphological characteristic observed was mainly Paris-type AMF, though Arum-type as well as intermediate forms occurred as well. In detail, coils were the most frequently counted structure (60%) (Fig. 5.1A) compared to a lower amount of intercellular hyphae (43%) and only few arbuscules (7%) and vesicles (9%).

Intraradical fungal structures other than AMF were rarely observed, never exceeding a colonization rate of 10%, although the root surface was continuously covered with brownish and aseptate fungi. These few observations mainly resembled either ectomycorrhizal Hartig net structures or microsclerotia of dark septate endophytes. In some cases, single septate hyphae were found within intercellular spaces.

Table 5.1 Parameters observed in the organic layer of a montane tropical forest in southern Ecuador across its depth profile (linear-mixed effect models, $P < 0.05$).

	Composition of the organic layer		AM fungi					Non-AM fungi		
Depth	Leaf weight (mg g^{-1} soil)	Root length (cm g^{-1} soil)	Root colonization (%)	Colonized root length (cm g^{-1} soil)	Total hyphal length (m g^{-1} soil)	Density on root surface (mm cm^{-2} root)	Density on leaf surface (mm cm^{-2} leaf)	Total hyphal length (m g^{-1} soil)	Density on root surface (mm cm^{-2} root)	Density on leaf surface (mm cm^{-2} leaf)
upper layer (0-5 cm)	186 ± 43^{a}	68 ± 12^{ab}	37 ± 12^{a}	31 ± 11^{ab}	16 ± 4^{a}	439 ± 111^{a}	263 ± 54^{a}	27 ± 9^{a}	585 ± 66^{a}	587 ± 102^{ab}
middle layer (5-10 cm)	16 ± 8^{b}	88 ± 11^{a}	46 ± 5^{a}	43 ± 10^{a}	10 ± 4^{ab}	376 ± 109^{a}	443 ± 150^{a}	16 ± 4^{b}	576 ± 109^{a}	509 ± 135^{b}
lower layer (10-15 cm)	57 ± 30^{b}	44 ± 10^{b}	46 ± 3^{a}	20 ± 4b	7 ± 2^{b}	349 ± 68^{a}	300 ± 61^{a}	14 ± 2^{b}	461 ± 74^{a}	686 ± 181^{a}

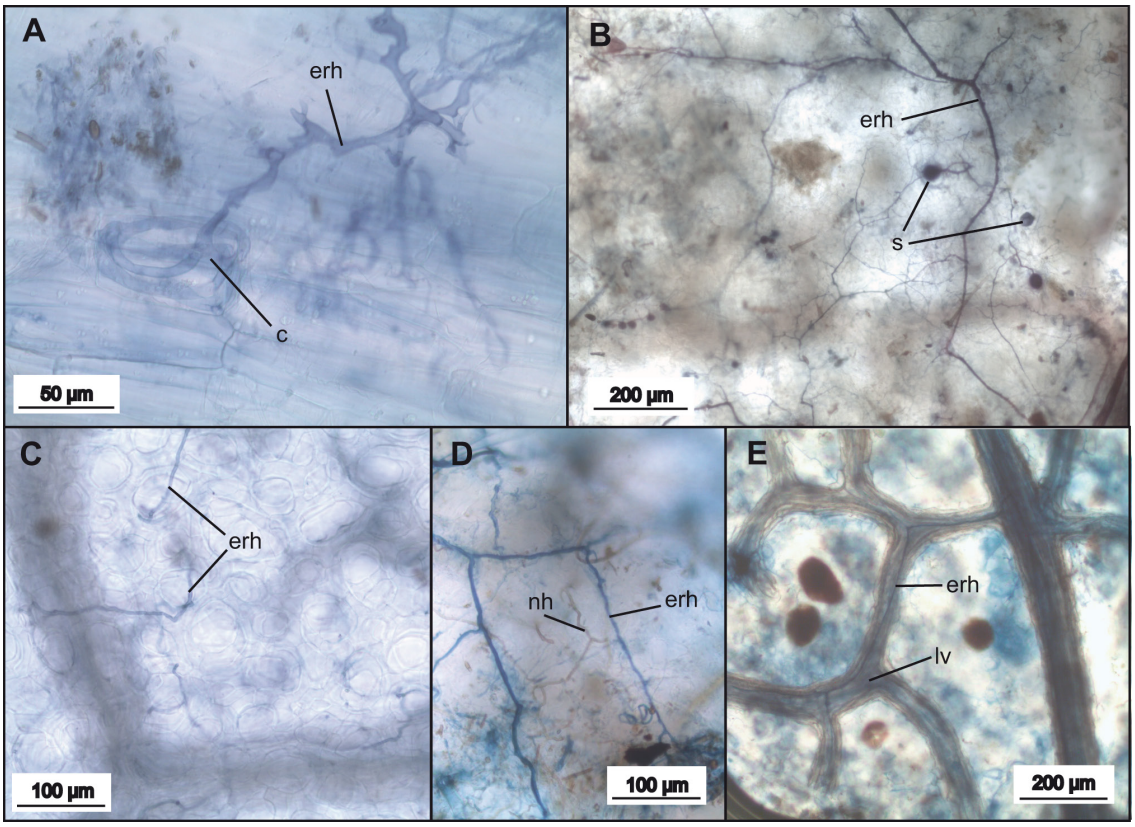


Fig. 5.1 Arbuscular mycorrhizal (AM) hyphae associated with roots and decomposing leaves. Staining was done with Trypan Blue. Photographs were taken with a microscopic camera (DFC290, Leica Microsystems, Wetzlar, Germany). A: Extraradical AM hypha at the root surface, invading the root cortex and forming an intracellular coil. B-E Fungal hyphae attached to decomposing leaves. B: AM hyphae and spores, C: AM hyphae apparently invading inner leaf tissues, D: septate brown non-AM hyphae and AM hyphae, E: AM hyphae within leaf veins. *Erh* extraradical AM hyphae, *c* coil, *s* spores, *nh* non-AM hyphae, *lv* leaf vein.

AM leaf litter colonization

On average, 29% of AM hyphae in the soil were attached to the surface of decomposing leaves (Fig. 5.2), in the upper layer this proportion even rose up to 73%. AM hyphae were mainly loosely attached to the leaf surface, though in some cases thin hyphae invaded the leaves subsequently following intercellular spaces (Fig. 5.1B, C, D). Spores were found in many cases (Fig. 5.1B), however, no vesicles or arbuscule-like structures. In a few samples, AM hyphae were observed within leaf veins, with hyphae spreading from there into the surrounding leaf tissues (Fig. 5.1E).

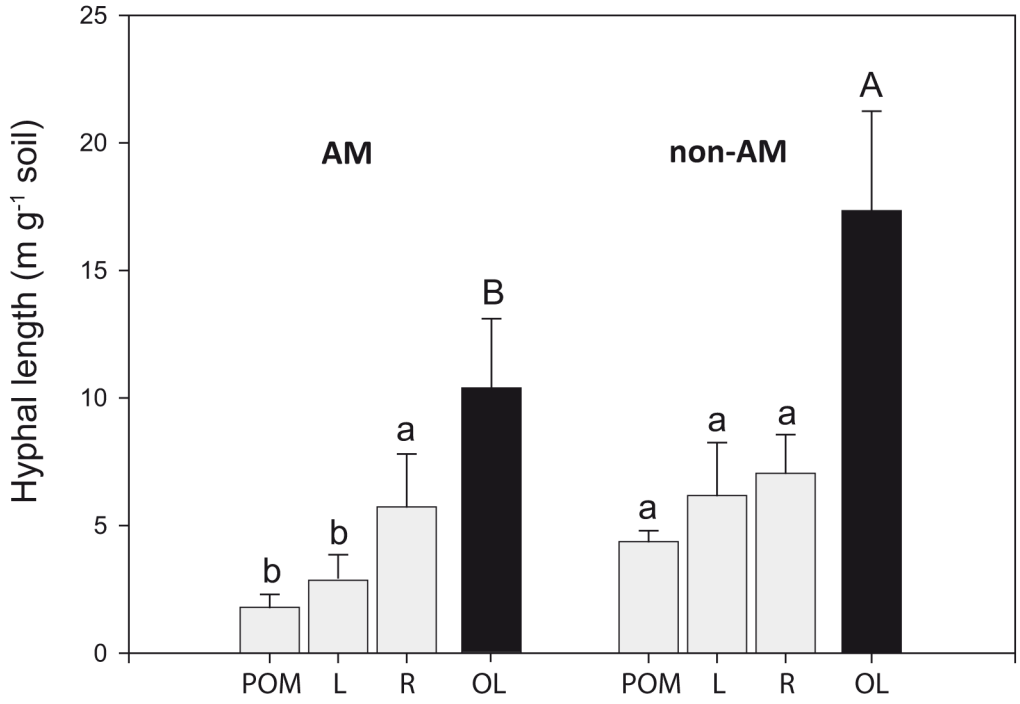


Fig. 5.2 Distribution of arbuscular mycorrhizal (AM) and non-AM hyphae in the organic layer (hyphae attached to every soil fraction: particulate organic matter (POM), leaves (L), roots (R); and the total amount of hyphae in the organic layer (OL)) . Mean values are presented, error bars show s.e.m. Lowercase letters indicate significant differences among groups and substrates; capital letters show differences in total hyphal length (linear-mixed effect models, $P < 0.05$).

Hyphal biomass partitioning in the organic layer

Summing hyphae quantified for every soil fraction, the amount of non-AM fungal hyphae in the organic layer was significantly higher than the amount of AM fungal hyphae ($P < 0.001$) (Fig. 5.2). Non-AM fungal hyphae were more evenly distributed across the soil fractions than AM hyphae (Fig. 5.2). On average, more than half (55%) of AM fungal hyphae were attached to the root surface. Comparing the extra- and intraradical phase of AM hyphae, hyphal length values in soil were significantly correlated with the colonized root length by AMF ($P = 0.013$) (Fig. S5.2).

Hyphal length values were significantly correlated with the amount of leaves and roots in the samples ($P < 0.05$). In order to exclude the confounding factor of substrate composition, hyphal densities on root and leaf surfaces were evaluated. Hyphal densities on leaf and root surfaces revealed an equal amount of hyphae attached to root and leaf surfaces. However, the density of non-AM fungal hyphae attached to root and leaf surfaces was higher than that of AMF (leaf surface: $P = 0.036$; root surface: $P = 0.07$) (Table 5.1).

In the depth profile both AM and non-AM fungal hyphae significantly decreased from the upper to the lower layer (Table 5.1). However, this pattern was mainly driven by a shift in the composition of the organic layer, since hyphal density per fraction remained similar. In fact, the density of non-AM hyphae attached to decomposing leaves was even highest in the lowest soil layer (Table 5.1).

The total amount of AM and non-AM fungal hyphae in the organic layer was significantly correlated (Fig. S5.3). Even by the removal of a value with high leverage the correlation remained significant ($P < 0.001$). However, both values were highly correlated with leaf weight as well as root length in the respective sample ($P < 0.001$). Consequently, eliminating the impact of leaf and root biomass on the analysis, the strong correlation disappeared: the density of AM and non-AM hyphae attached to leaves was independent ($P = 0.14$). Only on the root surface there appeared to be a linked occurrence of AM and non-AM fungal hyphae ($P = 0.01$).

Discussion

Our study fails to support the hypothesis that arbuscular mycorrhizal fungi are not well adapted to highly organic soils (Read, 1991; Brundrett & Abbott, 1995), which is in line with previous research from tropical forest soils characterized by a thick organic layer (Béreau *et al.*, 1997; Moyersoen *et al.*, 2001; Kottke *et al.*, 2004) and a global comparison across biomes (Treseder & Cross, 2006). Additionally, we provide evidence that the extraradical mycelium of AM fungi in tropical montane soils represents a substantial part of soil fungal mycelium, exceeding root length about 13-fold, with values of up to 25m cm^{-3} soil (Fig. S5.1). So far, in tropical forests only very few studies have dealt with the extraradical part of the symbiosis, in contrast to the comparatively much better documented intraradical predominance of AMF (Alexander & Lee, 2005) with typically high root colonization rates (e.g. Louis & Lim, 1987; Shi *et al.*, 2006; Becerra *et al.*, 2007; Zangaro *et al.*, 2012). Also, the various studies done in Colombian tropical forests (Aristizabal *et al.*, 2004 and references therein; Posada *et al.*, 2012) focus exclusively on AM hyphae attached to litter samples and give no data on AM hyphae in the soil. This data gap may give rise to misleading conclusions, since studies in temperate regions show that extraradical hyphae represent a large amount of microbial biomass in the soil (Leake *et al.*, 2004). Comparing across biomes, the highest values of AM fungal hyphal length are found in temperate grasslands (up to 90 m cm^{-3} soil) (Miller *et al.*, 1995; Rillig *et al.*, 1999; Rillig *et al.*, 2002; Wilson *et al.*, 2009). In contrast,

the few observations available from tropical forests yielded rather low values. Balser *et al.* (2005) and Guadarrama *et al.* (2008) report on average 4 and 15 m g^{-1} soil from Hawaiian and Mexican tropical forests, respectively. The comparison of Powers *et al.* (2005) of AM hyphal length at four tropical sites across South and Central America remains even below 0.2 m g^{-1} soil. The comparatively high amounts of hyphal biomass presented here may on the one hand result from the optimized quantification protocol, on the other hand hyphal length has never been quantified in the organic layer of tropical soils and might simply exceed values from mineral soils. This conclusion receives additional support by hyphal length values given for litter layers, which are comparatively high (Aristizabal *et al.*, 2004; Posada *et al.*, 2012).

Methodological approach

Generally, the comparison of values presented in different studies is rather difficult due to values mainly expressed by soil weight, taking into account a highly variable soil bulk density (Brady, 1984). Additionally, as shown in this study, the hyphal quantification method applied highly affects the result. Here we found that the widely used aqueous filtration extraction method greatly underestimates the extraradical hyphal length as has been reported before (Balser *et al.*, 2005). The calculated (pseudo-)efficiency factor indicates that the amount of extractable hyphae is about 13% for AMF and 30% for non-AMF (for this specific soil). The underlying mechanism of hyphal detachment obviously is ineffective in an organic soil layer with hyphae closely attached to decomposing leaves and roots. In contrast, a modified protocol based on Aristizábal *et al.* (2004), originally applied to tropical leaf litter, appears to be more suitable. Based on direct staining of the substrate high values of hyphal length were obtained, suggesting even higher extraradical hyphal length than presented above (Fig. S5.1).

Generally, hyphal quantification methods based on morphological identification require extensive research experience and remain to a certain amount subjective (Stahl *et al.*, 1995; Smith & Read, 2008). Therefore, we tried to reduce the risk of misidentification by analyzing AMF inside and attached to roots first, since here the direct connection of easily-recognizable intraradical AMF structures with extraradical hyphae facilitates the differentiation of AM and non-AM fungal hyphae. However, the risk of including dead hyphae in the analysis remains (Sylvia, 1992; Staddon *et al.*, 2003). Besides these constraints, morphological methods are primarily applied in AMF research and will remain an important technique, since alternatives, e.g. NLFA analysis (neutral lipid fatty acids quantification),

also have drawbacks. The method provides a reliable AMF marker and eliminates the bias by the incorporation of dead hyphae, however, the correlation of the quantified storage lipids either to spore or hyphal abundance is variable and also depends on the substrate and fungal species involved (Olsson & Wilhelmsson, 2000; van Aarle & Olsson, 2003; Ngosong *et al.*, 2012).

AM hyphae attached to decomposing leaves

As hypothesized, a large amount of extraradical AM hyphae were in fact attached to decomposing leaves. This phenomenon has so far only been described for leaf litter of distinct plant species from tropical forests in Colombia (reviewed in Aristizabal *et al.*, 2004). In line with these findings, several experimental studies report an enhanced proliferation of AMF following the addition of organic material (Ravnskov *et al.*, 1999; Albertsen *et al.*, 2006; Gryndler *et al.*, 2009) as well as a generally intimate contact with SOM particles (St. John *et al.*, 1983; Hodge & Fitter, 2010). The close association of AM hyphae with decomposing leaves and the partly direct colonization of inner leaf tissues points to an either direct or indirect role in the decomposition process. However, without further physiological analyses of the actual nutrient pathways, no definitive conclusions are possible. Nevertheless, several authors have reported enhanced decomposition in the presence of AM fungal hyphae (Hodge *et al.*, 2001; Atul-Nayyar *et al.*, 2009) and N and P transfer to the plant originating from organic residues (Koide & Kabir, 2000; Hodge *et al.*, 2001; Cheng & Baumgartner, 2006; Kahkola *et al.*, 2012). An indirect contribution of AMF by interactions with the microbial community and enhanced delivery of root exudates appears most likely (Andrade *et al.*, 1997; Marschner *et al.*, 2001; Wamberg *et al.*, 2003; Herman *et al.*, 2012).

AM hyphae attached to the root surface

Generally, AM extraradical hyphae are regarded as an extension of the root surface in order to improve nutrient uptake capacities beyond the root depletion zones (Smith & Read, 2008). Therefore, our findings that more than half of AM hyphal biomass is attached to root surfaces appears rather contradictory. However, similar characteristics were reported by Beck *et al.* (2007), who described in detail the distinctive characters of *„prominent supraradical mycelia“* attached *„in large amounts“* to tree roots sampled in the same area; however, this has never been quantified. From a morphological point of view, this pattern is most likely attributed to the root colonization trait observed here, predominantly assigned to Paris-type AMF (Gallaud, 1905; Smith & Smith, 1997). In contrast to Arum-type, the intraradical spreading of AM hyphae occurs from one cell to the next instead of hyphae directly growing within intercellular spaces. Thus, subsequent colonization of distant parts of the root are presumably rather accessed by

extraradical hyphae. In the functional context, this patterns appears to be counterintuitive in terms of nutrient uptake, however, other functional traits of the AM symbiosis, e.g. pathogen protection (Sikes *et al.*, 2009), may play an important role. Current knowledge on the functional role of AMF in this tropical soil remains vague, however, a worthwhile topic for further exploration.

Conclusions

In summary, we are able to show that AMF form a high extraradical biomass within the organic layer of a tropical montane soil and associate intimately with decomposing material. This study provides a basic morphological quantification method for hyphae in highly organic soils and highlights the problems of applying standardized methods across biomes. The direct association of AMF with organic material most likely points to an indirect contribution to decomposition as indicated by our findings and previous research in this field. However, experimental manipulations of the system and observations of nutrient flows are necessary to test these ideas.

Acknowledgements

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Chapter 6

General Discussion

AMF in the study system

The present work highlights several aspects of AMF in a tropical montane forest, elucidated by a combination of observational and experimental approaches. Beside the direct outcome of the nutrient manipulation experiment, interesting general patterns of AMF morphology and diversity emerged related to this complex ecosystem. First of all, we confirmed that AMF indeed represent the dominant mycorrhizal type in the study area, which has previously been reported by Kottke *et al.* (2004) at 2000m. This is in line with findings from other tropical forests (Janos, 1980a; Averill *et al.*, 2014), also from tropical mountain sites (Barnola & Montilla, 1997; Schmidt *et al.*, 2008), again disproving Read´s paradigm (1991) on the preferential occurrence of AMF in mineral soils of lower altitudes. In this context, we even found an increase towards higher altitudes in AMF root colonization rates as well as AMF extraradical biomass. This altitudinal shift in AMF and also root length is most likely related to decreasing nutrient availability with elevation, as predicted by the functional equilibrium model (Bloom *et al.*, 1985; Leuschner *et al.*, 2007; Johnson, 2010). AMF diversity was evaluated at 2000m: the deep sequencing approach revealed a high diversity with a total of 74 OTUs. Compared to richness estimates provided by temperate studies with comparable designs, the observed number of OTUs was relatively high but still within the upper range (Öpik *et al.*, 2009; Lumini *et al.*, 2010; Dumbrell *et al.*, 2011). The same pattern was found by Haug *et al.* (2013) using cloning and sequencing techniques in this area. Nevertheless, a sampling design including variation throughout the year as well as a broader geographic range might reveal even higher OUT numbers (Dumbrell *et al.*, 2011; Moora *et al.*, 2011). Interestingly, a large proportion of OTUs belonged to the Diversisporales, whereas in most molecular community analyses Glomerales clearly dominate AMF communities (e.g. Öpik *et al.*, 2010; Schnoor *et al.*, 2011; Lekberg *et al.*, 2013). However, spore surveys from other tropical regions likewise show high abundances especially of the genus *Acaulospora* (Lovelock *et al.*, 2003; Stürmer & Siqueira, 2011; reviewed in Leal *et al.*, 2013), which has been proposed to be favored by acidic soil conditions (Porter *et al.*, 1987; Veresoglou *et al.*, 2013). Focusing on AMF morphology, within the roots we found mainly Paris type AMF as previously reported from other tropical forests (Torti *et al.*, 1997; Whitbeck, 2001), though this pattern

is also common in temperate areas (Cavagnaro *et al.*, 2003). Typically the observed type of AMF was related to root characteristics: thin roots mostly were solely colonized by coils, whereas thicker roots - depending on root cellular structures - either solely harbored coils or additionally within the inner cortical cells intercellular hyphae and arbuscules. Interestingly, intraradical spores, characteristic for the worldwide occurring *Rhizophagus intraradices* but also other species, were not found (Stockinger *et al.*, 2009; Öpik *et al.*, 2010). By contrast, the overall extraradical spore abundance was exceptionally high, especially in comparison to lowland tropical habitats (Lovelock *et al.*, 2003; Chubo *et al.*, 2009; Leal *et al.*, 2013; Freitas *et al.*, 2014), though Soteras *et al.* (2015) and Barnola and Montilla (1997) give comparable numbers for tropical montane forests. Concerning the extraradical AMF mycelium, in the tropical literature only few hyphal length descriptions can be found with rather low values (Balser *et al.*, 2005; Powers *et al.*, 2005; Guadarrama *et al.*, 2008), which reflects the generally scarce incorporation of this part of the symbiosis in analyses (Leake *et al.*, 2004). Hyphal length values observed in mineral soils at 1000m were similarly low, whereas values calculated for the organic layer at 2000m were much higher, fitting within the range of the highest estimates typically found in grasslands (Miller *et al.*, 1995; Rillig *et al.*, 1999; Leake *et al.*, 2004). This adaptation of AMF to organic soils has been indicated by other studies, though the extraradical phase has so far been neglected (Béreau *et al.*, 1997; Moyersoen *et al.*, 2001). Here, a detailed description of hyphal distribution revealed a close contact of AMF hyphae with decomposing leaves, as has been previously described from litter layers of Colombian rainforests (Aristizabal *et al.*, 2004; Posada *et al.*, 2012). These findings point towards an indirect impact of AMF on decomposition processes and the rapid uptake of mineralized nutrients from organic material (Atul-Nayyar *et al.*, 2009; Nuccio *et al.*, 2013). The majority of hyphae, however, was found on root surfaces, as also described by Beck *et al.* (2007) in this region: this pattern is unusual and the physiological function of hyphae in close root association is unclear. Potentially, this is primarily related to the formation of Paris type AMF which is characterized by the lack of intercellular growth (Smith & Smith, 1997). In general, working in such a special study system with little prior knowledge uncovers interesting new insights on AMF, but also requires the development of new or adopted methodologies. Standard morphological methods on hyphal and spore extractions were shown to be insufficient , especially when examining the organic layer Furthermore, the staining of dark and rather thick tree roots was challenging and needed to be refined. Hence, as part of this work new methods were elaborated and all applied methods tested thoroughly.

Responses in AMF abundance to nutrient additions

Liebig´s law of the minimum states that plant productivity is restricted by the most limiting nutrient. Tropical forests have been shown to be primarily limited by N and P, though co-limitation by several nutrients also occurs (Vitousek *et al.*, 2010; Santiago *et al.*, 2012). A direct proof which nutrient is limiting in a system can only be obtained by experimental nutrient additions (Tanner *et al.*, 1998): in the Ecuadorian NUMEX project already short-term effects after one year indicated a co-limitation of N and P at 2000m (see Chapter 2, Wullaert *et al.*, 2010), which was confirmed by data gathered in the following years (Homeier, personal communication). Along the altitudinal gradient, at 1000m nutrient addition effects on plant growth and plant nutrient cycling also indicate N/P co-limitation, whereas at 3000m N appears to be the limiting factor (Homeier, 2013). These findings are further supported by leaf N:P ratios found along the gradient (Gusewell, 2004; Homeier, personal communication).

Shifts in AMF abundance along the altitudinal gradient did not match predictions made based on this gradient, especially since no effects were found at 3000m, a site characterized by strong nutrient limitations accompanied by high AMF root colonization. Here, most likely moderate nutrient amendments did not compensate for nutrient limitation, either of the plant or the fungus (Treseder & Allen, 2002), or other nutrients play an important role (Krashevska *et al.*, 2010; Santiago *et al.*, 2012). However, concurrent responses at 1000 and 2000m match the assumption of similar limitations at these sites, though effects were much more pronounced at 2000m. This site is characterized by lower nutrient availability parallel to higher AMF abundance, which indicates higher AMF dependency of the system (Treseder, 2013) potentially causing increased sensitivity of the symbiosis to alterations in nutrient availability. Since AMF show particular adaptations to the organic layer at 2000m, the mechanism behind these effects might differ among sites.

The overall effect of increases in AMF abundance following P additions in contrast to decreases following N additions contradicts previous findings (Treseder, 2004). In general, the direction of responses to nutrient additions was stable among sampling times but also regarding the extra- and intraradical phase, giving strong support to the detected results. These effects can be interpreted in two ways: from a plant perspective according to the functional equilibrium model (Johnson *et al.*, 2003; Johnson, 2010) or from the point of view of direct fungal nutrient limitations (Treseder & Allen, 2002). Additionally, since our knowledge on this ecosystem is scarce unknown influential

parameters might exist. However, the detailed evaluation of results including responses in root length, effects on differential AMF structures and a comparison of extra- versus intraradical AMF changes allow the preliminary conclusion that direct P-limitation of the fungus is most likely, which can be tested in further experimental settings. At the 2000m site the highest resolution was achieved by the incorporation of a whole time series. Interestingly, negative responses in AMF root colonization to N additions became apparent after two years, whereas the positive effect of P additions emerged later. The rapid effects of one-year nutrient additions described for other processes like plant growth, nutrient cycling but also microbial biomass and activity could not be observed in intraradical AMF abundance. Changes over time, which have been described also for other systems (see Treseder & Vitousek, 2001; Treseder & Allen, 2002), potentially illustrate strong interactions with other processes developing over time of soil microbes but also plants, e.g. shifts in the whole plant community (see Chapter 2; Homeier, 2013; Krashevska *et al.*, 2013). These shifts over time underline the necessity of long-term observations in ecological research (Müller *et al.*, 2010).

Responses in AMF molecular diversity and community composition in response to nutrient additions

From a methodological perspective, this study represents the first 454-pyrosequencing analysis in nutrient manipulation experiments (except for one agricultural study: Lin *et al.*, 2012). This method which enabled us to analyze AMF diversity in-depth and also to reveal effects on rare species (Öpik *et al.*, 2009). Distinct detrimental effects of all fertilization treatments were observed at 2000m on AMF richness, an effect which has been previously shown in fertilization studies in other ecosystems (Johnson, 1993; e.g. Egerton-Warburton *et al.*, 2007; Liu, YJ *et al.*, 2012). The main impact could be determined for rare species, which also may be relevant for ecosystem processes (Volkov *et al.*, 2003; Davies *et al.*, 2004). This loss in species numbers is not accompanied by decreasing AMF abundance. Therefore, it is most likely related to an increasing dominance of certain species favored by fertilized conditions (Eom *et al.*, 1999; Egerton-Warburton *et al.*, 2007), but potentially also a dropout of more “sensitive” ones (Johnson *et al.*, 2003). Multivariate analyses of AMF community composition, indicator species analysis and the detailed examination of phylogenetic groups revealed that indeed AMF community composition was affected especially by P additions. However, shifts rather occurred at the level of phylogenetic groups than at the

species level: Glomerales were negatively affected by P additions, whereas Diversisporales solely by N. To my knowledge such group-specific responses to nutrient additions or other environmental impacts have not been shown before, though certain traits in AMF indeed have been specified to be conserved at a group level (Powell *et al.*, 2009; Maherali & Klironomos, 2012). However, information on differential AMF traits is scarce, neither covering a large set of species nor most relevant traits which would allow further interpretations (Helgason *et al.*, 2002; Werner & Kiers, 2014). Additionally, in a tropical ecosystem traits and functionality might differ, but also the involved species pool since we found a large amount of OTUs which did not cluster with known sequences. Likewise, previous AMF community studies in the tropics reported a large amount of spore morphological types (Gavito *et al.*, 2008; Cuenca & Lovera, 2010; Stürmer & Siqueira, 2011) and OTUs (Husband *et al.*, 2002; Haug *et al.*, 2013) unassignable to known AMF species.

The direct and indirect mechanisms underlying the observed changes in AMF diversity and community composition but also in AMF abundance are difficult to disentangle, since ecosystem processes strongly interact in their response to nutrient additions (Liu, YJ *et al.*, 2012). As part of the NUMEX project effects on several ecosystem components were observed: dominant trees showed differential responses indicating plant community shifts (regarding seedling recruitment, tree growth and nutrient cycling; Homeier & Caraté, personal communication; Homeier, 2013), microbial biomass, activity and community composition was affected (see Chapter 2, Krashevska *et al.*, 2013) as well as soil nutrient cycles (Wullaert *et al.*, 2010; Martinson *et al.*, 2013; Velescu & Wilcke, personal communication). As a consequence, a great variety of complex interactions might occur. AMF community composition may directly influence AMF abundance, since AMF species differ in their extent of root and soil colonization (Helgason *et al.*, 2002; Maherali & Klironomos, 2012). Plant community structure may influence AMF abundance, since the dominant trees differ in AMF root colonization (Camenzind *et al.*, in preparation), but also potentially AMF community composition, in case host specificity plays a role in this system (Vandenkoornhuyse *et al.*, 2003; Hausmann & Hawkes, 2009; Haug *et al.*, 2013) and vice versa (van der Heijden *et al.*, 1998; Maherali & Klironomos, 2007; Wagg *et al.*, 2011). Finally, interactions with soil microbes may occur since AMF are known to affect microbial community composition and activity (Marschner *et al.*, 2001; Wamberg *et al.*, 2003; Nuccio *et al.*, 2013). These examples underscore the complexity of ecosystem processes but also the value of multidisciplinary experiments which allow the integration of single datasets into a larger context.

Conclusions

This work allows insights on AMF in a highly diverse and heterogeneous ecosystem that is so far understudied and thus starts to fill a gap in tropical research on AMF (Öpik *et al.*, 2010; Soudzilovskaia *et al.*, 2015). Especially in the organic layer at 2000m, some clear patterns were found regarding hyphal proliferation but also responses in abundance and community composition to N and P additions. These patterns point towards potential distinct limitations and functionalities of the symbiosis in this system, which might differ from temperate grasslands (the "standard" ecosystem for basic AMF research). This is further supported by the finding of a special AMF community structure dominated by so far unknown species (based on the LSU primer region). In this experimental setting the underlying mechanisms causing observed results can be discussed, though not clearly shown. Thus, further experimental designs are currently developed to test the proposed assumptions based on these results: experiments will focus on the role of AMF in decomposition processes and N uptake of plants, but also on differences in direct nutrient limitations of plants versus fungi along the altitudinal gradient. Nevertheless, this work reveals clear responses in AMF but also other ecosystem components to N and P additions, which is important in the context of expected nutrient depositions in the future, especially in the case of f N whose deposition rates in the study area has been shown to be higher than predicted (Galloway *et al.*, 2008; Wilcke *et al.*, 2013a; Wilcke *et al.*, 2013b).

References

Abbott LK, Robson AD, Deboer G. 1984. The effect of phosphorus on the formation of hyphae in soil by the vesicular arbuscular mycorrhizal fungus, *Glomus fasciculatum*. *New Phytologist* **97**: 437-446.

Adamek M, Corre MD, Hoelscher D. 2009. Early effect of elevated nitrogen input on above-ground net primary production of a lower montane rain forest, Panama. *Journal of Tropical Ecology* **25**: 637-647.

Aguilar-Fernandez M, Jaramillo VJ, Varela-Fregoso L, Gavito ME. 2009. Short-term consequences of slash-and-burn practices on the arbuscular mycorrhizal fungi of a tropical dry forest. *Mycorrhiza* **19**: 179-186.

Albertsen A, Ravnskov S, Green H, Jensen DF, Larsen J. 2006. Interactions between the external mycelium of the mycorrhizal fungus Glomus intraradices and other soil microorganisms as affected by organic matter. *Soil Biology & Biochemistry* **38**: 1008-1014.

Aldrich-Wolfe L. 2007. Distinct mycorrhizal communities on new and established hosts in a transitional tropical plant community. *Ecology* **88**: 559-566.

Alexander I, Selosse MA. 2009. Mycorrhizas in tropical forests: a neglected research imperative. *New Phytologist* **182**: 14-16.

Alexander IJ, Lee SS 2005. Mycorrhizas and ecosystem processes in tropical rain forest: implications for diversity. In: M. A. P. D.F. Burslem, S.E. Hartley ed. *Biotic interactions in the tropics: Their role in the maintenance of species diversity*. Cambridge: Cambridge University Press, 165-203.

Alguacil MD, Lozano Z, Campoy MJ, Roldan A. 2010. Phosphorus fertilisation management modifies the biodiversity of AM fungi in a tropical savanna forage system. *Soil Biology & Biochemistry* **42**: 1114-1122.

Allen EB, Allen ME, Egerton-Warburton L, Corkidi L, Gomez-Pompa A. 2003. Impacts of early- and late-seral mycorrhizae during restoration in seasonal tropical forest, Mexico. *Ecological Applications* **13**: 1701-1717.

Allen EB, Rincon E, Allen MF, Perez-Jimenez A, Huante P. 1998. Disturbance and seasonal dynamics of mycorrhizae in a tropical deciduous forest in Mexico. *Biotropica* **30**: 261-274.

Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**: 3389-3402.

Amend AS, Seifert KA, Bruns TD. 2010. Quantifying microbial communities with 454 pyrosequencing: does read abundance count? *Molecular Ecology* **19**: 5555-5565.

Anderson JPE, Domsch KH. 1978. Physiological method for quantitative measurement of microbial biomass in soils. *Soil Biology & Biochemistry* **10**: 215-221.

Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* **26**: 32-46.

Anderson RC, Liberta AE, Dickman LA. 1984. Interaction of vascular plants and vesicular-arbuscular mycorrhizal fungi across a soil moisture-nutrient gradient. *Oecologia* **64**: 111-117.

Andrade G, Mihara KL, Linderman RG, Bethlenfalvay GJ. 1997. Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. *Plant and Soil* **192**: 71-79.

Antoninka A, Reich PB, Johnson NC. 2011. Seven years of carbon dioxide enrichment, nitrogen fertilization and plant diversity influence arbuscular mycorrhizal fungi in a grassland ecosystem. *New Phytologist* **192**: 200-214.

Aristizabal C, Rivera EL, Janos DP. 2004. Arbuscular mycorrhizal fungi colonize decomposing leaves of Myrica parvifolia, M-pubescens and Paepalanthus sp. *Mycorrhiza* **14**: 221-228.

Arnold AE, Maynard Z, Gilbert GS, Coley PD, Kursar TA. 2000. Are tropical fungal endophytes hyperdiverse? *Ecology Letters* **3**: 267-274.

Atul-Nayyar A, Hamel C, Hanson K, Germida J. 2009. The arbuscular mycorrhizal symbiosis links N mineralization to plant demand. *Mycorrhiza* **19**: 239-246.

Averill C, Finzi AC. 2011. Increasing plant use of organic nitrogen with elevation is reflected in nitrogen uptake rates and ecosystem delta N-15. *Ecology* **92**: 883-891.

Averill C, Turner BL, Finzi AC. 2014. Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature* **505**: 543-545.

Avissar R, Werth D. 2005. Global hydroclimatological teleconnections resulting from tropical deforestation. *Journal of Hydrometeorology* **6**: 134-145.

Balser TC, Treseder KK, Ekenler M. 2005. Using lipid analysis and hyphal length to quantify AM and saprotrophic fungal abundance along a soil chronosequence. *Soil Biology & Biochemistry* **37**: 601-604.

Bardgett RD. 1991. The use of the membrane-filter technique for comparative measurements of hyphal lengths in different grassland sites. *Agriculture Ecosystems & Environment* **34**: 115-119.

Baribault TW, Kobe RK, Finley AO. 2012. Tropical tree growth is correlated with soil phosphorus, potassium, and calcium, though not for legumes. *Ecological Monographs* **82**: 189-203.

Barnola LG, Montilla MG. 1997. Vertical distribution of mycorrhizal colonization, root hairs, and belowground biomass in three contrasting sites from the Tropical high Mountains, Merida, Venezuela. *Arctic and Alpine Research* **29**: 206-212.

Barrett G, Campbell CD, Fitter AH, Hodge A. 2011. The arbuscular mycorrhizal fungus Glomus hoi can capture and transfer nitrogen from organic patches to its associated host plant at low temperature. *Applied Soil Ecology* **48**: 102-105.

Bartolomeesteban H, Schenck NC. 1994. Spore germination and hyphal growth of arbuscular mycorrhizal fungi in relation to soil aluminium saturation. *Mycologia* **86**: 217-226.

Bates D, Maechler M, Bolker B. 2012. lme4: Linear mixed-effects models using S4 classes. R package version 0.999999-0. http://CRAN.R-project.org/package=lme4.

Becerra A, Cabello M, Chiarini F. 2007. Arbuscular mycorrhizal colonization of vascular plants from the Yungas forests, Argentina. *Annals of Forest Science* **64**: 765-772.

Beck A, Haug I, Oberwinkler F, Kottke I. 2007. Structural characterization and molecular identification of arbuscular mycorrhiza morphotypes of Alzatea verticillata (Alzateaceae), a prominent tree in the tropical mountain rain forest of South Ecuador. *Mycorrhiza* **17**: 607-625.

Beck E, Bendix J, Kottke I, Makeschin F, Mosandl R. 2008. *Gradients in a tropical mountain ecosystem of Ecuador*. Berlin, Heidelberg, New York: Springer

Beck T, Joergensen RG, Kandeler E, Makeschin F, Nuss E, Oberholzer HR, Scheu S. 1997. An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C. *Soil Biology & Biochemistry* **29**: 1023-1032.

Becklin KM, Hertweck KL, Jumpponen A. 2012. Host identity impacts rhizosphere fungal communities associated with three alpine plant species. *Microbial Ecology* **63**: 682-693.

Bedison JE, McNeil BE. 2009. Is the growth of temperate forest trees enhanced along an ambient nitrogen deposition gradient? *Ecology* **90**: 1736-1742.

Bendix J, Beck E, Bräuning A, Makeschin F, Mosandl R, Scheu S, Wilcke W. 2013. *Ecosystem Services, Biodiversity and Environmental Change in a Tropical Mountain Ecosystem of South Ecuador*. Heidelberg, New York, Dordrecht, London: Springer.

Béreau M, Gazel M, Garbaye J. 1997. Mycorhizal symbiosis in trees of the tropical rainforest of French Guiana. *Canadian Journal of Botany* **75**: 711-716.

Bever JD, Richardson SC, Lawrence BM, Holmes J, Watson M. 2009. Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. *Ecology Letters* **12**: 13-21.

Blackwell M. 2011. The fungi: 1, 2, 3 ... 5.1 million species? *American Journal of Botany* **98**: 426-438.

Blanke V, Bassin S, Volk M, Fuhrer J. 2012. Nitrogen deposition effects on subalpine grassland: The role of nutrient limitations and changes in mycorrhizal abundance. *Acta Oecologica-*

International Journal of Ecology **45**: 57-65.

Blanke V, Renker C, Wagner M, Fullner K, Held M, Kuhn AJ, Buscot F. 2005. Nitrogen supply affects arbuscular mycorrhizal colonization of *Artemisia vulgaris* in a phosphate-polluted field site. *New Phytologist* **166**: 981-992.

Bloom AJ, Chapin FS, Mooney HA. 1985. Resource limitation in plants - an economic analogy. *Annual Review of Ecology and Systematics* **16**: 363-392.

Bobbink R, Hicks K, Galloway J, Spranger T, Alkemade R, Ashmore M, Bustamante M, Cinderby S, Davidson E, Dentener F, Emmett B, Erisman JW, Fenn M, Gilliam F, Nordin A, Pardo L, De Vries W. 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecological Applications* **20**: 30-59.

Bohrer G, Kagan-Zur V, Roth-Bejerano N, Ward D. 2001. Effects of environmental variables on vesicular-arbuscular mycorrhizal abundance in wild populations of Vangueria infausta. *Journal of Vegetation Science* **12**: 279-288.

Bolan NS, Robson AD, Barrow NJ. 1984. Increasing phosphorus supply can increase the infection of plant roots by vesicular arbuscular mycorrhizal fungi. *Soil Biology & Biochemistry* **16**: 419-420.

Borneman J, Triplett EW. 1997. Molecular microbial diversity in soils from eastern Amazonia: Evidence for unusual microorganisms and microbial population shifts associated with deforestation. *Applied and Environmental Microbiology* **63**: 2647-2653.

Bouwman L, Goldewijk KK, Van Der Hoek KW, Beusen AHW, Van Vuuren DP, Willems J, Rufino MC, Stehfest E. 2013. Exploring global changes in nitrogen and phosphorus cycles in agriculture induced by livestock production over the 1900-2050 period. *Proceedings of the National Academy of Sciences of the United States of America* **110**: 20882-20887.

Boy J, Rollenbeck R, Valarezo C, Wilcke W. 2008. Amazonian biomass burning-derived acid and nutrient deposition in the north Andean montane forest of Ecuador. *Global Biogeochemical Cycles* **22**.

Bracken MES, Low NHN. 2012. Realistic losses of rare species disproportionately impact higher trophic levels. *Ecology Letters* **15**: 461-467.

Brady NC. 1984. *The nature and properties of soils*. New York: Macmillan Publishing Company.

Brehm G, Homeier J, Fiedler K, Kottke I, Illig J, Nöske N, Werner F, Breckle S 2008. Moutain rain forests in southern Ecuador as a hotspot of biodiversity - limited knowledge and diverging patterns. In: E. Beck, J. Bendix, I. Kottke, F. MakeschinR. Mosandl eds. *Gradients in a Tropical Mountain Ecosystem of Ecuador*: Berlin, Heidelberg, New York, Springer., 15-23.

Brehm G, Pitkin L, Hilt N, Fiedler K. 2005. Montane Andean rain forests are a global diversity hotspot of geometrid moths. *Journal of Biogeography* **32**: 1621-1627.

Breuillin F, Schramm J, Hajirezaei M, Ahkami A, Favre P, Druege U, Hause B, Bucher M, Kretzschmar T, Bossolini E, Kuhlemeier C, Martinoia E, Franken P, Scholz U, Reinhardt D. 2010. Phosphate systemically inhibits development of arbuscular mycorrhiza in Petunia hybrida and represses genes involved in mycorrhizal functioning. *Plant Journal* **64**: 1002-1017.

Brummitt N, Lughadha EN. 2003. Biodiversity: Where's hot and where's not. *Conservation Biology* **17**: 1442-1448.

Brundrett MC, Abbott LK. 1995. Mycorrhizal fungus propagules in the jarrah forest .2. Spatial variability in inoculum levels. *New Phytologist* **131**: 461-469.

Brundrett MC, Ashwath N. 2013. Glomeromycotan mycorrhizal fungi from tropical Australia III. Measuring diversity in natural and disturbed habitats. *Plant and Soil* **370**: 419-433.

Camenzind T, Hempel S, Homeier J, Horn S, Velescu A, Wilcke W, Rillig MC. 2014. Nitrogen and phosphorus additions impact arbuscular mycorrhizal abundance and molecular diversity in a tropical montane forest. *Global Change Biology* **20**: 3646-3659.

Camenzind T, Rillig MC. 2013. Extraradical arbuscular mycorrhizal fungal hyphae in an organic tropical montane forest soil. *Soil Biology & Biochemistry* **64**: 96-102.

Carbonnel S, Gutjahr C. 2014. Control of arbuscular mycorrhiza development by nutrient signals. *Frontiers in Plant Science* **5**.

Caruso T, Taormina M, Migliorini M. 2012. Relative role of deterministic and stochastic determinants of soil animal community: a spatially explicit analysis of oribatid mites. *Journal of Animal Ecology* **81**: 214-221.

Cavagnaro TR, Smith FA, Ayling SM, Smith SE. 2003. Growth and phosphorus nutrition of a Paris-type arbuscular mycorrhizal symbiosis. *New Phytologist* **157**: 127-134.

Cavelier J, Tanner E, Santamaria J. 2000. Effect of water, temperature and fertilizers on soil nitrogen net transformations and tree growth in an elfin cloud forest of Colombia. *Journal of Tropical Ecology* **16**: 83-99.

Ceulemans T, Stevens CJ, Duchateau L, Jacquemyn H, Gowing DJG, Merckx R, Wallace H, van Rooijen N, Goethem T, Bobbink R, Dorland E, Gaudnik C, Alard D, Corcket E, Muller S, Dise NB, Dupre C, Diekmann M, Honnay O. 2014. Soil phosphorus constrains biodiversity across European grasslands. *Global Change Biology* **20**: 3814-3822.

Chagnon PL, Bradley RL, Klironomos JN. 2012. Using ecological network theory to evaluate the causes and consequences of arbuscular mycorrhizal community structure. *New Phytologist* **194**: 307-312.

Cheng XM, Baumgartner K. 2006. Effects of mycorrhizal roots and extraradical hyphae on N-15 uptake from vineyard cover crop litter and the soil microbial community. *Soil Biology & Biochemistry* **38**: 2665-2675.

Chubo JK, Huat OK, Jais HM, Mardatin NF, Majid N. 2009. Genera of arbuscular mycorrhiza occurring within the rhizospheres of Octomeles sumatrana and Anthocephalus chinensis in Niah, Sarawak, Malaysia. *Scienceasia* **35**: 340-345.

Clapp JP, Young JPW, Merryweather JW, Fitter AH. 1995. Diversity of fungal symbionts in arbuscular mycorrhizas from a natural community. *New Phytologist* **130**: 259-265.

Clark RB. 1997. Arbuscular mycorrhizal adaptation, spore germination, root colonization, and host plant growth and mineral acquisition at low pH. *Plant and Soil* **192**: 15-22.

Cleveland CC, Townsend AR. 2006. Nutrient additions to a tropical rain forest drive substantial soil carbon dioxide losses to the atmosphere. *Proceedings of the National Academy of Sciences of the United States of America* **103**: 10316-10321.

Corre MD, Veldkamp E, Arnold J, Wright SJ. 2010. Impact of elevated N input on soil N cycling and losses in old-growth lowland and montane forests in Panama. *Ecology* **91**: 1715-1729.

Croll D, Sanders IR. 2009. Recombination in Glomus intraradices, a supposed ancient asexual arbuscular mycorrhizal fungus. *Bmc Evolutionary Biology* **9**.

Cuenca G, De Andrade Z, Escalante G. 1998. Diversity of Glomalean spores from natural, disturbed and revegetated communities growing on nutrient-poor tropical soils. *Soil Biology & Biochemistry* **30**: 711-719.

Cuenca G, Lovera M. 2010. Seasonal variation and distribution at different soil depths of arbuscular mycorrhizal fungi spores in a tropical sclerophyllous shrubland. *Botany-Botanique* **88**: 54-64.

Cusack DF, Silver WL, Torn MS, McDowell WH. 2011. Effects of nitrogen additions on above- and belowground carbon dynamics in two tropical forests. *Biogeochemistry* **104**: 203-225.

da Silva IR, Aragao de Mello CM, Ferreira Neto RA, Alves da Silva DK, de Melo AL, Oehl F, Maia LC. 2014. Diversity of arbuscular mycorrhizal fungi along an environmental gradient in the Brazilian semiarid. *Applied Soil Ecology* **84**: 166-175.

Daniels BA, Skipper HD 1982. Methods for the Recovery and quantitative estimation of propagules from soil. In: N. C. Schenck ed. *Methods and Principles of Mycorrhizal Research.* St Paul, Minnesota: American Phytopathological Society, 29-36.

Davies KF, Margules CR, Lawrence JF. 2004. A synergistic effect puts rare, specialized species at greater risk of extinction. *Ecology* **85**: 265-271.

Davison J, Opik M, Daniell TJ, Moora M, Zobel M. 2011. Arbuscular mycorrhizal fungal communities in plant roots are not random assemblages. *Fems Microbiology Ecology* **78**: 103-115.

De Caceres M, Legendre P. 2009. Associations between species and groups of sites: indices and statistical inference. *Ecology* **90**: 3566-3574.

de Carvalho F, de Souza FA, Carrenho R, Moreira FMD, Jesus ED, Fernandes GW. 2012. The mosaic of habitats in the high-altitude Brazilian rupestrian fields is a hotspot for arbuscular mycorrhizal fungi. *Applied Soil Ecology* **52**: 9-19.

Dickie IA. 2010. Insidious effects of sequencing errors on perceived diversity in molecular surveys. *New Phytologist* **188**: 916-918.

Dickie IA, Martinez-Garcia LB, Koele N, Grelet GA, Tylianakis JM, Peltzer DA, Richardson SJ. 2013. Mycorrhizas and mycorrhizal fungal communities throughout ecosystem development. *Plant and Soil* **367**: 11-39.

Dickson S. 2004. The Arum-Paris continuum of mycorrhizal symbioses. *New Phytologist* **163**: 187-200.

Dickson S, Smith FA, Smith SE. 2007. Structural differences in arbuscular mycorrhizal symbioses: more than 100 years after Gallaud, where next? *Mycorrhiza* **17**: 375-393.

Dixon RK, Brown S, Houghton RA, Solomon AM, Trexler MC, Wisniewski J. 1994. Carbon pools and flux of global forest ecosystems. *Science* **263**: 185-190.

Dodd JC, Boddington CL, Rodriguez A, Gonzalez-Chavez C, Mansur I. 2000. Mycelium of arbuscular mycorrhizal fungi (AMF) from different genera: form, function and detection. *Plant and Soil* **226**: 131-151.

Dormann CF, Gruber B, Fruend J. 2009. Introducing the bipartite Package: Analysing Ecological Networks. *R News* **8**: 8-11.

Douds DD, Schenck NC. 1990. Relationship of colonization and sporulation by VA mycorrhizal fungi to plant nutrient and carbohydrate contents. *New Phytologist* **116**: 621-627.

Dray S. 2010. spacemakeR: Spatial modelling. R package version 0.0-5/r101. http://R-Forge.R-project.org/projects/sedar/.

Dray S, Legendre P, Peres-Neto PR. 2006. Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). *Ecological Modelling* **196**: 483-493.

Dumbrell AJ, Ashton PD, Aziz N, Feng G, Nelson M, Dytham C, Fitter AH, Helgason T. 2011. Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. *New Phytologist* **190**: 794-804.

Dumbrell AJ, Nelson M, Helgason T, Dytham C, Fitter AH. 2010a. Idiosyncrasy and overdominance in the structure of natural communities of arbuscular mycorrhizal fungi: is there a role for stochastic processes? *Journal of Ecology* **98**: 419-428.

Dumbrell AJ, Nelson M, Helgason T, Dytham C, Fitter AH. 2010b. Relative roles of niche and neutral processes in structuring a soil microbial community. *ISME Journal* **4**: 337-345.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**: 1792-1797.

Edwards PJ, Grubb PJ. 1977. Studies on mineral cycling in a montane rain-forest in New-Guinea. 1. Distribution of organic-matter in vegetation and soil. *Journal of Ecology* **65**: 943-969.

Egerton-Warburton LM, Allen EB. 2000. Shifts in arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient. *Ecological Applications* **10**: 484-496.

Egerton-Warburton LM, Johnson NC, Allen EB. 2007. Mycorrhizal community dynamics following nitrogen fertilization: A cross-site test in five grasslands. *Ecological Monographs* **77**: 527-544.

Ehinger MO, Croll D, Koch AM, Sanders IR. 2012. Significant genetic and phenotypic changes arising from clonal growth of a single spore of an arbuscular mycorrhizal fungus over multiple generations. *New Phytologist* **196**: 853-861.

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters* **10**: 1135-1142.

Eom AH, Hartnett DC, Wilson GWT, Figge DAH. 1999. The effect of fire, mowing and fertilizer amendment on arbuscular mycorrhizas in tallgrass prairie. *American Midland Naturalist* **142**: 55-70.

Ericsson T. 1995. Growth and shoot-root ratio of seedlings in relation to nutrient availability. *Plant and Soil* **168**: 205-214.

Everitt BS, Hothorn T. 2010. *A Handbook of Statistical Analyses Using R*. Boca Raton, Florida, USA: CRC Press, 2006.

Fabian P, Kohlpaintner M, Rollenbeck R. 2005. Biomass burning in the Amazon-fertilizer for the mountaineous rain forest in Ecuador. *Environmental Science and Pollution Research* **12**: 290-296.

Fischer CR, Janos DP, Perry DA, Linderman RG. 1994. Mycorrhiza inoculum potentials in tropical secondary succession. *Biotropica* **26**: 369-377.

Fisher JB, Malhi Y, Cuba Torres I, Metcalfe DB, van de Weg MJ, Meir P, Silva-Espejo JE, Huaraca Huasco W. 2013a. Nutrient limitation in rainforests and cloud forests along a 3,000-m elevation gradient in the Peruvian Andes. *Oecologia* **172**: 889-902.

Fisher JB, Malhi Y, Torres IC, Metcalfe DB, van de Weg MJ, Meir P, Silva-Espejo JE, Huasco WH. 2013b. Nutrient limitation in rainforests and cloud forests along a 3,000-m elevation gradient in the Peruvian Andes. *Oecologia* **172**: 889-902.

Fitzsimons MS, Miller RM, Jastrow JD. 2008. Scale-dependent niche axes of arbuscular mycorrhizal fungi. *Oecologia* **158**: 117-127.

Fog K. 1988. THE EFFECT OF ADDED NITROGEN ON THE RATE OF DECOMPOSITION OF ORGANIC-MATTER. *Biological Reviews of the Cambridge Philosophical Society* **63**: 433-462.

Freitas RdO, Buscardo E, Nagy L, dos Santos Maciel AB, Carrenho R, Luizao RCC. 2014. Arbuscular mycorrhizal fungal communities along a pedo-hydrological gradient in a Central Amazonian terra firme forest. *Mycorrhiza* **24**: 21-32.

Frostegård A, Tunlid A, Baath E. 1993. Phospholipid fatty-acid composition, biomass and activity of microbial communities from two soil types experimentally exposed to different heavy-metals. *Applied and Environmental Microbiology* **59**: 3605-3617.

Gai JP, Christie P, Cai XB, Fan JQ, Zhang JL, Feng G, Li XL. 2009. Occurrence and distribution of arbuscular mycorrhizal fungal species in three types of grassland community of the Tibetan Plateau. *Ecological Research* **24**: 1345-1350.

Gale J. 2004. Plants and altitude - Revisited. *Annals of Botany* **94**: 199-199.

Gallaud I. 1905. Études sur les mycorrhizes endotrophes. *Revue générale de Botanique* **17**: 5-8, 66-83, 123-136, 223-239, 313-323, 425-433, 479-500.

Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai ZC, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA. 2008. Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science* **320**: 889-892.

Garcia MO, Ovasapyan T, Greas M, Treseder KK. 2008. Mycorrhizal dynamics under elevated CO2 and nitrogen fertilization in a warm temperate forest. *Plant and Soil* **303**: 301-310.

Gardes M, Dahlberg A. 1996. Mycorrhizal diversity in arctic and alpine tundra: An open question. *New Phytologist* **133**: 147-157.

Gavito ME, Perez-Castillo D, Gonzalez-Monterrubio CF, Vieyra-Hernandez T, Martinez-Trujillo M. 2008. High compatibility between arbuscular mycorrhizal fungal communities and seedlings of different land use types in a tropical dry ecosystem. *Mycorrhiza* **19**: 47-60.

Gehring CA, Connell JH. 2006. Arbuscular mycorrhizal fungi in the tree seedlings of two Australian rain forests: occurrence, colonization, and relationships with plant performance. *Mycorrhiza* **16**: 89-98.

Gerdemann JW, Nicolson TH. 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet-sieving and decanting. *Transactions of the British Mycological Society* **46**: 235-244.

Girardin CAJ, Malhi Y, Aragao L, Mamani M, Huasco WH, Durand L, Feeley KJ, Rapp J, Silva-Espejo JE, Silman M, Salinas N, Whittaker RJ. 2010. Net primary productivity allocation and cycling of carbon along a tropical forest elevational transect in the Peruvian Andes. *Global Change Biology* **16**: 3176-3192.

Goller R, Wilcke W, Fleischbein K, Valarezo C, Zech W. 2006. Dissolved nitrogen, phosphorus, and sulfur forms in the ecosystem fluxes of a montane forest in ecuador. *Biogeochemistry* **77**: 57-89.

Gomez SK, Javot H, Deewatthanawong P, Torres-Jerez I, Tang Y, Blancaflor EB, Udvardi MK, Harrison MJ. 2009. Medicago truncatula and Glomus intraradices gene expression in cortical cells harboring arbuscules in the arbuscular mycorrhizal symbiosis. *Bmc Plant Biology* **9**.

Gosling P, Mead A, Proctor M, Hammond JP, Bending GD. 2013. Contrasting arbuscular mycorrhizal communities colonizing different host plants show a similar response to a soil phosphorus concentration gradient. *New Phytologist* **198**: 546-556.

Govindarajulu M, Pfeffer PE, Jin HR, Abubaker J, Douds DD, Allen JW, Bucking H, Lammers PJ, Shachar-Hill Y. 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature* **435**: 819-823.

Gower ST, Vitousek PM. 1989. Effects of nutrient amendments on fine root biomass in a primary successional forest in Hawaii. *Oecologia* **81**: 566-568.

Graefe S, Hertel D, Leuschner C. 2010. N, P and K limitation of fine root growth along an elevation transect in tropical mountain forests. *Acta Oecologica-International Journal of Ecology* **36**: 537-542.

Graefe S, Leuschner C, Coners H, Hertel D. 2011. Root functioning in tropical high-elevation forests: Environmental vs. biological control of root water absorption. *Environmental and Experimental Botany* **71**: 329-336.

Gress SE, Nichols TD, Northcraft CC, Peterjohn WT. 2007. Nutrient limitation in soils exhibiting differing nitrogen availabilities: What lies beyond nitrogen saturation? *Ecology* **88**: 119-130.

Grieve IC, Proctor J, Cousins SA. 1990. Soil variation with altitude on volcan Barva, Costa-Rica. *Catena* **17**: 525-534.

Gruber N, Galloway JN. 2008. An Earth-system perspective of the global nitrogen cycle. *Nature* **451**: 293-296.

Gryndler M, Hrselova H, Cajthaml T, Havrankova M, Rezacova V, Gryndlerova H, Larsen J. 2009. Influence of soil organic matter decomposition on arbuscular mycorrhizal fungi in terms of asymbiotic hyphal growth and root colonization. *Mycorrhiza* **19**: 255-266.

Guadarrama P, Alvarez-Sanchez J, Briones O. 2004a. Seedling growth of two pioneer tropical tree species in Competition: The role of arbuscular mycorrhizae. *Euphytica* **138**: 113-121.

Guadarrama P, Alvarez-Sanchez J, Estrada-Torres A. 2004b. Phosphorus dependence in seedlings of a tropical pioneer tree: The role of arbuscular mycorrhizae. *Journal of Plant Nutrition* **27**: 2159-2174.

Guadarrama P, Castillo-Arguero S, Ramos-Zapata J, Camargo-Ricalde SL, Alvarez-Sanchez J. 2008. Propagules of arbuscular mycorrhizal fungi in a secondary dry forest of Oaxaca, Mexico. *Revista De Biologia Tropical* **56**: 269-277.

Guether M, Neuhaeuser B, Balestrini R, Dynowski M, Ludewig U, Bonfante P. 2009. A Mycorrhizal-Specific Ammonium Transporter from Lotus japonicus Acquires Nitrogen Released by Arbuscular Mycorrhizal Fungi. *Plant Physiology* **150**: 73-83.

Gullison RE, Frumhoff PC, Canadell JG, Field CB, Nepstad DC, Hayhoe K, Avissar R, Curran LM, Friedlingstein P, Jones CD, Nobre C. 2007. Tropical forests and climate policy. *Science* **316**: 985-986.

Guo SL, Wu JS, Coleman K, Zhu HH, Li Y, Liu WZ. 2012. Soil organic carbon dynamics in a dryland cereal cropping system of the Loess Plateau under long-term nitrogen fertilizer applications. *Plant and Soil* **353**: 321-332.

Gusewell S. 2004. N : P ratios in terrestrial plants: variation and functional significance. *New Phytologist* **164**: 243-266.

Güsewell S, Gessner MO. 2009. N : P ratios influence litter decomposition and colonization by fungi and bacteria in microcosms. *Functional Ecology* **23**: 211-219.

Gutjahr C, Parniske M. 2013. Cell and Developmental Biology of Arbuscular Mycorrhiza Symbiosis. *Annual Review of Cell and Developmental Biology* **29**: 593-617.

Hall SJ, Matson PA. 2003. Nutrient status of tropical rain forests influences soil N dynamics after N additions. *Ecological Monographs* **73**: 107-129.

Hao XL, Jiang R, Chen T. 2011. Clustering 16S rRNA for OTU prediction: a method of unsupervised Bayesian clustering. *Bioinformatics* **27**: 611-618.

Harpole WS, Ngai JT, Cleland EE, Seabloom EW, Borer ET, Bracken MES, Elser JJ, Gruner DS, Hillebrand H, Shurin JB, Smith JE. 2011. Nutrient co-limitation of primary producer communities. *Ecology Letters* **14**: 852-862.

Hart MM, Reader RJ. 2002. Does percent root length colonization and soil hyphal length reflect the extent of colonization for all AMF? *Mycorrhiza* **12**: 297-301.

Hart SC, Stark JM, Davidson EA, Firestone MK 1994. Nitrogen mineralization, immobilization and nitrification. In: P. S. Bottomley, J. S. AngleR. W. Weaver eds. *Methods of Analysis Part 2. Microbiological and Biochemical Properties*: Soil Science Society of America, 985-1018.

Haselwandter K. 1987. Mycorrhizal infection and its possible ecological significance in climatically and nutritionally stressed alpine plant communities. *Angewandte Botanik* **61**: 107-114.

Haug I, Setaro S, Suarez JP. 2013. Reforestation sites show similar and nested AMF communities to an adjacent pristine forest in a tropical mountain area of South Ecuador. *PloS one* **8**: e63524.

Haug I, Wubet T, Weiss M, Aguirre N, Weber M, Gunter S, Kottke I. 2010. Species-rich but distinct arbuscular mycorrhizal communities in reforestation plots on degraded pastures and in neighboring pristine tropical mountain rain forest. *Tropical Ecology* **51**: 125-148.

Hausmann NT, Hawkes CV. 2009. Plant neighborhood control of arbuscular mycorrhizal community composition. *New Phytologist* **183**: 1188-1200.

Hedin LO, Brookshire ENJ, Menge DNL, Barron AR 2009. The Nitrogen Paradox in Tropical Forest Ecosystems. *Annual Review of Ecology Evolution and Systematics*, 613-635.

Helgason T, Merryweather JW, Denison J, Wilson P, Young JPW, Fitter AH. 2002. Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland. *Journal of Ecology* **90**: 371-384.

Herman DJ, Firestone MK, Nuccio E, Hodge A. 2012. Interactions between an arbuscular mycorrhizal fungus and a soil microbial community mediating litter decomposition. *Fems Microbiology Ecology* **80**: 236-247.

Herrera R, Merida T, Stark N, Jordan CF. 1978. Direct phosphorus transfer from leaf litter to roots. *Naturwissenschaften* **65**: 208-209.

Herrera R, Rodriguez A, Furrazola E 1986. Método para determinar la biomasa de micelio extramátrico vesiculo-arbuscular.In. *Ciclo lectivo sobre el tema: Técnicas de investigación en micorriza*. Turrialba, Costa Rica: Fundación Internacional para la Ciencia.

Hertel D, Leuschner C, Holscher D. 2003. Size and structure of fine root systems in old-growth and secondary tropical montane forests (Costa Rica). *Biotropica* **35**: 143-153.

Hodge A. 2003. Plant nitrogen capture from organic matter as affected by spatial dispersion, interspecific competition and mycorrhizal colonization. *New Phytologist* **157**: 303-314.

Hodge A, Campbell CD, Fitter AH. 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature* **413**: 297-299.

Hodge A, Fitter AH. 2010. Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. *Proceedings of the National Academy of Sciences of the United States of America* **107**: 13754-13759.

Hodge A, Helgason T, Fitter AH. 2010. Nutritional ecology of arbuscular mycorrhizal fungi. *Fungal Ecology* **3**: 267-273.

Hodge A, Storer K. 2015. Arbuscular mycorrhiza and nitrogen: implications for individual plants through to ecosystems. *Plant and Soil* **386**: 1-19.

Hofstetter V, Clemencon H, Vilgalys R, Moncalvo JM. 2002. Phylogenetic analyses of the Lyophylleae (Agaricales, Basidiomycota) based on nuclear and mitochondrial rDNA sequences. *Mycological Research* **106**: 1043-1059.

Högberg P, Fan HB, Quist M, Binkley D, Tamm CO. 2006. Tree growth and soil acidification in response to 30 years of experimental nitrogen loading on boreal forest. *Global Change Biology* **12**: 489-499.

Homeier J 2013. Effects of nutrient addition on tree growth.In. *Tropical Mountain Forest (TMF)*

Newsletter: DFG Research Unit 816. 6.

Homeier J, Breckle S-W, Guenter S, Rollenbeck RT, Leuschner C. 2010. Tree Diversity, Forest Structure and Productivity along Altitudinal and Topographical Gradients in a Species-Rich Ecuadorian Montane Rain Forest. *Biotropica* **42**: 140-148.

Homeier J, Hertel D, Camenzind T, Cumbicus NL, Maraun M, Martinson GO, Poma LN, Rillig MC, Sandmann D, Scheu S, Veldkamp E, Wilcke W, Wullaert H, Leuschner C. 2012. Tropical andean forests are highly susceptible to nutrient inputs-rapid effects of experimental N and p addition to an ecuadorian montane forest. *PloS one* **7**: e47128.

Homeier J, Leuschner C, Bräuning A, Cumbicus NL, Hertel D, Matrinson GO, Spannl S, Veldkamp E 2013. Effects of Nutrient Addition on the Productivity of Montane Forests and Implications for the Carbon Cycle. In: J. Bendix, E. Beck, A. Bräuning, F. Makeschin, R. Mosandl, S. ScheuW. Wilcke eds. *Ecosystem Services, Biodiversity and Environmental Change in a Tropical Mountain Ecosystem of South Ecuador*. Berlin Heidelberg: Springer-Verlag.

Homeier J, Werner FA. 2007. Spermatophyta. Checklist of the Reserva Biológica San Francisco (Prov. Zamora-Chinchipe, S-Ecuador). *Ecotropical Monographs* **4**: 15-58.

Homeier J, Werner FA, Gradstein SR, Breckle SW, Richter M 2008. Potential vegetation and floristic composition of Andean forests in South Ecuador, with a focus on the RBSF. In: E. Beck, J. Bendix, I. Kottke, F. MakeschinR. Mosandl eds. *Ecological Studies*: Springer, 233 Spring Street, New York, Ny 10013, United States, 87-100.

Hothorn T, Bretz F, Westfall P. 2008. Simultaneous inference in general parametric models. *Biometrical Journal* **50**: 346-363.

Husband R, Herre EA, Turner SL, Gallery R, Young JPW. 2002. Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. *Molecular Ecology* **11**: 2669-2678.

Huson DH, Mitra S, Ruscheweyh H-J, Weber N, Schuster SC. 2011. Integrative analysis of environmental sequences using MEGAN4. *Genome Research* **21**: 1552-1560.

Ijdo M, Schtickzelle N, Cranenbrouck S, Declerck S. 2010. Do arbuscular mycorrhizal fungi with contrasting life-history strategies differ in their responses to repeated defoliation? *Fems Microbiology Ecology* **72**: 114-122.

Isbell F, Calcagno V, Hector A, Connolly J, Harpole WS, Reich PB, Scherer-Lorenzen M, Schmid B, Tilman D, van Ruijven J, Weigelt A, Wilsey BJ, Zavaleta ES, Loreau M. 2011. High plant diversity is needed to maintain ecosystem services. *Nature* **477**: 199-U196.

Jackson BE, Wright RD, Alley MM. 2009. Comparison of Fertilizer Nitrogen Availability, Nitrogen Immobilization, Substrate Carbon Dioxide Efflux, and Nutrient Leaching in Peat-lite, Pine Bark, and Pine Tree Substrates. *Hortscience* **44**: 781-790.

Janos DP. 1980a. Mycorrhizae influence tropical succession. *Biotropica* **12**: 56-64.

Janos DP. 1980b. Vesicular-arbuscular mycorrhizae affect lowland tropical rain-forest plant-growth. *Ecology* **61**: 151-162.

Janssens IA, Dieleman W, Luyssaert S, Subke JA, Reichstein M, Ceulemans R, Ciais P, Dolman AJ, Grace J, Matteucci G, Papale D, Piao SL, Schulze ED, Tang J, Law BE. 2010. Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience* **3**: 315-322.

Ji BM, Bentivenga SP, Casper BB. 2012. Comparisons of AM fungal spore communities with the same hosts but different soil chemistries over local and geographic scales. *Oecologia* **168**: 187-197.

Johansen A, Jensen ES. 1996. Transfer of N and P from intact or decomposing roots of pea to barley interconnected by an arbuscular mycorrhizal fungus. *Soil Biology & Biochemistry* **28**: 73-81.

Johnson NC. 1993. Can fertilization of soil select less mutualistic mycorrhizae. *Ecological Applications* **3**: 749-757.

Johnson NC. 2010. Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. *New Phytologist* **185**: 631-647.

Johnson NC, Rowland DL, Corkidi L, Allen EB. 2008. Plant winners and losers during grassland N-

eutrophication differ in biomass allocation and mycorrhizas. *Ecology* **89**: 2868-2878.
Johnson NC, Rowland DL, Corkidi L, Egerton-Warburton LM, Allen EB. 2003. Nitrogen enrichment alters mycorrhizal allocation at five mesic to semiarid grasslands. *Ecology* **84**: 1895-1908.
Johnson NC, Wedin DA. 1997. Soil carbon, nutrients, and mycorrhizae during conversion of dry tropical forest to grassland. *Ecological Applications* **7**: 171-182.
Johnson SD, Steiner KE. 2000. Generalization versus specialization in plant pollination systems. *Trends in Ecology & Evolution* **15**: 140-143.
Kahkola AK, Nygren P, Leblanc HA, Pennanen T, Pietikainen J. 2012. Leaf and root litter of a legume tree as nitrogen sources for cacaos with different root colonisation by arbuscular mycorrhizae. *Nutrient Cycling in Agroecosystems* **92**: 51-65.
Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772-780.
Kessler M, Guedel R, Salazar L, Homeier J, Kluge J. 2014. Impact of mycorrhization on the abundance, growth and leaf nutrient status of ferns along a tropical elevational gradient. *Oecologia* **175**: 887-900.
Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A, Palmer TM, West SA, Vandenkoornhuyse P, Jansa J, Bucking H. 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* **333**: 880-882.
Kirschbaum MUF. 1995. The temperature-dependence of soil organic-matter decomposition, and the effect of global warming on soil organic-C storage. *Soil Biology & Biochemistry* **27**: 753-760.
Kivlin SN, Hawkes CV, Treseder KK. 2011. Global diversity and distribution of arbuscular mycorrhizal fungi. *Soil Biology & Biochemistry* **43**: 2294-2303.
Klironomos JN, McCune J, Hart M, Neville J. 2000. The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. *Ecology Letters* **3**: 137-141.
Klironomos JN, Ursic M, Rillig M, Allen MF. 1998. Interspecific differences in the response of arbuscular mycorrhizal fungi to *Artemisia tridentata* grown under elevated atmospheric CO2. *New Phytologist* **138**: 599-605.
Kobe RK, Lepczyk CA, Iyer M. 2005. Resorption efficiency decreases with increasing green leaf nutrients in a global data set. *Ecology* **86**: 2780-2792.
Koehler B, Corre MD, Veldkamp E, Wullaert H, Wright SJ. 2009. Immediate and long-term nitrogen oxide emissions from tropical forest soils exposed to elevated nitrogen input. *Global Change Biology* **15**: 2049-2066.
Kohout P, Sudova R, Janouskova M, Ctvrtlikova M, Hejda M, Pankova H, Slavikova R, Stajerova K, Vosatka M, Sykorova Z. 2014. Comparison of commonly used primer sets for evaluating arbuscular mycorrhizal fungal communities: Is there a universal solution? *Soil Biology & Biochemistry* **68**: 482-493.
Koide RT. 1991. Nutrient supply, nutrient demand and plant-response to mycorrhizal infection. *New Phytologist* **117**: 365-386.
Koide RT, Kabir Z. 2000. Extraradical hyphae of the mycorrhizal fungus Glomus intraradices can hydrolyse organic phosphate. *New Phytologist* **148**: 511-517.
Körner C. 1999. *Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystems*. Berlin, Heidelberg: Springer-Verlag.
Korner C, Renhardt U. 1987. Dry-matter partitioning and root length - leaf area ratios in herbaceous perennial plants with diverse altitudinal distribution. *Oecologia* **74**: 411-418.
kottke I, Beck A, Haug I, Setaro S, Jeske V, Suárez JP, Pazmiño L, Preußling M, Nebel M, Oberwinkler F 2008. Mycorrhizal state and new and special features of mycorrhizae of trees, ericads, orchids, ferns, and liverworts. In: E. Beck, J. Bendix, I. Kottke, F. MakeschinR. Mosandl eds. *Ecological Studies*: Springer, 233 Spring Street, New York, Ny 10013, United States.
Kottke I, Beck A, Oberwinkler F, Homeier J, Neill D. 2004. Arbuscular endomycorrhizas are dominant in the organic soil of a neotropical montane cloud forest. *Journal of Tropical*

Ecology **20**: 125-129.

Krashevska V, Maraun M, Ruess L, Scheu S. 2010. Carbon and nutrient limitation of soil microorganisms and microbial grazers in a tropical montane rain forest. *Oikos* **119**: 1020-1028.

Krashevska V, Sandmann D, Maraun M, Scheu S. 2012. Consequences of exclusion of precipitation on microorganisms and microbial consumers in montane tropical rainforests. *Oecologia* **170**: 1067-1076.

Krashevska V, Sandmann D, Maraun M, Scheu S. 2013. Moderate changes in nutrient input alter tropical microbial and protist communities and belowground linkages. *ISME Journal*.

Krüger M, Krüger C, Walker C, Stockinger H, Schüßler A. 2012. Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. *New Phytologist* **193**: 970-984.

Krüger M, Stockinger H, Krüger C, Schussler A. 2009. DNA-based species level detection of Glomeromycota: one PCR primer set for all arbuscular mycorrhizal fungi. *New Phytologist* **183**: 212-223.

Kuhn G, Hijri M, Sanders IR. 2001. Evidence for the evolution of multiple genomes in arbuscular mycorrhizal fungi. *Nature* **414**: 745-748.

Leake JR, Johnson D, Donnelly DP, Muckle GE, Boddy L, Read DJ. 2004. Networks of power and influence: the role of mycorrhizal mycelium in controlling plant communities and agroecosystem functioning. *Canadian Journal of Botany-Revue Canadienne De Botanique* **82**: 1016-1045.

Leal PL, Siqueira JO, Stuermer SL. 2013. Switch of tropical Amazon forest to pasture affects taxonomic composition but not species abundance and diversity of arbuscular mycorrhizal fungal community. *Applied Soil Ecology* **71**: 72-80.

Leal PL, Sturmer SL, Siqueira JO. 2009. Occurrence and diversity of arbuscular mycorrhizal fungi in trap cultures from soils under different land use systems in the Amazon, Brazil. *Brazilian Journal of Microbiology* **40**: 111-121.

LeBauer DS, Treseder KK. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* **89**: 371-379.

Lehmann A, Veresoglou SD, Leifheit EF, Rillig MC. 2014. Arbuscular mycorrhizal influence on zinc nutrition in crop plants - A meta-analysis. *Soil Biology & Biochemistry* **69**: 123-131.

Leigh J, Hodge A, Fitter AH. 2009. Arbuscular mycorrhizal fungi can transfer substantial amounts of nitrogen to their host plant from organic material. *New Phytologist* **181**: 199-207.

Lekberg Y, Gibbons SM, Rosendahl S, Ramsey PW. 2013. Severe plant invasions can increase mycorrhizal fungal abundance and diversity. *ISME Journal* **7**: 1424-1433.

Lekberg Y, Koide RT, Rohr JR, Aldrich-Wolfe L, Morton JB. 2007. Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. *Journal of Ecology* **95**: 95-105.

Lekberg Y, Schnoor T, Kjoller R, Gibbons SM, Hansen LH, Al-Soud WA, Sorensen SJ, Rosendahl S. 2012. 454-sequencing reveals stochastic local reassembly and high disturbance tolerance within arbuscular mycorrhizal fungal communities. *Journal of Ecology* **100**: 151-160.

Leuschner C, Hertel D, Coners H, Buttner V. 2001. Root competition between beech and oak: a hypothesis. *Oecologia* **126**: 276-284.

Leuschner C, Moser G, Bertsch C, Roederstein M, Hertel D. 2007. Large altitudinal increase in tree root/shoot ratio in tropical mountain forests of Ecuador. *Basic and Applied Ecology* **8**: 219-230.

Lewis SL, Malhi Y, Phillips OL. 2004. Fingerprinting the impacts of global change on tropical forests. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **359**: 437-462.

Li LF, Li T, Zhang Y, Zhao ZW. 2010. Molecular diversity of arbuscular mycorrhizal fungi and their distribution patterns related to host-plants and habitats in a hot and arid ecosystem, southwest China. *Fems Microbiology Ecology* **71**: 418-427.

Li YQ, Xu M, Zou XM. 2006. Effects of nutrient additions on ecosystem carbon cycle in a Puerto

Rican tropical wet forest. *Global Change Biology* **12**: 284-293.
Lin X, Feng Y, Zhang H, Chen R, Wang J, Zhang J, Chu H. 2012. Long-term balanced fertilization decreases arbuscular mycorrhizal fungal diversity in an arable soil in North China revealed by 454 pyrosequencing. *Environmental Science & Technology* **46**: 5764-5771.
Lindahl BD, Nilsson RH, Tedersoo L, Abarenkov K, Carlsen T, Kjoller R, Koljalg U, Pennanen T, Rosendahl S, Stenlid J, Kauserud H. 2013. Fungal community analysis by high-throughput sequencing of amplified markers - a user's guide. *New Phytologist* **199**: 288-299.
Liu K-L, Porras-Alfaro A, Kuske CR, Eichorst SA, Xie G. 2012. Accurate, rapid taxonomic classification of fungal large-subunit rRNA genes. *Applied and Environmental Microbiology* **78**: 1523-1533.
Liu YJ, He JX, Shi GX, An LZ, Opik M, Feng HY. 2011. Diverse communities of arbuscular mycorrhizal fungi inhabit sites with very high altitude in Tibet Plateau. *Fems Microbiology Ecology* **78**: 355-365.
Liu YJ, Shi GX, Mao L, Cheng G, Jiang SJ, Ma XJ, An LZ, Du GZ, Johnson NC, Feng HY. 2012. Direct and indirect influences of 8 yr of nitrogen and phosphorus fertilization on Glomeromycota in an alpine meadow ecosystem. *New Phytologist* **194**: 523-535.
Loftfield N, Flessa H, Augustin J, Beese F. 1997. Automated gas chromatographic system for rapid analysis of the atmospheric trace gases methane, carbon dioxide, and nitrous oxide. *Journal of Environmental Quality* **26**: 560-564.
Lohse KA, Matson P. 2005. Consequences of nitrogen additions for soil losses from wet tropical forests. *Ecological Applications* **15**: 1629-1648.
Louis I, Lim G. 1987. Spore density and root colonization of vesicular-arbuscular mycorrhizas in tropical soil. *Transactions of the British Mycological Society* **88**: 207-212.
Lovelock CE, Andersen K, Morton JB. 2003. Arbuscular mycorrhizal communities in tropical forests are affected by host tree species and environment. *Oecologia* **135**: 268-279.
Lu M, Yang Y, Luo Y, Fang C, Zhou X, Chen J, Yang X, Li B. 2011. Responses of ecosystem nitrogen cycle to nitrogen addition: a meta-analysis. *New Phytologist* **189**: 1040-1050.
Lu X, Mo J, Gilliam FS, Zhou G, Fang Y. 2010. Effects of experimental nitrogen additions on plant diversity in an old-growth tropical forest. *Global Change Biology* **16**: 2688-2700.
Lumini E, Orgiazzi A, Borriello R, Bonfante P, Bianciotto V. 2010. Disclosing arbuscular mycorrhizal fungal biodiversity in soil through a land-use gradient using a pyrosequencing approach. *Environmental Microbiology* **12**: 2165-2179.
Lyons KG, Brigham CA, Traut BH, Schwartz MW. 2005. Rare species and ecosystem functioning. *Conservation Biology* **19**: 1019-1024.
Maherali H, Klironomos JN. 2007. Influence of Phylogeny on fungal community assembly and ecosystem functioning. *Science* **316**: 1746-1748.
Maherali H, Klironomos JN. 2012. Phylogenetic and trait-based assembly of arbuscular mycorrhizal fungal communities. *PloS one* **7**.
Mahowald N, Jickells TD, Baker AR, Artaxo P, Benitez-Nelson CR, Bergametti G, Bond TC, Chen Y, Cohen DD, Herut B, Kubilay N, Losno R, Luo C, Maenhaut W, McGee KA, Okin GS, Siefert RL, Tsukuda S. 2008. Global distribution of atmospheric phosphorus sources, concentrations and deposition rates, and anthropogenic impacts. *Global Biogeochemical Cycles* **22**.
Mahowald NM, Artaxo P, Baker AR, Jickells TD, Okin GS, Randerson JT, Townsend AR. 2005. Impacts of biomass burning emissions and land use change on Amazonian atmospheric phosphorus cycling and deposition. *Global Biogeochemical Cycles* **19**.
Marschner H, Dell B. 1994. Nutrient-uptake in mycorrhizal symbiosis. *Plant and Soil* **159**: 89-102.
Marschner P, Crowley DE, Lieberei R. 2001. Arbuscular mycorrhizal infection changes the bacterial 16 S rDNA community composition in the rhizosphere of maize. *Mycorrhiza* **11**: 297-302.
Martinez-Garcia LB, Pugnaire FI. 2011. Arbuscular mycorrhizal fungi host preference and site effects in two plant species in a semiarid environment. *Applied Soil Ecology* **48**: 313-317.
Martinson GO, Corre MD, Veldkamp E. 2013. Responses of nitrous oxide fluxes and soil nitrogen cycling to nutrient additions in montane forests along an elevation gradient in southern

Ecuador. *Biogeochemistry* **112**: 625-636.

Matson PA, McDowell WH, Townsend AR, Vitousek PM. 1999. The globalization of N deposition: ecosystem consequences in tropical environments. *Biogeochemistry* **46**: 67-83.

Matzner E, Zuber T, Lischeid G 2004. Response of soil solution chemistry and solute fluxes to changing deposition rates. In: Matzner E, editor. Biogeochemstry of forested catchments in a changing environment - A German case study. In: E. Matzner ed. *Ecological Studies*. Heidelberg, Berlin: Springer.

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA. 1990. A new method which gives an objective-measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. *New Phytologist* **115**: 495-501.

McGuire KL, Henkel TW, de la Cerda IG, Villa G, Edmund F, Andrew C. 2008. Dual mycorrhizal colonization of forest-dominating tropical trees and the mycorrhizal status of non-dominant tree and liana species. *Mycorrhiza* **18**: 217-222.

Meir P, Grace J, Miranda AC. 2001. Leaf respiration in two tropical rainforests: constraints on physiology by phosphorus, nitrogen and temperature. *Functional Ecology* **15**: 378-387.

Miller RM, Reinhardt DR, Jastrow JD. 1995. External hyphal production of vesicular-arbuscular mycorrhizal fungi in pasture and tallgrass prairie communities. *Oecologia* **103**: 17-23.

Mirmanto E, Proctor J, Green J, Nagy L, Suriantata. 1999. Effects of nitrogen and phosphorus fertilization in a lowland evergreen rainforest. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **354**: 1825-1829.

Montesinos-Navarro A, Segarra-Moragues JG, Valiente-Banuet A, Verdu M. 2012. The network structure of plant-arbuscular mycorrhizal fungi. *New Phytologist* **194**: 536-547.

Moora M, Berger S, Davison J, Opik M, Bommarco R, Bruelheide H, Kuhn I, Kunin WE, Metsis M, Rortais A, Vanatoa A, Vanatoa E, Stout JC, Truusa M, Westphal C, Zobel M, Walther GR. 2011. Alien plants associate with widespread generalist arbuscular mycorrhizal fungal taxa: evidence from a continental-scale study using massively parallel 454 sequencing. *Journal of Biogeography* **38**: 1305-1317.

Moser G, Hertel D, Leuschner C. 2007. Altitudinal change in LAI and stand leaf biomass in tropical montane forests: a transect shady in Ecuador and a pan-tropical meta-analysis. *Ecosystems* **10**: 924-935.

Moser G, Leuschner C, Hertel D, Graefe S, Soethe N, Iost S. 2011. Elevation effects on the carbon budget of tropical mountain forests (S Ecuador): the role of the belowground compartment. *Global Change Biology* **17**: 2211-2226.

Mosse B. 1959. Observations on the extramatrical mycelium of a vesicular-arbuscular endophyte. *Transactions of the British Mycological Society* **42**: 439-448.

Mouillot D, Bellwood DR, Baraloto C, Chave J, Galzin R, Harmelin-Vivien M, Kulbicki M, Lavergne S, Lavorel S, Mouquet N, Paine CET, Renaud J, Thuiller W. 2013. Rare Species Support Vulnerable Functions in High-Diversity Ecosystems. *Plos Biology* **11**.

Moyersoen B, Alexander IJ, Fitter AH. 1998a. Phosphorus nutrition of ectomycorrhizal and arbuscular mycorrhizal tree seedlings from a lowland tropical rain forest in Korup National Park, Cameroon. *Journal of Tropical Ecology* **14**: 47-61.

Moyersoen B, Becker P, Alexander IJ. 2001. Are ectomycorrhizas more abundant than arbuscular mycorrhizas in tropical heath forests? *New Phytologist* **150**: 591-599.

Moyersoen B, Fitter AH, Alexander IJ. 1998b. Spatial distribution of ectomycorrhizas and arbuscular mycorrhizas in Korup National Park rain forest, Cameroon, in relation to edaphic parameters. *New Phytologist* **139**: 311-320.

Müller F, Baessler C, Schubert H, Klotz S. 2010. *Long-Term Ecological Research: Between Theory and Application*. Dordrecht, Heidelberg, London, New York: Springer.

Mummey DL, Rillig MC. 2008. Spatial characterization of arbuscular mycorrhizal fungal molecular diversity at the submetre scale in a temperate grassland. *Fems Microbiology Ecology* **64**: 260-270.

Munkvold L, Kjoller R, Vestberg M, Rosendahl S, Jakobsen I. 2004. High functional diversity within species of arbuscular mycorrhizal fungi. *New Phytologist* **164**: 357-364.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J. 2000. Biodiversity hotspots for conservation priorities. *Nature* **403**: 853-858.
Nadelhoffer KJ. 2000. The potential effects of nitrogen deposition on fine-root production in forest ecosystems. *New Phytologist* **147**: 131-139.
Newman EI. 1966. A method of estimating total length of root in a sample. *Journal of Applied Ecology* **3**: 139-145.
Newsham KK, Upson R, Read DJ. 2009. Mycorrhizas and dark septate root endophytes in polar regions. *Fungal Ecology* **2**: 10-20.
Ngosong C, Gabriel E, Ruess L. 2012. Use of the signature Fatty Acid 16:1omega5 as a tool to determine the distribution of arbuscular mycorrhizal fungi in soil. *Journal of lipids* **2012**: 236807.
Nijjer S, Rogers WE, Siemann E. 2010. The Impacts of Fertilization on Mycorrhizal Production and Investment in Western Gulf Coast Grasslands. *American Midland Naturalist* **163**: 124-133.
Nomura N, Kikuzawa K. 2003. Productive phenology of tropical montane forests: Fertilization experiments along a moisture gradient. *Ecological Research* **18**: 573-586.
Nuccio EE, Hodge A, Pett-Ridge J, Herman DJ, Weber PK, Firestone MK. 2013. An arbuscular mycorrhizal fungus significantly modifies the soil bacterial community and nitrogen cycling during litter decomposition. *Environmental Microbiology* **15**: 1870-1881.
Oehl F, Laczko E, Bogenrieder A, Stahr K, Boesch R, van der Heijden M, Sieverding E. 2010. Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. *Soil Biology & Biochemistry* **42**: 724-738.
Okin GS, Mahowald N, Chadwick OA, Artaxo P. 2004. Impact of desert dust on the biogeochemistry of phosphorus in terrestrial ecosystems. *Global Biogeochemical Cycles* **18**.
Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2013. vegan: community ecology package. http://CRAN.R-project.org/package=vegan.
Olander LP, Vitousek PM. 2000. Regulation of soil phosphatase and chitinase activity by N and P availability. *Biogeochemistry* **49**: 175-190.
Olson DM, Dinerstein E, Wikramanayake ED, Burgess ND, Powell GVN, Underwood EC, D'Amico JA, Itoua I, Strand HE, Morrison JC, Loucks CJ, Allnutt TF, Ricketts TH, Kura Y, Lamoreux JF, Wettengel WW, Hedao P, Kassem KR. 2001. Terrestrial ecoregions of the worlds: A new map of life on Earth. *Bioscience* **51**: 933-938.
Olsson PA, Baath E, Jakobsen I. 1997. Phosphorus effects on the mycelium and storage structures of an arbuscular mycorrhizal fungus as studied in the soil and roots by analysis of fatty acid signatures. *Applied and Environmental Microbiology* **63**: 3531-3538.
Olsson PA, Eriksen BE, Dahlberg A. 2004. Colonization by arbuscular mycorrhizal and fine endophytic fungi in herbaceous vegetation in the Canadian High Arctic. *Canadian Journal of Botany-Revue Canadienne De Botanique* **82**: 1547-1556.
Olsson PA, Johansen A. 2000. Lipid and fatty acid composition of hyphae and spores of arbuscular mycorrhizal fungi at different growth stages. *Mycological Research* **104**: 429-434.
Olsson PA, Thingstrup I, Jakobsen I, Baath E. 1999. Estimation of the biomass of arbuscular mycorrhizal fungi in a linseed field. *Soil Biology & Biochemistry* **31**: 1879-1887.
Olsson PA, Wilhelmsson P. 2000. The growth of external AM fungal mycelium in sand dunes and in experimental systems. *Plant and Soil* **226**: 161-169.
Öpik M, Davison J, Moora M, Zobel M. 2014. DNA-based detection and identification of Glomeromycota: the virtual taxonomy of environmental sequences. *Botany-Botanique* **92**: 135-147.
Öpik M, Metsis M, Daniell TJ, Zobel M, Moora M. 2009. Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. *New Phytologist* **184**: 424-437.
Öpik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij JM, Reier U, Zobel M. 2010. The online database MaarjAM reveals global and ecosystemic distribution patterns in

arbuscular mycorrhizal fungi (Glomeromycota). *New Phytologist* **188**: 223-241.

Orme CDL, Davies RG, Burgess M, Eigenbrod F, Pickup N, Olson VA, Webster AJ, Ding TS, Rasmussen PC, Ridgely RS, Stattersfield AJ, Bennett PM, Blackburn TM, Gaston KJ, Owens IPF. 2005. Global hotspots of species richness are not congruent with endemism or threat. *Nature* **436**: 1016-1019.

Ostertag R. 2010. Foliar nitrogen and phosphorus accumulation responses after fertilization: an example from nutrient-limited Hawaiian forests. *Plant and Soil* **334**: 85-98.

Parniske M. 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nature Reviews Microbiology* **6**: 763-775.

Persson H. 1978. Root dynamics in a young Scots Pine stand in Central Sweden. *Oikos* **30**: 508-519.

Pett-Ridge JC. 2009. Contributions of dust to phosphorus cycling in tropical forests of the Luquillo Mountains, Puerto Rico. *Biogeochemistry* **94**: 63-80.

Phillips JM, Hayman DS. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**: 158-161.

Phoenix GK, Hicks WK, Cinderby S, Kuylenstierna JCI, Stock WD, Dentener FJ, Giller KE, Austin AT, Lefroy RDB, Gimeno BS, Ashmore MR, Ineson P. 2006. Atmospheric nitrogen deposition in world biodiversity hotspots: the need for a greater global perspective in assessing N deposition impacts. *Global Change Biology* **12**: 470-476.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. 2014. nlme: Linear and nonlinear mixed effects models. R package version 3.1-117. http://CRAN.R-project.org/package=nlme.

Poorter L, Bongers F. 2006. Leaf traits are good predictors of plant performance across 53 rain forest species. *Ecology* **87**: 1733-1743.

Porras-Alfaro A, Herrera J, Natvig DO, Sinsabaugh RL. 2007. Effect of long-term nitrogen fertilization on mycorrhizal fungi associated with a dominant grass in a semiarid grassland. *Plant and Soil* **296**: 65-75.

Porter WM, Robson AD, Abbott LK. 1987. Field survey of the distribution of vesicular arbuscular mycorrhizal fungi in relation to soil pH. *Journal of Applied Ecology* **24**: 659-662.

Posada RH, Madrinan S, Rivera EL. 2012. Relationships between the litter colonization by saprotrophic and arbuscular mycorrhizal fungi with depth in a tropical forest. *Fungal Biology* **116**: 747-755.

Powell JR, Parrent JL, Hart MM, Klironomos JN, Rillig MC, Maherali H. 2009. Phylogenetic trait conservatism and the evolution of functional trade-offs in arbuscular mycorrhizal fungi. *Proceedings of the Royal Society B-Biological Sciences* **276**: 4237-4245.

Powers JS, Treseder KK, Lerdau MT. 2005. Fine roots, arbuscular mycorrhizal hyphae and soil nutrients in four neotropical rain forests: patterns across large geographic distances. *New Phytologist* **165**: 913-921.

Quince C, Lanzen A, Curtis TP, Davenport RJ, Hall N, Head IM, Read LF, Sloan WT. 2009. Accurate determination of microbial diversity from 454 pyrosequencing data. *Nature Methods* **6**: 639-U627.

R Core Team. 2014. R: A language and environment for statistical computing. http://www.R-project.org/.

R Development Core Team. 2011. R: A language and environment for statistical computing.

Ravnskov S, Larsen J, Olsson PA, Jakobsen I. 1999. Effects of various organic compounds growth and phosphorus uptake of an arbuscular mycorrhizal fungus. *New Phytologist* **141**: 517-524.

Read DJ. 1991. Mycorrhizas in ecosystems. *Experientia* **47**: 376-391.

Read DJ, Haselwandter K. 1981. Observations on the mycorrhizal status of some alpine plant communities. *New Phytologist* **88**: 341-352.

Reay DS, Dentener F, Smith P, Grace J, Feely RA. 2008. Global nitrogen deposition and carbon sinks. *Nature Geoscience* **1**: 430-437.

Reynolds HL, Hartley AE, Vogelsang KM, Bever JD, Schultz PA. 2005. Arbuscular mycorrhizal fungi do not enhance nitrogen acquisition and growth of old-field perennials under low nitrogen

supply in glasshouse culture. *New Phytologist* **167**: 869-880.

Rillig MC. 2004. Arbuscular mycorrhizae and terrestrial ecosystem processes. *Ecology Letters* **7**: 740-754.

Rillig MC, Allen MF, Klironomos JN, Chiariello NR, Field CB. 1998. Plant species-specific changes in root-inhabiting fungi in a California annual grassland: responses to elevated CO2 and nutrients. *Oecologia* **113**: 252-259.

Rillig MC, Field CB, Allen MF. 1999. Soil biota responses to long-term atmospheric CO2 enrichment in two California annual grasslands. *Oecologia* **119**: 572-577.

Rillig MC, Wright SF, Eviner VT. 2002. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. *Plant and Soil* **238**: 325-333.

Rodriguez-Girones MA, Santamaria L. 2006. A new algorithm to calculate the nestedness temperature of presence-absence matrices. *Journal of Biogeography* **33**: 924-935.

Ruotsalainen AL, Tuomi J, Vare H. 2002. A model for optimal mycorrhizal colonization along altitudinal gradients. *Silva Fennica* **36**: 681-694.

Sanders IR. 2004. Plant and arbuscular mycorrhizal fungal diversity - are we looking at the relevant levels of diversity and are we using the right techniques? *New Phytologist* **164**: 415-418.

Santiago LS, Wright SJ, Harms KE, Yavitt JB, Korine C, Garcia MN, Turner BL. 2012. Tropical tree seedling growth responses to nitrogen, phosphorus and potassium addition. *Journal of Ecology* **100**: 309-316.

Santos JC, Finlay RD, Tehler A. 2006. Molecular analysis of arbuscular mycorrhizal fungi colonising a semi-natural grassland along a fertilisation gradient. *New Phytologist* **172**: 159-168.

Schechter SP, Bruns TD. 2013. A common garden test of host-symbiont specificity supports a dominant role for soil type in determining AMF assemblage structure in *Collinsia sparsiflora*. *PloS one* **8**.

Scheu S. 1992. Automated measurement of the respiratory response of soil microcompartments - active microbial biomass in earthworm feces. *Soil Biology & Biochemistry* **24**: 1113-1118.

Schlesinger WH. 2009. On the fate of anthropogenic nitrogen. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 203-208.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF. 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* **75**: 7537-7541.

Schmidt SK, Sobieniak-Wiseman LC, Kageyama SA, Halloy SRP, Schadt CW. 2008. Mycorrhizal and dark-septate fungi in plant roots above 4270 meters elevation in the Andes and Rocky Mountains. *Arctic Antarctic and Alpine Research* **40**: 576-583.

Schnoor TK, Lekberg Y, Rosendahl S, Olsson PA. 2011. Mechanical soil disturbance as a determinant of arbuscular mycorrhizal fungal communities in semi-natural grassland. *Mycorrhiza* **21**: 211-220.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Bolchacova E, Voigt K, Crous PW, Miller AN, Wingfield MJ, Aime MC, An KD, Bai FY, Barreto RW, Begerow D, Bergeron MJ, Blackwell M, Boekhout T, Bogale M, Boonyuen N, Burgaz AR, Buyck B, Cai L, Cai Q, Cardinali G, Chaverri P, Coppins BJ, Crespo A, Cubas P P, Cummings C, Damm U, de Beer ZW, de Hoog GS, Del-Prado R, Dentinger B, Dieguez-Uribeondo J, Divakar PK, Douglas B, Duenas M, Duong TA, Eberhardt U, Edwards JE, Elshahed MS, Fliegerova K, Furtado M, Garcia MA, Ge ZW, Griffith GW, Griffiths K, Groenewald JZ, Groenewald M, Grube M, Gryzenhout M, Guo LD, Hagen F, Hambleton S, Hamelin RC, Hansen K, Harrold P, Heller G, Herrera G, Hirayama K, Hirooka Y, Ho HM, Hoffmann K, Hofstetter V, Hognabba F, Hollingsworth PM, Hong SB, Hosaka K, Houbraken J, Hughes K, Huhtinen S, Hyde KD, James T, Johnson EM, Johnson JE, Johnston PR, Jones EB, Kelly LJ, Kirk PM, Knapp DG, Koljalg U, Kovacs GM, Kurtzman CP, Landvik S, Leavitt SD, Liggenstoffer AS, Liimatainen K, Lombard L, Luangsa-Ard JJ, Lumbsch HT, Maganti H, Maharachchikumbura SS, Martin MP, May TW, McTaggart AR, Methven AS, Meyer W,

Moncalvo JM, Mongkolsamrit S, Nagy LG, Nilsson RH, Niskanen T, Nyilasi I, Okada G, Okane I, Olariaga I, Otte J, Papp T, Park D, Petkovits T, Pino-Bodas R, Quaedvlieg W, Raja HA, Redecker D, Rintoul T, Ruibal C, Sarmiento-Ramirez JM, Schmitt I, Schussler A, Shearer C, Sotome K, Stefani FO, Stenroos S, Stielow B, Stockinger H, Suetrong S, Suh SO, Sung GH, Suzuki M, Tanaka K, Tedersoo L, Telleria MT, Tretter E, Untereiner WA, Urbina H, Vagvolgyi C, Vialle A, Vu TD, Walther G, Wang QM, Wang Y, Weir BS, Weiss M, White MM, Xu J, Yahr R, Yang ZL, Yurkov A, Zamora JC, Zhang N, Zhuang WY, Schindel D, Fungal Barcoding C. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences of the United States of America* **109**: 6241-6246.

Schüßler A, Schwarzott D, Walker C. 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research* **105**: 1413-1421.

Senes-Guerrero C, Torres-Cortes G, Pfeiffer S, Rojas M, Schuessler A. 2014. Potato-associated arbuscular mycorrhizal fungal communities in the Peruvian Andes. *Mycorrhiza* **24**: 405-417.

Senés-Guerrero C, Torres-Cortés G, Pfeiffer S, Rojas M, Schüßler A. 2013. Potato-associated arbuscular mycorrhizal fungal communities in the Peruvian Andes. *Mycorrhiza*.

Setaro SD, Garnica S, Herrera PI, Suarez JP, Goker M. 2012. A clustering optimization strategy to estimate species richness of Sebacinales in the tropical Andes based on molecular sequences from distinct DNA regions. *Biodiversity and Conservation* **21**: 2269-2285.

Shearer CA, Descals E, Kohlmeyer B, Kohlmeyer J, Marvanova L, Padgett D, Porter D, Raja HA, Schmit JP, Thorton HA, Voglymayr H. 2007. Fungal biodiversity in aquatic habitats. *Biodiversity and Conservation* **16**: 49-67.

Shi ZY, Chen YL, Feng G, Liu RJ, Christie P, Li XL. 2006. Arbuscular mycorrhizal fungi associated with the Meliaceae on Hainan island, China. *Mycorrhiza* **16**: 81-87.

Sikes BA, Cottenie K, Klironomos JN. 2009. Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. *Journal of Ecology* **97**: 1274-1280.

Siqueira JO, Carneiro MAC, Curi N, Rosado SCS, Davide AC. 1998. Mycorrhizal colonization and mycotrophic growth of native woody species as related to successional groups in Southeastern Brazil. *Forest Ecology and Management* **107**: 241-252.

Smith FA, Smith SE. 1997. Tansley Review No. 96 Structural diversity in (vesicular)-arbuscular mycorrhizal symbioses. *New Phytologist* **137**: 373-388.

Smith SE, Read DJ. 2008. *Mycorrhizal symbiosis*. New York: Academic Press.

Smith SE, Smith FA 2011. Roles of arbuscular mycorrhizas in plant nutrition and growth: New paradigms from cellular to ecosystem scales. In: S. S. Merchant, W. R. BriggsD. Ort eds. *Annual Review of Plant Biology, Vol 62*. Palo Alto: Annual Reviews, 227-250.

Soethe N, Lehmann J, Engels C. 2007. Carbon and nutrient stocks in roots of forests at different altitudes in the Ecuadorian Andes. *Journal of Tropical Ecology* **23**: 319-328.

Soethe N, Lehmann J, Engels C. 2008. Nutrient availability at different altitudes in a tropical montane forest in Ecuador. *Journal of Tropical Ecology* **24**: 397-406.

Soteras F, Grilli G, Cofre MN, Marro N, Becerra A. 2015. Arbuscular mycorrhizal fungal composition in high montane forests with different disturbance histories in central Argentina. *Applied Soil Ecology* **85**: 30-37.

Soudzilovskaia NA, Douma JC, Akhmetzhanova AA, van Bodegom PM, Cornwell WK, Moens EJ, Treseder KK, Tibbett M, Wang Y-P, Cornelissen JHC. 2015. Global patterns of plant root colonization intensity by mycorrhizal fungi explained by climate and soil chemistry. *Global Ecology and Biogeography*: doi: 10.1111/geb.1227.

St. John TV, Coleman DC, Reid CPP. 1983. Association of vesicular-arbuscular mycorrhizal hyphae with soil organic particles. *Ecology* **64**: 957-959.

Staddon PL, Ramsey CB, Ostle N, Ineson P, Fitter AH. 2003. Rapid turnover of hyphae of mycorrhizal fungi determined by AMS microanalysis of C-14. *Science* **300**: 1138-1140.

Stahl PD, Parkin TB, Eash NS. 1995. Sources of error in direct microscopic methods for estimation of fungal biomass in soil. *Soil Biology & Biochemistry* **27**: 1091-1097.

Stevens CJ, Dise NB, Mountford JO, Gowing DJ. 2004. Impact of nitrogen deposition on the species richness of grasslands. *Science* **303**: 1876-1879.

Stockinger H, Kruger M, Schussler A. 2010. DNA barcoding of arbuscular mycorrhizal fungi. *New Phytologist* **187**: 461-474.

Stockinger H, Walker C, Schuessler A. 2009. 'Glomus intraradices DAOM197198', a model fungus in arbuscular mycorrhiza research, is not Glomus intraradices. *New Phytologist* **183**: 1176-1187.

Stürmer SL, Siqueira JO. 2011. Species richness and spore abundance of arbuscular mycorrhizal fungi across distinct land uses in Western Brazilian Amazon. *Mycorrhiza* **21**: 255-267.

Sundqvist MK, Liu Z, Giesler R, Wardle DA. 2014. Plant and microbial responses to nitrogen and phosphorus addition across an elevational gradient in subarctic tundra. *Ecology* **95**: 1819-1835.

Sylvia DM. 1992. Quantification of external hyphae of vesicular-arbuscular mycorrhizal fungi. *Methods in Microbiology* **24**: 53-65.

Tamatamah RA, Hecky RE, Duthie HC. 2005. The atmospheric deposition of phosphorus in Lake Victoria (East Africa). *Biogeochemistry* **73**: 325-344.

Tanner EVJ, Kapos V, Franco W. 1992. Nitrogen and phosphorus fertilization effects on Venezuelan montane forest trunk growth and litterfall. *Ecology* **73**: 78-86.

Tanner EVJ, Kapos V, Freskos S, Healey JR, Theobald AM. 1990. Nitrogen and phosphorus fertilization of Jamaican montane forest trees. *Journal of Tropical Ecology* **6**: 231-238.

Tanner EVJ, Vitousek PM, Cuevas E. 1998. Experimental investigation of nutrient limitation of forest growth on wet tropical mountains. *Ecology* **79**: 10-22.

Tawaraya K, Takaya Y, Turjaman M, Tuah SJ, Limin SH, Tamai Y, Cha JY, Wagatsuma T, Osaki M. 2003. Arbuscular mycorrhizal colonization of tree species grown in peat swamp forests of Central Kalimantan, Indonesia. *Forest Ecology and Management* **182**: 381-386.

Tedersoo L, Bahram M, Polme S, Koljalg U, Yorou NS, Wijesundera R, Villarreal Ruiz L, Vasco-Palacios AM, Pham Quang T, Suija A, Smith ME, Sharp C, Saluveer E, Saitta A, Rosas M, Riit T, Ratkowsky D, Pritsch K, Poldmaa K, Piepenbring M, Phosri C, Peterson M, Parts K, Paertel K, Otsing E, Nouhra E, Njouonkou AL, Nilsson RH, Morgado LN, Mayor J, May TW, Majuakim L, Lodge DJ, Lee SS, Larsson K-H, Kohout P, Hosaka K, Hiiesalu I, Henkel TW, Harend H, Guo L-d, Greslebin A, Grelet G, Geml J, Gates G, Dunstan W, Dunk C, Drenkhan R, Dearnaley J, De Kesel A, Tan D, Chen X, Buegger F, Brearley FQ, Bonito G, Anslan S, Abell S, Abarenkov K. 2014. Global diversity and geography of soil fungi. *Science* **346**: 1078-+.

Tedersoo L, Sadam A, Zambrano M, Valencia R, Bahram M. 2010. Low diversity and high host preference of ectomycorrhizal fungi in Western Amazonia, a neotropical biodiversity hotspot. *ISME Journal* **4**: 465-471.

Thiery O, Moora M, Vasar M, Zobel M, Oepik M. 2012. Inter- and intrasporal nuclear ribosomal gene sequence variation within one isolate of arbuscular mycorrhizal fungus, Diversispora sp. *Symbiosis* **58**: 135-147.

Tian H, Drijber RA, Zhang JL, Li XL. 2013. Impact of long-term nitrogen fertilization and rotation with soybean on the diversity and phosphorus metabolism of indigenous arbuscular mycorrhizal fungi within the roots of maize (Zea mays L.). *Agriculture Ecosystems & Environment* **164**: 53-61.

Tilman D. 1993. Species richness of experimental productivity gradients - how important is colonization limitation. *Ecology* **74**: 2179-2191.

Tilman D, Fargione J, Wolff B, D'Antonio C, Dobson A, Howarth R, Schindler D, Schlesinger WH, Simberloff D, Swackhamer D. 2001. Forecasting agriculturally driven global environmental change. *Science* **292**: 281-284.

Toljander JF, Santos-Gonzalez JC, Tehler A, Finlay RD. 2008. Community analysis of arbuscular mycorrhizal fungi and bacteria in the maize mycorrhizosphere in a long-term fertilization trial. *Fems Microbiology Ecology* **65**: 323-338.

Torti SD, Coley PD, Janos DP. 1997. Vesicular-arbuscular mycorrhizae in two tropical

monodominant trees. *Journal of Tropical Ecology* **13**: 623-629.
Townsend AR, Asner GP, Cleveland CC. 2008. The biogeochemical heterogeneity of tropical forests. *Trends in Ecology & Evolution* **23**: 424-431.
Townsend AR, Cleveland CC, Asner GP, Bustamante MMC. 2007. Controls over foliar N : P ratios in tropical rain forests. *Ecology* **88**: 107-118.
Treseder KK. 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO2 in field studies. *New Phytologist* **164**: 347-355.
Treseder KK. 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecology Letters* **11**: 1111-1120.
Treseder KK. 2013. The extent of mycorrhizal colonization of roots and its influence on plant growth and phosphorus content. *Plant and Soil* **371**: 1-13.
Treseder KK, Allen MF. 2002. Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: a model and field test. *New Phytologist* **155**: 507-515.
Treseder KK, Cross A. 2006. Global distributions of arbuscular mycorrhizal fungi. *Ecosystems* **9**: 305-316.
Treseder KK, Turner KM, Mack MC. 2007. Mycorrhizal responses to nitrogen fertilization in boreal ecosystems: potential consequences for soil carbon storage. *Global Change Biology* **13**: 78-88.
Treseder KK, Vitousek PM. 2001. Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. *Ecology* **82**: 946-954.
Tripathi P, Khare PK. 2012. Occurrence of vesicular arbuscular mycorrhizal fungi in tropical forest communities of India. *Applied Ecology and Environmental Research* **10**: 561-571.
Tu C, Booker FL, Watson DM, Chen X, Rufty TW, Shi W, Hu SJ. 2006. Mycorrhizal mediation of plant N acquisition and residue decomposition: Impact of mineral N inputs. *Global Change Biology* **12**: 793-803.
Turrini A, Giovannetti M. 2012. Arbuscular mycorrhizal fungi in national parks, nature reserves and protected areas worldwide: a strategic perspective for their in situ conservation. *Mycorrhiza* **22**: 81-97.
Unger M, Leuschner C, Homeier J. 2010. Variability of indices of macronutrient availability in soils at different spatial scales along an elevation transect in tropical moist forests (NE Ecuador). *Plant and Soil* **336**: 443-458.
Urcelay C, Acho J, Joffre R. 2011. Fungal root symbionts and their relationship with fine root proportion in native plants from the Bolivian Andean highlands above 3,700 m elevation. *Mycorrhiza* **21**: 323-330.
Urgiles N, Strauss A, Lojan P, Schussler A. 2014. Cultured arbuscular mycorrhizal fungi and native soil inocula improve seedling development of two pioneer trees in the Andean region. *New Forests* **45**: 859-874.
van Aarle IM, Olsson PA. 2003. Fungal lipid accumulation and development of mycelial structures by two arbuscular mycorrhizal fungi. *Applied and Environmental Microbiology* **69**: 6762-6767.
van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* **396**: 69-72.
Vance ED, Nadkarni NM. 1992. Root biomass distribution in a moist tropical montane forest. *Plant and Soil* **142**: 31-39.
Vandenkoornhuyse P, Husband R, Daniell TJ, Watson IJ, Duck JM, Fitter AH, Young JPW. 2002. Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. *Molecular Ecology* **11**: 1555-1564.
Vandenkoornhuyse P, Ridgway KP, Watson IJ, Fitter AH, Young JPW. 2003. Co-existing grass species have distinctive arbuscular mycorrhizal communities. *Molecular Ecology* **12**: 3085-3095.
Väre H, Vestberg M, Ohtonen R. 1997. Shifts in mycorrhiza and microbial activity along an oroarctic altitudinal gradient in northern Fennoscandia. *Arctic and Alpine Research* **29**:

93-104.
Verbruggen E, van der Heijden MGA, Weedon JT, Kowalchuk GA, Roling WFM. 2012. Community assembly, species richness and nestedness of arbuscular mycorrhizal fungi in agricultural soils. *Molecular Ecology* **21**: 2341-2353.
Veresoglou SD, Caruso T, Rillig MC. 2013. Modelling the environmental and soil factors that shape the niches of two common arbuscular mycorrhizal fungal families. *Plant and Soil* **368**: 507-518.
Veresoglou SD, Chen B, Rillig MC. 2012. Arbuscular mycorrhiza and soil nitrogen cycling. *Soil Biology & Biochemistry* **46**: 53-62.
Vitousek PM. 1984a. Litterfall, nutrient cycling and nutrient limitation in tropical forest. *Ecology* **65**: 285-298.
Vitousek PM. 1984b. Litterfall, nutrient cycling and nutrient limitation in tropical forests. *Ecology* **65**: 285-298.
Vitousek PM, Farrington H. 1997. Nutrient limitation and soil development: Experimental test of a biogeochemical theory. *Biogeochemistry* **37**: 63-75.
Vitousek PM, Porder S, Houlton BZ, Chadwick OA. 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. *Ecological Applications* **20**: 5-15.
Vitousek PM, Turner DR, Kitayama K. 1995. Foliar nutrients during long-term soil development in Hawaiian montane rainforest. *Ecology* **76**: 712-720.
Vitousek PM, Walker LR, Whiteaker LD, Matson PA. 1993. Nutrient limitations to plant-growth during primary succession in Hawaii-Volcanos-National-Park. *Biogeochemistry* **23**: 197-215.
Volkov I, Banavar JR, Hubbell SP, Maritan A. 2003. Neutral theory and relative species abundance in ecology. *Nature* **424**: 1035-1037.
Wagg C, Jansa J, Schmid B, van der Heijden MGA. 2011. Belowground biodiversity effects of plant symbionts support aboveground productivity. *Ecology Letters* **14**: 1001-1009.
Walker TW, Syers JK. 1976. Fate of phosphorus during pedogenesis. *Geoderma* **15**: 1-19.
Wamberg C, Christensen S, Jakobsen I, Muller AK, Sorensen SJ. 2003. The mycorrhizal fungus (Glomus intraradices) affects microbial activity in the rhizosphere of pea plants (Pisum sativum). *Soil Biology & Biochemistry* **35**: 1349-1357.
Wang FY, Hu JL, Lin XG, Qin SW, Wang JH. 2011. Arbuscular mycorrhizal fungal community structure and diversity in response to long-term fertilization: a field case from China. *World Journal of Microbiology & Biotechnology* **27**: 67-74.
Wehner J, Powell JR, Muller LAH, Caruso T, Veresoglou SD, Hempel S, Rillig MC. 2013. Determinants of root-associated fungal communities within Asteraceae in a semi-arid grassland.
Went FW, Stark N. 1968. Biological and mechanical role of soil fungi. *Proceedings of the National Academy of Sciences of the United States of America* **60**: 497-504.
Werner GDA, Kiers TE. 2014. Partner selection in the mycorrhizal mutualism. *New Phytologist*: doi:10.1111/nph.13113.
Whitbeck JL. 2001. Effects of light environment on vesicular-arbuscular mycorrhiza development in Inga leiocalycina, a tropical wet forest tree. *Biotropica* **33**: 303-311.
Wieder WR, Cleveland CC, Townsend AR. 2009. Controls over leaf litter decomposition in wet tropical forests. *Ecology* **90**: 3333-3341.
Wilcke W, Leimer S, Peters T, Emck P, Rollenbeck R, Trachte K, Valarezo C, Bendix J. 2013a. The nitrogen cycle of tropical montane forest in Ecuador turns inorganic under environmental change. *Global Biogeochemical Cycles* **27**: 1194-1204.
Wilcke W, Leimer S, Valarezo C 2013b. Environmental change: Increased nitrogen availability and litterfall.In. *Tropical Mountain Forest (TMF) Newsletter*: DFG Research Unit 816. 11.
Wilcke W, Oelmann Y, Schmitt A, Valarezo C, Zech W, Horneier J. 2008. Soil properties and tree growth along an altitudinal transect in Ecuadorian tropical montane forest. *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde* **171**:

220-230.

Wilcke W, Yasin S, Abramowski U, Valarezo C, Zech W. 2002. Nutrient storage and turnover in organic layers under tropical montane rain forest in Ecuador. *European Journal of Soil Science* **53**: 15-27.

Wilson GWT, Rice CW, Rillig MC, Springer A, Hartnett DC. 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. *Ecology Letters* **12**: 452-461.

Wolf K, Veldkamp E, Homeier J, Martinson GO. 2011. Nitrogen availability links forest productivity, soil nitrous oxide and nitric oxide fluxes of a tropical montane forest in southern Ecuador. *Global Biogeochemical Cycles* **25**.

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas ML, Niinemets U, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R. 2004. The worldwide leaf economics spectrum. *Nature* **428**: 821-827.

Wright RF, Rasmussen L. 1998. Introduction to the NITREX and EXMAN projects. *Forest Ecology and Management* **101**: 1-7.

Wright SJ. 2002. Plant diversity in tropical forests: a review of mechanisms of species coexistence. *Oecologia* **130**: 1-14.

Wright SJ. 2005. Tropical forests in a changing environment. *Trends in Ecology & Evolution* **20**: 553-560.

Wright SJ 2010. The future of tropical forests. In: R. S. OstfeldW. H. Schlesinger eds. *Year in Ecology and Conservation Biology 2010*. Malden: Wiley-Blackwell, 1-27.

Wright SJ, Yavitt JB, Wurzburger N, Turner BL, Tanner EVJ, Sayer EJ, Santiago LS, Kaspari M, Hedin LO, Harms KE, Garcia MN, Corre MD. 2011. Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest. *Ecology* **92**: 1616-1625.

Wu BY, Isobe K, Ishii R. 2004. Arbuscular mycorrhizal colonization of the dominant plant species in primary successional volcanic deserts on the Southeast slope of Mount Fuji. *Mycorrhiza* **14**: 391-395.

Wullaert H, Bigalke M, Homeier J, Cumbicus N, Valarezo C, Wilcke W. 2013. Short-term response of the Ca cycle of a montane forest in Ecuador to low experimental CaCl2 additions. *Journal of Plant Nutrition and Soil Science* **176**: 892-903.

Wullaert H, Homeier J, Valarezo C, Wilcke W. 2010. Response of the N and P cycles of an old-growth montane forest in Ecuador to experimental low-level N and P amendments. *Forest Ecology and Management* **260**: 1434-1445.

Xia J, Wan S. 2008. Global response patterns of terrestrial plant species to nitrogen addition. *New Phytologist* **179**: 428-439.

Yuan ZY, Chen HYH. 2012. A global analysis of fine root production as affected by soil nitrogen and phosphorus. *Proceedings of the Royal Society B-Biological Sciences* **279**: 3796-3802.

Zangaro W, Alves RdA, de Souza PB, Rostirola LV, Azevedo Marques Lescano LE, Lirio Rondina AB, Nogueira MA. 2014. Succession and environmental variation influence soil exploration potential by fine roots and mycorrhizal fungi in an Atlantic ecosystem in southern Brazil. *Journal of Tropical Ecology* **30**: 237-248.

Zangaro W, Ansanelo AP, Lescano L, Alves RD, Rondina ABL, Nogueira MA. 2012. Infection intensity, spore density and inoculum potential of arbuscular mycorrhizal fungi decrease during secondary succession in tropical Brazilian ecosystems. *Journal of Tropical Ecology* **28**: 453-462.

Zangaro W, Bononi VLR, Trufen SB. 2000. Mycorrhizal dependency, inoculum potential and habitat preference of native woody species in South Brazil. *Journal of Tropical Ecology* **16**: 603-621.

Zhu YG, Miller RM. 2003. Carbon cycling by arbuscular mycorrhizal fungi in soil-plant systems. *Trends in Plant Science* **8**: 407-409.

Contribution to the publications

I. Homeier, J., Hertel, D., Camenzind, T., Cumbicus, N.L., Maraun, M., Martinson, G.O., Poma, L.N., Rillig, M.C., Sandmann, D., Scheu, S., Veldkamp, E., Wilcke, W., Wullaert, H., Leuschner, C. (2012) Tropical andean forests are highly susceptible to nutrient inputs - rapid effects of experimental N and P addition to an ecuadorian montane forest. *PloS one* 7: e47128.

Own contribution: I added the dataset on AMF root colonization and performed statistical analyses on the whole dataset of this paper. I also wrote the respective parts in the manuscript.

II. Camenzind, T., Hempel, S., Homeier, J., Horn, S., Velescu, A., Wilcke, W., Rillig, M.C. (2014) Nitrogen and phosphorus additions impact arbuscular mycorrhizal abundance and molecular diversity in a tropical montane forest. *Global Change Biology* 20: 3646-3659.

Own contribution: I did the sampling and all morphological analyses by myself. I also performed part of the molecular laboratory work and the complete bioinformatic and statistical analyses. I wrote the manuscript.

III. Camenzind, T., Dietrich, K., Hertel, D., Homeier, J., Krohn, A., Oelmann, Y., Olsson, P.A., Suárez, J.P., Rillig, M.C. (2016) Opposing effects of nitrogen versus phosphorus additions on mycorrhizal fungal abundance along an elevational gradient in tropical montane forests. *Soil Biology & Biochemistry* 94: 37-47.

Own contribution: I performed partly the sampling in the field and the complete laboratory work on AMF analyses by myself (except for spore counts). Furthermore, I generated certain data on soil parameters (C/N and pH measurements). I did all statistical analyses and wrote the manuscript.

IV. Camenzind, T., Rillig, M.C. (2013) Extraradical arbuscular mycorrhizal fungal hyphae in an organic tropical montane forest soil. *Soil Biology & Biochemistry* 64: 96-102.

Own contribution: I conceptualized the sampling design and the applied methodologies. I did all laboratory work as well as statistical analyses and wrote the manuscript.

Appendix A

Supplementary Material for Chapter 2

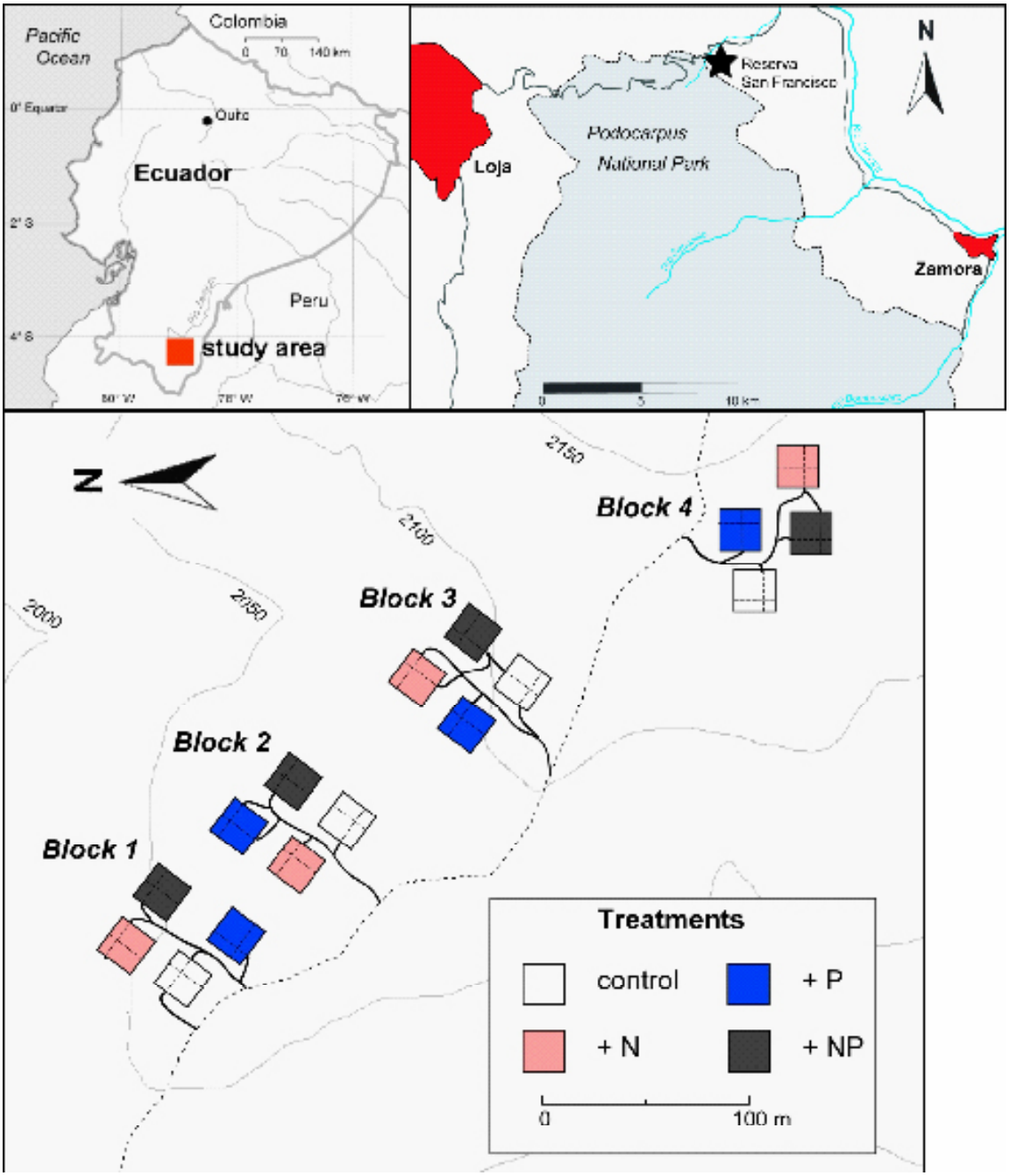


Figure S2.1. Location of the study area in southern Ecuador and outline of the Ecuadorian Nutrient Manipulation EXperiment (NUMEX). **A.** and **B.** Location of the study area in southern Ecuador. **C.** Outline of the Ecuadorian Nutrient Manipulation EXperiment (NUMEX). The study was conducted in 16 plots à 400 m² (20m x 20m) consisting of four treatments (control, N, P, N+P); the replicates were randomly arranged in four blocks at ~ 2100 m a.s.l..

Table S2.1. Soil nutrient status of the experimental plots in July 2007 before the first fertilization.

		Control	+N	+P	+NP
Org. layer depth (cm)	O-horizon	28.8 ± 10.8	26.5 ± 3.9	23.8 ± 4.5	35.0 ± 4.4
pH (H_2O)	O-horizon	3.25 ± 0.25	3.33 ± 0.17	3.28 ± 0.08	3.36 ± 0.09
	A-horizon	3.66 ± 0.12	3.69 ± 0.09	3.63 ± 0.06	3.67 ± 0.03
	B-horizon	4.05 ± 0.20	3.95 ± 0.19	3.95 ± 0.19	3.93 ± 0.21
Total C	O-horizon	42.90 ± 3.14	42.90 ± 1.13	43.78 ± 1.78	43.47 ± 0.96
(%)	A-horizon	1.05 ± 0.24	1.20 ± 0.26	1.56 ± 0.28	1.20 ± 0.40
	B-horizon	0.67 ± 0.29	0.71 ± 0.31	0.52 ± 0.02	0.95 ± 0.39
Total N	O-horizon	2.03 ± 0.14	2.11 ± 0.20	2.03 ± 0.18	2.21 ± 0.09
(%)	A-horizon	0.06 ± 0.02	0.08 ± 0.02	0.08 ± 0.02	0.08 ± 0.03
	B-horizon	0.04 ± 0.01	0.05 ± 0.02	0.04 ± 0.01	0.07 ± 0.03
C:N ratio	O-horizon	21.1 ±1.4	20.5 ±1.4	21.7 ± 1.5	19.7 ± 0.5
	A-horizon	17.0 ± 1.6	16.0 ± 1.8	19.5 ± 2.6	15.4 ± 2.6
	B-horizon	16.2 ± 4.8	14.4 ± 0.2	14.9 ± 4.8	14.7 ± 5.4
Total P	mineral topsoil				
(mg/g)	(0-10cm)	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.02	0.06 ± 0.02
	(10-20cm)	0.10 ± 0.03	0.09 ± 0.02	0.13 ± 0.05	0.12 ± 0.07
ECEC	A-horizon	3.0 ± 1.4	3.9 ± 0.8	3.5 ± 1.3	3.3 ± 0.8
(cmol c/kg)	B-horizon	3.2 ± 1.1	3.8 ± 0.5	3.5 ± 1.5	4.3 ± 1.3
Base	A-horizon	4.1 ± 3.1	3.7 ± 1.3	4.4 ± 2.6	3.4 ± 0.4
saturation (%)	B-horizon	1.8 ± 0.9	1.4 ± 0.8	1.6 ± 0.5	1.2 ± 0.7

Shown are means (± SD) of the four plots per treatment (one composite sample per plots).

Table S2.2. Effects of nutrient addition on different ecosystem parameters (Figures 2 – 6) of a tropical montane forest ecosystem.

	Time of measurement	Control	+N	+P	+NP
Org. layer N pool (Mg N ha^{-1})	April 2009	3.78 (2.96–4.40)	3.45 (2.61–4.41)	3.51 (2.50–4.66)	4.29 (3.84–4.87)
Org. layer P pool (kg P ha^{-1})	April 2009	98.6 (78.8–109.5)	92.2 (81.4–107.6)	101.9 (81.4–131.2)	125.6 (110.1–142.1)
Soil microbial biomass (µg C_{mic} g^{-1} soil dry mass)	May 2009	5881 (5826–5930)	4699 (3747–5723)	6178 (5826–6524)	5991 (5573–6749)
Respiration of soil microorganisms (µl O_2 mg C_{mic}^{-1} h^{-1})	May 2009	5.07 (4.74–6.14)	9.25 (7.26–15.2)	5.78 (4.00–6.60)	6.97 (5.03–10.83)
Net N min. (ng N cm^2 h^{-1})	September 2008	23.4 (2.8–30.4)	44.4 (15.5–67.3)	11.2 (4.1–19.3)	40.5 (28.0–55.5)
Net N nitrification (ng N cm^2 h^{-1})	September 2008	0 (0–0)	-1.68 (-5.04–0)	0 (0–0)	7.5 (0–14.9)
N_2O emission (kg N ha^{-1} yr^{-1})	Feb 2008 – Jan 2009	0.245 (0.206–0.306)	0.390 (0.232–0.572)	0.123 (0.009–0.250)	0.427 (0.231–0.564)
NH4/NO3 ratio (org. layer percolate)	Feb 2008 – Jan 2009	15.7 (4.4–28.9)	11.0 (1.6–33.6)	16.5 (0.9–27.6)	4.8 (1.5–6.8)
NH4/NO3 ratio (soil solution)	Feb 2008 – Jan 2009	8.9 (3.0–11.9)	7.8 (0.9–13.7)	6.9 (1.0–11.5)	2.5 (1.1–4.3)
Litterfall N conc. (% N)	January 2009	0.870 (0.816–0.945)	1.018 (0.943–1.182)	0.926 (0.903–0.956)	0.981 (0.924–1.064)
Litterfall P conc. (mg g^{-1})	January 2009	0.251 (0.176–0.425)	0.275 (0.241–0.335)	0.338 (0.311–0.365)	0.338 (0.260–0.392)
Annual return of N with litterfall (kg N ha^{-1} yr^{-1})	Feb 2008 – Jan 2009	41.7 (33.5–54.5)	53.0 (27.7–70.5)	43.5 (32.9–45.7)	54.2 (44.5–62.4)
Annual return of P with litterfall (kg P ha^{-1} yr^{-1})	Feb 2008 – Jan 2009	1.48 (1.14–2.18)	1.85 (0.94–2.77)	1.80 (1.18–2.33)	1.95 (1.61–2.33)
Annual return of N with throughfall (kg N ha^{-1} yr^{-1})	Feb 2008 – Jan 2009	10.09 (8.15–12.44)	11.47 (10.02–12.61)	10.64 (10.10–11.29)	10.92 (10.50–11.47)
Annual return of P with throughfall (kg P ha^{-1} yr^{-1})	Feb 2008 – Jan 2009	0.151 (0.098–0.233)	0.158 (0.132–0.187)	0.210 (0.140–0.283)	0.290 (0.203–0.370)
Org. layer percolate N (kg N ha^{-1} yr^{-1})	Feb 2008 – Jan 2009	14.65 (12.53–18.08)	22.51 (19.07–35.59)	14.16 (12.68–15.14)	18.37 (16.99–19.58)
Org. layer percolate P (kg P ha^{-1} yr^{-1})	Feb 2008 – Jan 2009	0.13 (0.09–0.19)	0.20 (0.11–0.31)	0.21 (0.10–0.44)	0.24 (0.12–0.39)

Table S2: continued

	Time of measurement	Control	+N	+P	+NP
Min. soil solution N (kg N ha^{-1} yr^{-1})	Feb 2008 – Jan 2009	3.26 (2.08–4.88)	3.92 (3.48–4.48)	3.55 (2.72–4.46)	3.70 (2.96–4.24)
Min. soil solution P (kg P ha^{-1} yr^{-1})	Feb 2008 – Jan 2009	0.03 (0.02–0.04)	0.04 (0.03–0.06)	0.02 (0.02–0.03)	0.03 (0.02–0.06)
LAI change (%)	Jan 2008 – Jan 2009	2.6 (-5.5–14.5)	10.9 (1.1–20.9)	11.7 (-4.7–24.7)	5.3 (-8.2–18.9)
Tree basal area increment (m^2 ha^{-1})	Feb 2008 – Jan 2009	0.111 (0.081–0.162)	0.109 (0.073–0.147)	0.120 (0.052–0.175)	0.160 (0.109–0.222)
Leaf litter production (Mg ha^{-1} yr^{-1})	Feb 2008 – Jan 2009	3.46 (2.71–4.75)	3.65 (2.63–4.90)	3.28 (2.44–4.47)	3.99 (3.43–4.42)
Fine root biomass (g m^{-2})	January 2009	443 (337–567)	375 (277–474)	320 (245–398)	346 (180–434)
Fine root necromass (g m^{-2})	January 2009	426 (307–527)	447 (370–578)	607 (570–631)	441 (423–468)
Mycorrhiza colonization (%)	January 2009	53.3 (41.5–70.7)	49.9 (34.6–66.4)	42.8 (10.2–63.0)	52.6 (39.4–59.4)
N use efficiency (g g^{-1} N)	January 2009	117 (106–124)	100 (86–107)	110 (106–114)	104 (97–109)
P use efficiency (g g^{-1} P)	January 2009	4751 (2441–5895)	4181 (3056–4929)	3505 (2954–4200)	3388 (2577–4306)

Shown are means and ranges (in parentheses) of the four replicates (plots) per treatment. The nutrient manipulation experiment started in January 2008.

Appendix B

Supplementary Material for Chapter 3

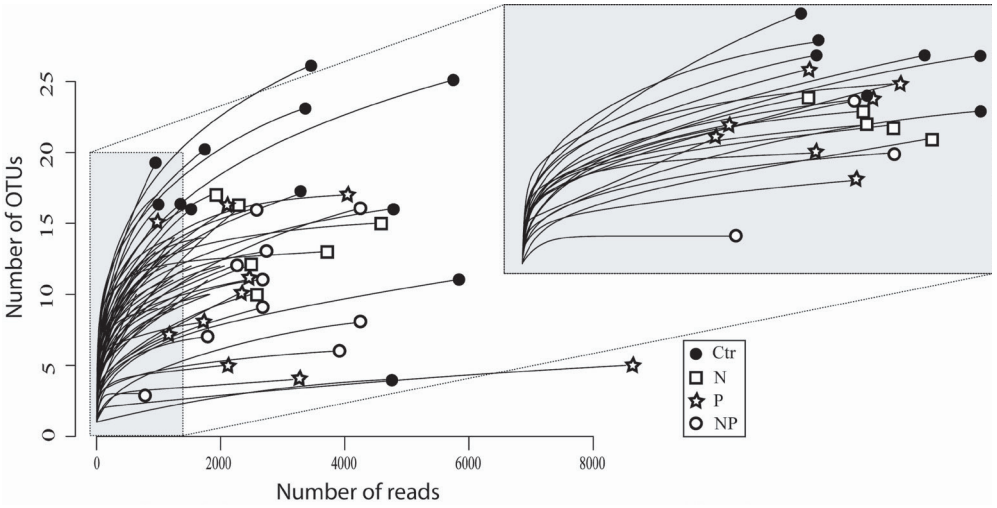


Fig. S3.1: Lines represent rarefaction curves drawn separately for every sample - plotting the number of OTUs (operational taxonomic units) versus read numbers. Control treatments (closed circles) as well as nitrogen (open squares), phosphorus (open asterisks) and combined treatments (open circles) are illustrated with the respective symbols. Samples with read numbers below 1600 sequences were plotted separately again.

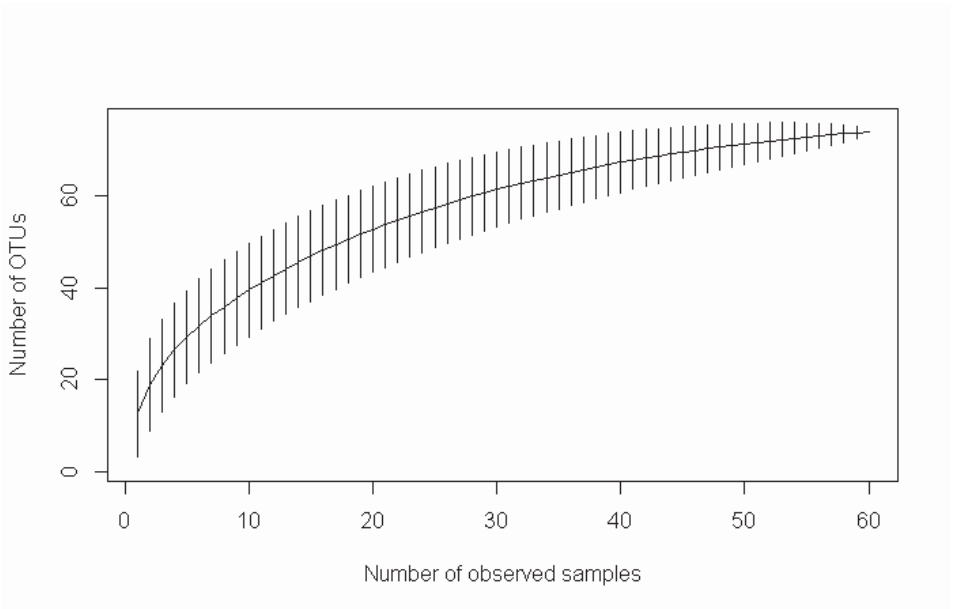
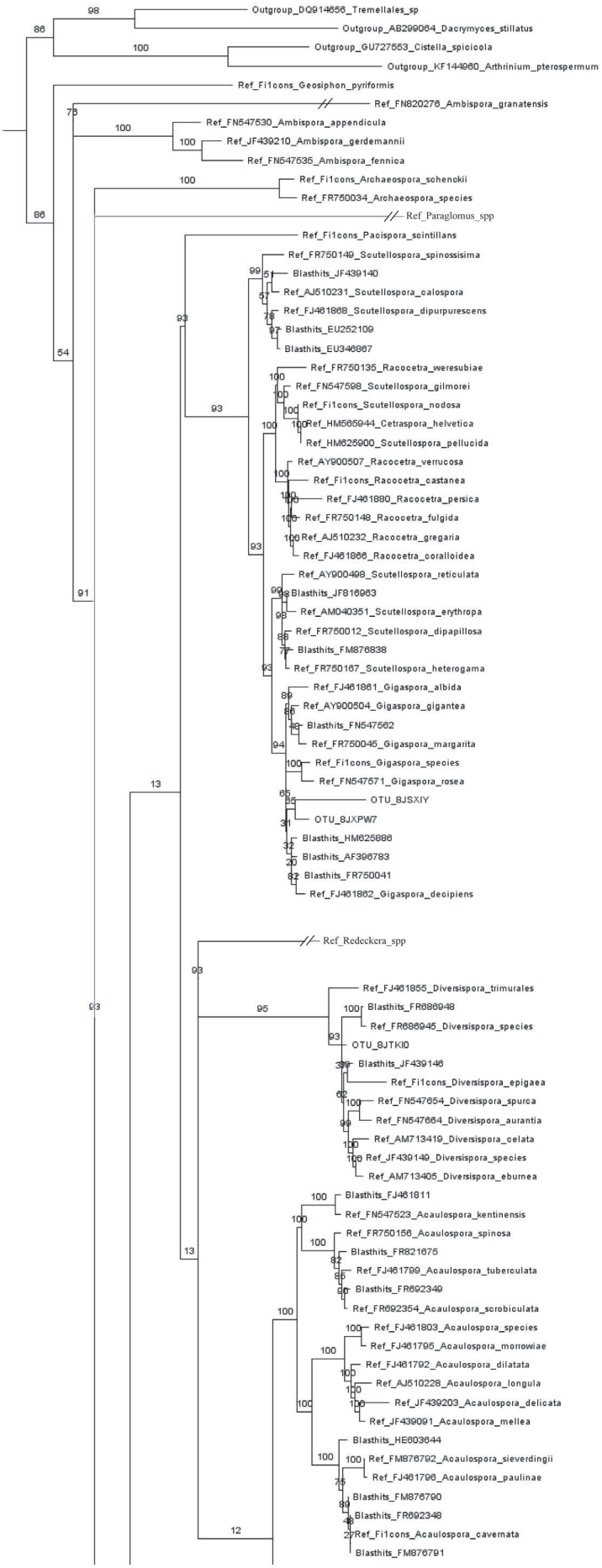


Fig. S3.2: Rarefaction curve of the number of OTUs (operational taxonomic units) in non-resampled data plotted against the number of sites sampled.


Outgroup_DQ914656_Tremellales_sp
Outgroup_AB299064_Dacrymyces_stillatus
Outgroup_GU727553_Cistella_spicicola
Outgroup_KF144960_Arthrinium_pterospermum
Ref_Fi1cons_Geosiphon_pyriformis
Ref_FN820276_Ambispora_granatensis
Ref_FN547530_Ambispora_appendicula
Ref_JF439210_Ambispora_gerdemannii
Ref_FN547535_Ambispora_fennica
Ref_Fi1cons_Archaeospora_schenckii
Ref_FR750034_Archaeospora_species
Ref_Paraglomus_spp
Ref_Fi1cons_Pacispora_scintillans
Ref_FR750149_Scutellospora_spinossisima
Blasthits_JF439140
Ref_AJ510231_Scutellospora_calospora
Ref_FJ461868_Scutellospora_dipurpurescens
Blasthits_EU252109
Blasthits_EU348867
Ref_FR750135_Racocetra_weresubiae
Ref_FN547598_Scutellospora_gilmorei
Ref_Fi1cons_Scutellospora_nodosa
Ref_HM585944_Cetraspora_helvetica
Ref_HM625900_Scutellospora_pellucida
Ref_AY900507_Racocetra_verrucosa
Ref_Fi1cons_Racocetra_castanea
Ref_FJ461880_Racocetra_persica
Ref_FR750148_Racocetra_fulgida
Ref_AJ510232_Racocetra_gregaria
Ref_FJ461866_Racocetra_coralloidea
Ref_AY900498_Scutellospora_reticulata
Blasthits_JF816963
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Ref_FR750012_Scutellospora_dipapillosa
Blasthits_FM876838
Ref_FR750167_Scutellospora_heterogama
Ref_FJ461861_Gigaspora_albida
Ref_AY900504_Gigaspora_gigantea
Blasthits_FN547562
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Ref_Fi1cons_Gigaspora_species
Ref_FN547571_Gigaspora_rosea
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OTU_8JXPW7
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Ref_FN547654_Diversispora_spurca
Ref_FN547664_Diversispora_aurantia
Ref_AM713419_Diversispora_celata
Ref_JF439149_Diversispora_species
Ref_AM713405_Diversispora_eburnea
Blasthits_FJ461811
Ref_FN547523_Acaulospora_kentinensis
Ref_FR750156_Acaulospora_spinosa
Blasthits_FR821675
Ref_FJ461799_Acaulospora_tuberculata
Blasthits_FR692349
Ref_FR692354_Acaulospora_scrobiculata
Ref_FJ461803_Acaulospora_species
Ref_FJ461795_Acaulospora_morrowiae
Ref_FJ461792_Acaulospora_dilatata
Ref_AJ510228_Acaulospora_longula
Ref_JF439203_Acaulospora_delicata
Ref_JF439091_Acaulospora_mellea
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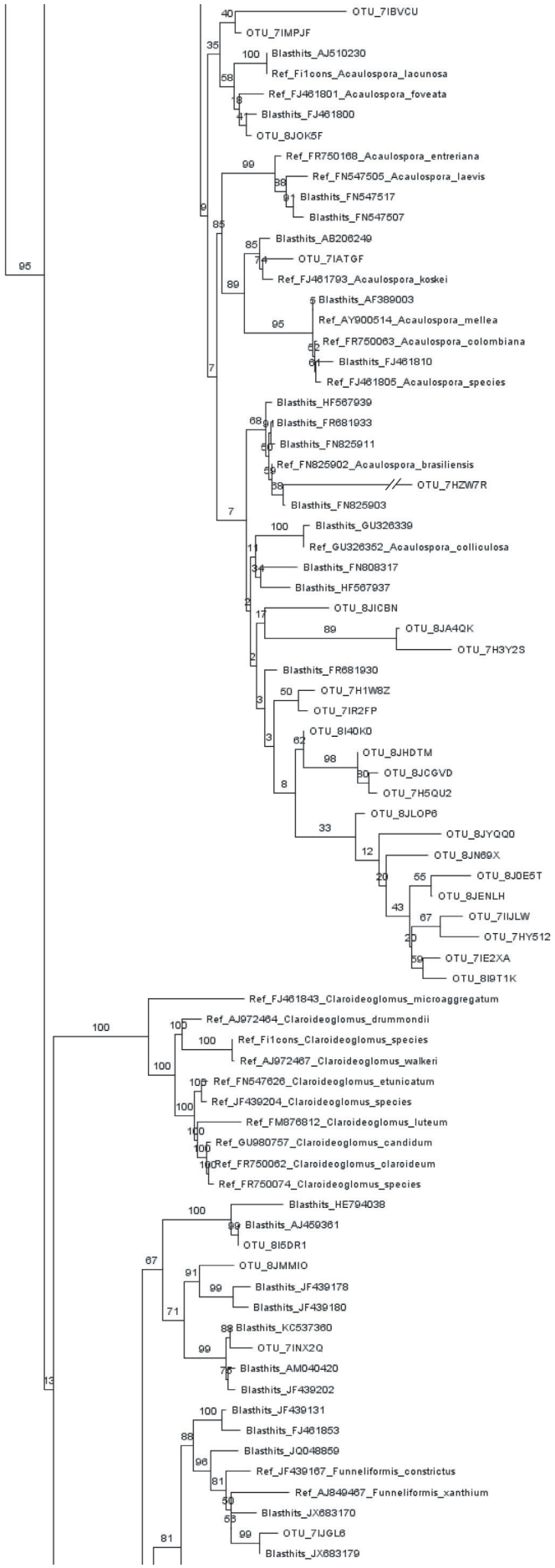
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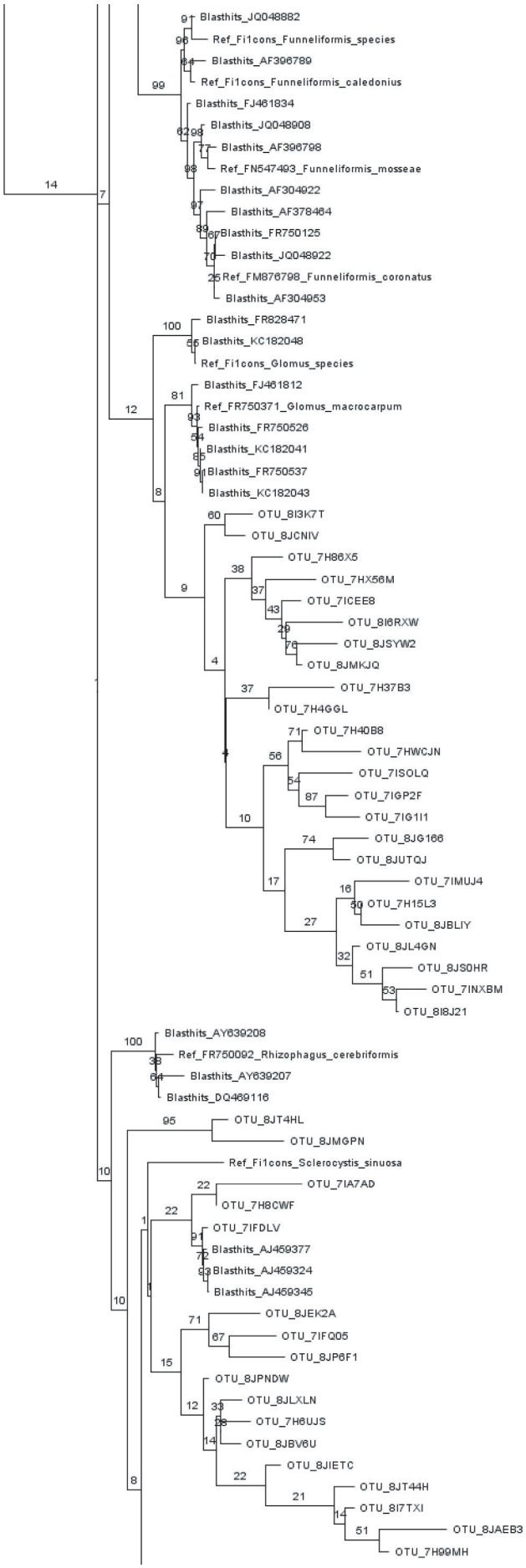
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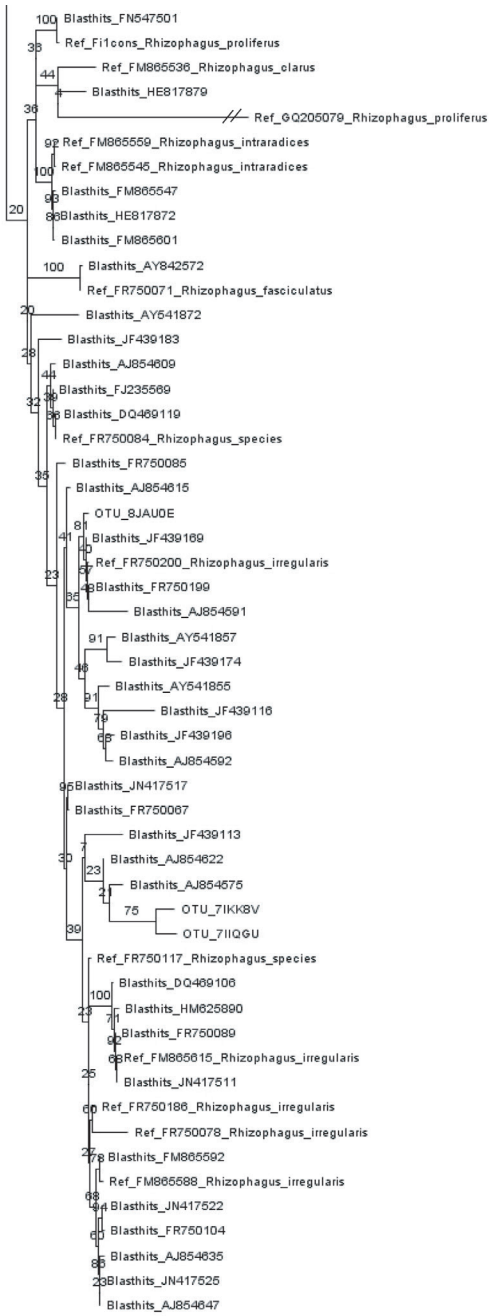

Fig. S3.3: Complete maximum likelihood tree including bootstrap supports

Table S3.1: Mean numbers of reads and read lengths summarized per treatment (mean ± standard deviation) are presented. Read numbers include all Glomeromycota sequences included in the analysis before resampling. Read lengths were extracted from flowgrams generated in MOTHUR already subjected to quality control, encompassing all sequences of samples included in the analysis.

Treatment	Read numbers	Read lengths (bp)
Ctr	2549 ± 1695	375 ± 32
N	2069 ± 1045	393 ± 31
P	2124 ± 1813	377 ± 38
NP	2414 ± 1125	382 ± 38

Table S3.2: Detailed statistical results of nitrogen (N) and phosphorus (P) addition effects on AMF species richness (number of OTUs (operational taxonomic units)) and AMF root colonization (two-way linear-mixed effects model). N:P refers to the interaction term given by the full factorial analysis. The broad taxonomic classification into Diversisporales and Glomerales is based on their placement within a maximum-likelihood tree.

OTUs

	Richness				Shannon´s H			
	Estimate	Std. Error	t-value	p-value	Estimate	Std. Error	t-value	p-value
N	-3.034	1.069	-2.838	**0.005**	-0.313	0.162	-1.930	*0.054*
P	-4.587	0.987	-4.647	**<0.001**	-0.305	0.150	-2.033	**0.042**
N:P	2.642	1.483	1.781	*0.075*	0.166	0.225	0.737	0.46

OTUs$_{97}$

	Richness				Shannon´s H			
	Estimate	Std. Error	t-value	p-value	Estimate	Std. Error	t-value	p-value
N	-6.277	2.162	-2.903	**0.004**	-0.3352	0.1857	-1.805	*0.071*
P	-9.2	1.995	-4.612	**<0.001**	-0.4444	0.1717	-2.588	**0.009**
N:P	5.738	2.998	1.914	*0.056*	0.2732	0.2579	1.059	**0.046**

□□□□□**s**□**s**□□□□□□**s**

	Richness				□□undance			
	Estimate	Std. Error	t-value	p-value	Estimate	Std. Error	t-value	p-value
N	-1.512	0.667	-2.270	**0.02**□	-119.06	94.34	-1.262	0.21
P	-1.093	0.618	-1.768	*0.0*□	124.92	87.04	1.435	0.15
N:P	0.951	0.923	1.031	0.3	18.62	130.81	0.142	0.89

□□□□□□□□**s**

	Richness				□□undance			
	Estimate	Std. Error	t-value	p-value	Estimate	Std. Error	t-value	p-value
N	-1.590	0.966	-1.646	*0.1*	125.97	101.29	1.244	0.21
P	-3.889	0.891	-4.364	**<0.001**	-122.11	93.45	-1.307	0.19
N:P	1.812	1.339	1.353	0.18	-25.74	140.45	-0.183	0.86

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	Estimate	Std. Error	t-value	p-value	Estimate	Std. Error	t-value	p-value
N	-1.1466	0.4542	-2.524	**0.012**	-0.62153	0.28758	-2.161	**0.0□1**
P	0.1393	0.4595	0.303	0.76	0.03781	0.28912	0.131	0.9
N:P	0.4244	0.6461	0.657	0.51	0.56376	0.40675	1.386	0.17

Table S3.3: Results of the multivariate analyses of AMF community composition in response to nitrogen (N) and phosphorus (P) additions, presented on the basis of operational taxonomic units (OTUs). *P*-values are based on a two-way permutational multivariate analysis of variance (perMANOVA), including spatial eigenvectors as covariates. Differences in community composition are expressed as Bray-Curtis dissimilarity, a distance index of abundance data, and Jaccard similarity coefficient based on the presence/absence community matrix. N:P refers to the interaction term given by the two-way analysis.

OTU matrix

	Bray-Curtis (abundance)			Jaccard (pres/abs)		
	pseudo-F	R^2-value	*P*-value	pseudo-F	R^2-value	*P*-value
N	1.28	0.021	0.28	0.94	0.016	0.46
P	1.38	0.023	0.22	2.22	0.037	**0.015**
N:P	0.80	0.013	0.59	1.03	0.017	0.41

OTU_{97} matrix

	Bray-Curtis (abundance)			Jaccard (pres/abs)		
	pseudo-F	R^2-value	*P*-value	pseudo-F	R^2-value	*P*-value
N	1.27	0.021	0.25	1.18	0.02	0.25
P	1.76	0.029	*0.09*	2.00	0.034	**0.005**
N:P	1.13	0.019	0.33	0.96	0.016	0.49

Table S3.4: Important environmental parameters included in the statistical analysis are displayed, showing the mean values per treatment (control (Ctr), nitrogen (N) and phosphorus (P) additions as well as the combined treatment (NP)) ± standard deviation. Subscript "Lys" depicts measurements originating from litter leachate samples. The subscript "org" refers to total element concentrations measured in the organic layer. Lys results are representative for the whole year 2010 and organic layer samples were collected in 2009.

Treatment	N_{org} (%)	P_{org} (mg kg^{-1})	Ca_{org} (mg kg^{-1})	Zn_{org} (mg kg^{-1})	pH_{org}	C/N_{org}	C/S_{org}	C/P_{org}
Ctr	0.96 ± 0.05	344 ± 31	1405 ± 198	22.9 ± 3	4.56	48.6 ± 4	1543 ± 72	1345 ± 153
N	1.14 ± 0.10	358 ± 23	1263 ± 306	24.3 ± 3	4.48	42.3 ± 3	1615 ± 491	1407 ± 122
P	1.00 ± 0.06	478 ± 50	2126 ± 873	23.5 ± 3	4.61	46.2 ± 3	1368 ± 389	1027 ± 137
NP	1.13 ± 0.06	484 ± 41	1915 ± 777	25.7 ± 4	4.60	43.6 ± 2	1292 ± 206	1075 ± 167

Treatment	$PO4_{Lys}$ (mg l^{-1})	P_{Lys} (mg l^{-1})	NH_{4Lys} (mg l^{-1})	NO_{3Lys} (mg l^{-1})	TOC_{Lys} (mg l^{-1})	pH_{Lys}
Ctr	0.060 ± 0.02	0.44 ± 0.1	0.89 ± 0.04	0.05 ± 0.03	50.3 ± 16	4.00
N	0.069 ± 0.05	0.46 ± 0.3	1.03 ± 0.19	0.11 ± 0.01	45.2 ± 6	4.03
P	0.144 ± 0.16	0.57 ± 0.2	0.95 ± 0.03	0.05 ± 0.01	41.9 ± 8	4.09
NP	0.082 ± 0.03	0.52 ± 0.4	1.05 ± 0.18	0.10 ± 0.03	52.5 ± 9.3	3.99

Table S3.5: Detailed output of envfit() (R package "vegan") – plotting variables onto the ordination of the redundancy analysis, modeling the effect of nitrogen and phosphorus additions on presence/absence AMF community data including spatial eigenvectors as conditional parameters. Subscript "Lys" depicts measurements originating from litter leachate samples, "org" total element concentrations measured in the organic layer.

Vectors	RDA1	RDA2	r2	**Pr(>r)**
Alchornea_lojaensis	-0.87762	0.479364	0.0675	0.132
Dictyocaryum_lamarckianum	-0.99527	-0.09718	0.0464	0.27
Graffenrieda_emarginata	-0.98464	-0.17461	0.0595	0.159
Hieronyma_fendleri	0.969292	-0.24591	0.0413	0.313
Myrcia_sp	-0.39742	0.917636	0.0051	0.881
dist$x	0.899678	-0.43656	0.0016	0.941
dist$y	0.764325	-0.64483	0.0461	0.259
N_{org}	0.752794	-0.65826	0.1495	**0.009**
P_{org}	0.999865	-0.01643	0.3635	**0.001**
Ca_{org}	0.959652	-0.28119	0.1066	**0.045**
Zn_{org}	0.848893	-0.52857	0.0467	0.276
pH_{org}	0.929828	0.367995	0.0097	0.764
C/N_{org}	-0.87003	0.492996	0.1422	**0.015**
C/S_{org}	-0.90788	-0.41923	0.0265	0.483
C/O_{org}	-0.99665	-0.08174	0.2866	**0.001**
PO_{4Lys}	0.744896	-0.66718	0.0363	0.373
P_{Lys}	0.983219	0.182432	0.0461	0.277
NH_{4Lys}	0.833928	-0.55187	0.1186	**0.046**
NO_{3Lys}	0.201034	-0.97958	0.2172	**0.002**
TOC_{Lys}	-0.12216	-0.99251	0.0215	0.556
pH_{Lys}	0.662991	0.748627	0.0401	0.348

Appendix C

Supplementary Material for Chapter 4

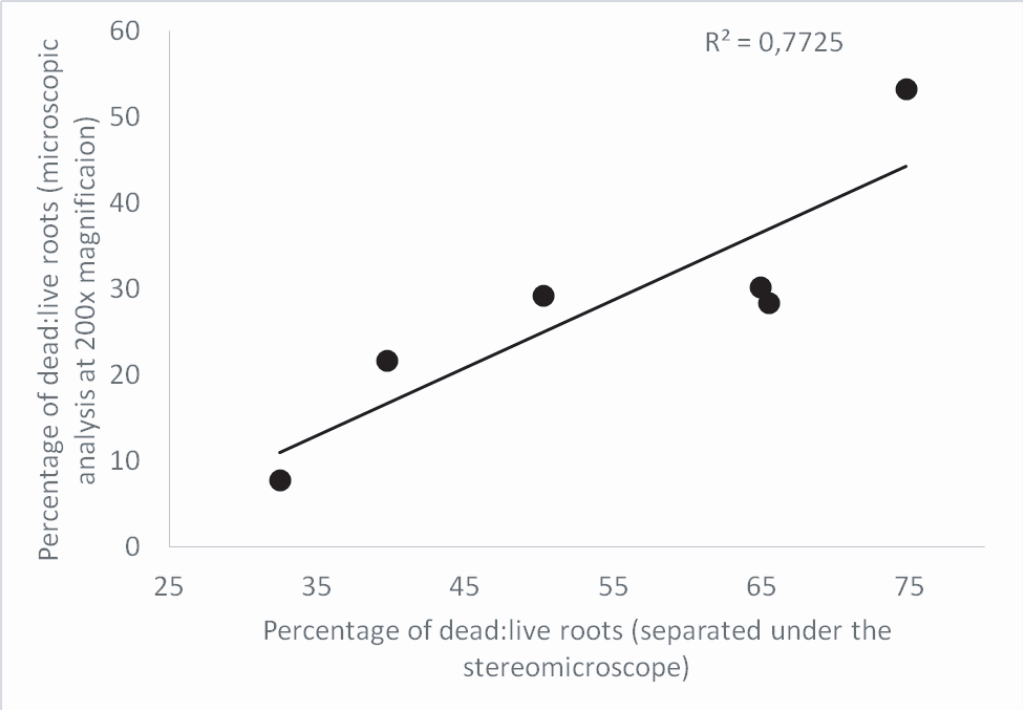

Fig. S4.1: Correlation of the two different applied methodologies to separate live and dead roots. Six samples were used for direct comparisons.

Table S4.1: Effects of nitrogen (N) and phosphorus (P) additions on intra- and extraradical AMF abundance as well as root length along an altitudinal gradient.

1000m

	F-value						
	N df = 1,9	**P** df = 1,9	**N:P** df = 1,9	**Ctr**	**N**	**P**	**NP**
Root length (cm cm^{-3} soil)	0.3	0.3	0.2	0.76 ± 0.16	1.09 ± 0.20	0.71 ± 0.17	0.86 ± 0.16
Colonized root length by AMF (cm cm^{-3} soil)	0	0.8	0.1	0.20 ± 0.07	0.17 ± 0.05	0.22 ± 0.06	0.28 ± 0.07
AMF root colonization (%)	0.2	3.9	1.8	25.4 ± 4.8	15.4 ± 2.9	29.0 ± 3.9	34.3 ± 4.0
Extraradical AMF biomass (NLFA 16:15 nmol cm^{-3} soil)	2.8	6.5*	0.7	1.92 ± 0.15	1.76 ± 0.16	2.55 ± 0.19*	2.08 ± 0.14
AMF spore abundance (spores cm^{-3} soil)	-	-	-	184 ± 23	-	138 ± 12	-
AMF hyphal length (m cm^{-3} soil)	-	-	-	12.4 ± 1.6	-	10.7 ± 1.75	-

2000m

	F-value						
	N df = 1,9	**P** df = 1,9	**N:P** df = 1,9	**Ctr**	**N**	**P**	**NP**
Root length (cm cm^{-3} soil)	3.5	0.1	1	0.86 ± 0.18	1.68 ± 0.24·	1.31 ± 0.29	1.47 ± 0.20
Colonized root length by AMF (cm cm^{-3} soil)	0	2.3	2.6	0.39 ± 0.08	0.63 ± 0.11	0.75 ± 0.15	0.66 ± 0.17
AMF root colonization (%)	8.3*	4.1	2.4	46.0 ± 3.9	40.4 ± 4.1	61.3 ± 3.4*	42.4 ± 5.4
Extraradical AMF biomass (NLFA 16:15 nmol cm^{-3} soil)	2.4	136***	3.6	6.63 ± 0.51	4.81 ± 0.41*	16.86 ± 1.44***	18.68 ± 2.12***
AMF spore abundance (spores cm^{-3} soil)	-	-	-	92 ± 15	-	78 ± 21	-
AMF hyphal length (m cm^{-3} soil)	-	-	-	19.2 ± 1.5	-	19.3 ± 1.4	-

	3000m						
	F-value						
	N df=1,9	P df=1,9	N:P df=1,9	Ctr	N	P	NP
Root length (cm cm^{-3} soil)	0.1	0.8	2	1.51 ± 0.24	0.67 ± 0.17	1.00 ± 0.22	1.30 ± 0.38
Colonized root length by AMF (cm cm^{-3} soil)	0.1	0	4.5	0.87 ± 0.14	0.45 ± 0.14	0.51 ± 0.11	0.85 ± 0.30
AMF root colonization (%)	0.4	0.3	0	61.1 ± 4.5	58.8 ± 3.9	59.3 ± 4.0	55.8 ± 4.8
Extraradical AMF biomass (NLFA 16:15 nmol cm^{-3} soil)	0.1	0.1	2.2	6.10 ± 0.60	7.84 ± 0.86	7.81 ± 0.77	6.75 ± 0.80
AMF spore abundance (spores cm^{-3} soil)	-	-	-	-	-	-	-
AMF hyphal length (m cm^{-3} soil)	-	-	-	-	-	-	-

[1] Mean values ± standard errors are presented.

[2] Asterisks indicate significant differences compared to the control treatment within the respective study site (linear mixed-effects model; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Supplementary Material S4.2:

Detailed description of the quantification of AMF hyphae and spores

Spore abundance and hyphal length were evaluated at 1000 and 2000m in control and phosphorus fertilized plots. In all cases roots were extracted from soil prior to measurements.

In the mineral soil at 1000m standard methods were applied: Hyphae were extracted by a modified aqueous filtration extraction method (Bardgett, 1991; Camenzind & Rillig, 2013). 5 g of soil was soaked in water, 80 ml of sodium hexametaphosphate (39g l^{-1}) added and shaken vigorously. After one hour, soil material (including hyphae) kept on a 20 µm sieve were collected, stirred and a 2ml aliquot was transferred to a membrane filter (0.45 µm pore size) and stained with 0.05% Trypan Blue. Hyphae were quantified at 200x magnification using the grindline-intersects method (Tennant, 1975). Spores were isolated from 8 g of soil by a modified wet-sieving and decanting method

(Gerdemann & Nicolson, 1963) followed by centrifugation in a 20/60% sucrose gradient (Daniels & Skipper, 1982). In order to detach spores from soil material 200 ml sodium hexametaphosphate (20g l^{-1}) were added to soil samples, blended and left for 30 minutes. Additionally, before wet-sieving two minutes UV were applied. Material was poured through a 500 µm mesh and material kept on a 38 µm sieve used for sucrose centrifugation. Spores were collected on a filter paper and counted under the dissecting microscope. We did not observe detrimental effects of blending and UV application on spores.

In the organic layer at 2000m methods had to be adopted to this substrate, mainly consisting of decomposing plant material (see Camenzind & Rillig, 2013): Hyphae were quantified based on a method originally described for hyphal quantification in tropical leaf litter (Aristizabal *et al.*, 2004; Camenzind & Rillig, 2013), counting AMF hyphae directly in stained soil. 2 g of soil was bleached in 20% H_2O_2 for 30 minutes, blended and poured through a 500 µm sieve directly onto a filter paper (pore size: 4-7 µm). After drying two subsamples were transferred to a slide and directly stained with 0.05% Trypan Blue. Hyphae were quantified at 200x magnification using the gridline-intersects method (Tennant, 1975). For spore quantification, a similar protocol as described above was applied. However, during wet-sieving an additional 250 µm mesh was used in addition to 500 µm, in order to exclude large litter fragments which impede the centrifugation step. Both fractions were checked for AMF spores, however, spores kept on the 250 µm mesh were negligible.

Discussion on applied methodologies

Standard methods on the measurement of AMF abundance have been developed in temperate systems, mainly grasslands. Thus, especially in the organic layer of tropical montane forest, consisting of hardly decomposed plant material, methods have to be adopted to this special substrate and the validity of methods tested thoroughly (Camenzind & Rillig, 2013).

We found opposing trends in the responses to nutrient additions measuring different components of the extraradical AMF biomass: whereas the amount of NLFA 16:1ω5 reacted strongly to P additions, spore abundance as well as hyphal length showed no response. Likewise, spore numbers were lower at 2000m compared to the 1000m site, contrary to the general increase in AMF abundance with elevation. Opposing trends in spore abundances might be related to species-specific sporulation patterns regulated by environmental conditions and not inherently correlated with AMF abundance (Allen *et al.*, 1998; Sanders, 2004). Still, the lack of correlation between NLFA 16:1ω5 biomass and spore numbers, but

also extraradical hyphal length raise doubts about the significance of NLFA 16:1ω5 in this study system, though we found a significant correlation of NLFA 16:1ω5 and intraradical AMF abundance at 1000 and 2000m. The applicability of NLFA 16:1ω5 has been extensively investigated under controlled conditions (Olsson, 1999; Olsson & Wilhelmsson, 2000), where direct correlations with microscopic spore counts and hyphal measurements were demonstrated (Olsson *et al.*, 1997; Olsson *et al.*, 1998). Nevertheless, available correlations in natural systems reveal more heterogeneous results, even partly resulting in negative trends (Balser *et al.*, 2005; Gryndler *et al.*, 2006; Ngosong *et al.*, 2012). Lekberg *et al.* (2013) proposed an interesting hypothesis that NLFA 16:1ω5 amounts rather reflect the storage status of AMF instead of total biomass, which fits the potential mechanism suggested here of alleviated fungal nutrient deficiency following P additions. Nevertheless, experiments under more controlled conditions are needed to critically test the significance of NLFA 16:1ω5 in this system, also to eliminate the possibility of background noise as found in PLFA marker 16:1ω5 (Larsen *et al.*, 1998; van Aarle & Olsson, 2003), since we observed parallel to NLFA 16:1ω5 an increase and decrease of other NLFA markers as 16:0, 18:2ω6 and 18:1ω9 following P and N additions, respectively (data not shown).

Allen EB, Rincon E, Allen MF, Perez-Jimenez A, Huante P. 1998. Disturbance and seasonal dynamics of mycorrhizae in a tropical deciduous forest in mexico. *Biotropica* **30**: 261-274.

Aristizabal C, Rivera EL, Janos DP. 2004. Arbuscular mycorrhizal fungi colonize decomposing leaves of myrica parvifolia, m-pubescens and paepalanthus sp. *Mycorrhiza* **14**: 221-228.

Balser TC, Treseder KK, Ekenler M. 2005. Using lipid analysis and hyphal length to quantify am and saprotrophic fungal abundance along a soil chronosequence. *Soil Biology & Biochemistry* **37**: 601-604.

Bardgett RD. 1991. The use of the membrane-filter technique for comparative measurements of hyphal lengths in different grassland sites. *Agriculture Ecosystems & Environment* **34**: 115-119.

Camenzind T, Rillig MC. 2013. Extraradical arbuscular mycorrhizal fungal hyphae in an organic tropical montane forest soil. *Soil Biology & Biochemistry* **64**: 96-102.

Daniels BA, Skipper HD 1982. Methods for the recovery and quantitative estimation of propagules from soil. In: N. C. Schenck ed. *Methods and principles of mycorrhizal research.* St Paul, Minnesota: American Phytopathological Society, 29-36.

Gerdemann JW, Nicolson TH. 1963. Spores of mycorrhizal *endogone* species extracted from soil by wet-sieving and decanting. *Transactions of the British Mycological Society* **46**: 235-244.

Gryndler M, Larsen J, Hrselova H, Rezacova V, Gryndlerova H, Kubat J. 2006. Organic and mineral fertilization, respectively, increase and decrease the development of external mycelium of arbuscular mycorrhizal fungi in a long-term field experiment. *Mycorrhiza* **16**: 159-166.

Larsen J, Olsson PA, Jakobsen I. 1998. The use of fatty acid signatures to study mycelial

interactions between the arbuscular mycorrhizal fungus glomus intraradices and the saprotrophic fungus fusarium culmorum in root-free soil. *Mycological Research* **102**: 1491-1496.

Lekberg Y, Rosendahl S, Michelsen A, Olsson PA. 2013. Seasonal carbon allocation to arbuscular mycorrhizal fungi assessed by microscopic examination, stable isotope probing and fatty acid analysis. *Plant and Soil* **368**: 547-555.

Ngosong C, Gabriel E, Ruess L. 2012. Use of the signature fatty acid 16:1omega5 as a tool to determine the distribution of arbuscular mycorrhizal fungi in soil. *Journal of lipids* **2012**: 236807.

Olsson PA. 1999. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. *Fems Microbiology Ecology* **29**: 303-310.

Olsson PA, Baath E, Jakobsen I. 1997. Phosphorus effects on the mycelium and storage structures of an arbuscular mycorrhizal fungus as studied in the soil and roots by analysis of fatty acid signatures. *Applied and Environmental Microbiology* **63**: 3531-3538.

Olsson PA, Francis R, Read DJ, Soderstrom B. 1998. Growth of arbuscular mycorrhizal mycelium in calcareous dune sand and its interaction with other soil microorganisms as estimated by measurement of specific fatty acids. *Plant and Soil* **201**: 9-16.

Olsson PA, Wilhelmsson P. 2000. The growth of external am fungal mycelium in sand dunes and in experimental systems. *Plant and Soil* **226**: 161-169.

Sanders IR. 2004. Plant and arbuscular mycorrhizal fungal diversity - are we looking at the relevant levels of diversity and are we using the right techniques? *New Phytologist* **164**: 415-418.

Tennant D. 1975. Test of a modified line intersect method of estimating root length. *Journal of Ecology* **63**: 995-1001.

van Aarle IM, Olsson PA. 2003. Fungal lipid accumulation and development of mycelial structures by two arbuscular mycorrhizal fungi. *Applied and Environmental Microbiology* **69**: 6762-6767.

Appendix D

Supplementary Material for Chapter 5

Material S5.1: Detailed descriptions of the hyphal quantification protocols tested

Modified aqueous filtration extraction method: This standard protocol is a slightly modified aqueous filtration extraction method (Bardgett, 1991). 80 ml of sodium hexametaphosphate (39g l^{-1}) were added to 1.5 – 3 g of soil and shaken vigorously for 30 seconds. The sample was directly sonicated for 20 seconds as proposed by Treseder et al. (2007) and left for 1 hour. Hyphae and substrate kept on a 38 µm mesh were filled up to 200 ml into an Erlenmeyer flask, stirred for 5 min. and after 90 seconds a 4 ml aliquot was transferred on a 25 mm Millipore-filter paper and stained with 0.05% Trypan Blue. Hyphae were counted at 200x magnification. The number of hyphae crossing the lines incorporated in the lens (x10) of a compound microscope was inserted into Newman´s formula (equation 1). For the calculation of the efficiency factor see *2.5.3.*

Modified direct litter staining method: The second protocol is based on a method described for hyphal quantification in tropical leaf litter (Aristizabal et al., 2004; Posada et al., 2012). 100 ml 20% H_2O_2 were added to 1.5 – 3 g of soil and stirred for 30 minutes. The samples were blended for 15 seconds and poured through a 500 µm sieve directly onto a filter paper (pore size: 4-7 µm). After two days drying at RT, three subsamples of soil material on the filter paper were transferred onto microscopic slides (approx. 5 mg) with a few drops of 0.05% Trypan Blue (in lactoglycerol). After two days (at which time the Trypan Blue staining is optimal) hyphal length was counted as described above. We counted 100 intersects per slide. The cover slide area (A) and the respective soil weight were inserted into Newman´s formula (equation 1). The (pseudo-)efficiency factor of this protocol was calculated for three samples. The hyphal loss through the filter paper was determined (hyphae in the flow-through were quantified) as well as the proportion of hyphae attached to soil kept on the 500 µm sieve (remaining soil was dried and slightly milled in order to add subsamples on a microscopic slide, quantifying hyphae as described above).

Aristizabal, C., Rivera, E.L., Janos, D.P., 2004. Arbuscular mycorrhizal fungi colonize decomposing leaves of Myrica parvifolia, M-pubescens and Paepalanthus sp. Mycorrhiza 14, 221-228.

Bardgett, R.D., 1991. The use of the membrane-filter technique for comparative measurements of hyphal lengths in different grassland sites. Agriculture Ecosystems & Environment 34, 115-119.

Posada, R.H., Madrinan, S., Rivera, E.L., 2012. Relationships between the litter colonization by saprotrophic and arbuscular mycorrhizal fungi with depth in a tropical forest. Fungal Biology 116, 747-755.

Treseder, K.K., Turner, K.M., Mack, M.C., 2007. Mycorrhizal responses to nitrogen fertilization in boreal ecosystems: potential consequences for soil carbon storage. Global Change Biology 13, 78-88.

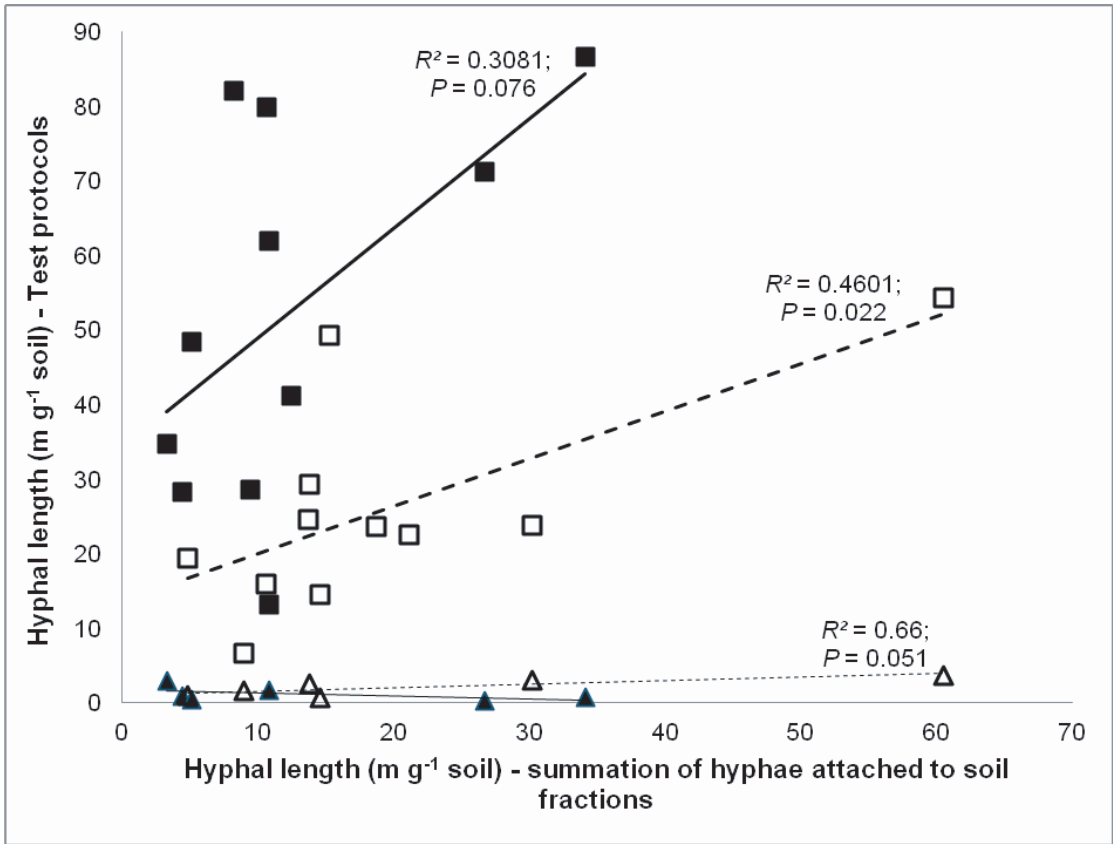

Fig.S5.1. Correlation of the applied hyphal quantification protocols (modified aqueous filtration extraction method (triangles) and modified direct litter staining method (rectangles)) with the summation of hyphae attached to every soil fraction. Open dots: non-AM hyphae, closed dots: AM hyphae. (Statistical model: linear regression)

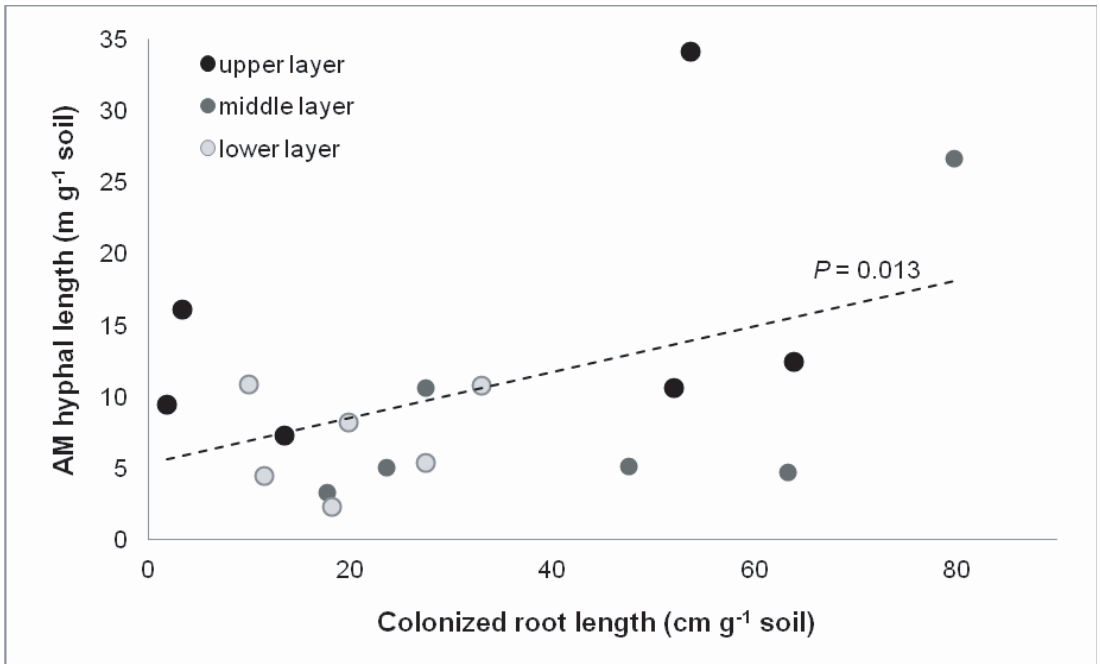

Fig. S5.2. Correlation of hyphal length data (calculated by the summation of hyphae attached to every soil fraction) and the colonized root length by AMF is shown. Roots were stained with Trypan Blue and colonization was counted by a magnified intersect method (200x magnification). Statistical model: ancova including "depth" as categorical independent variable and „sample" as random effect.

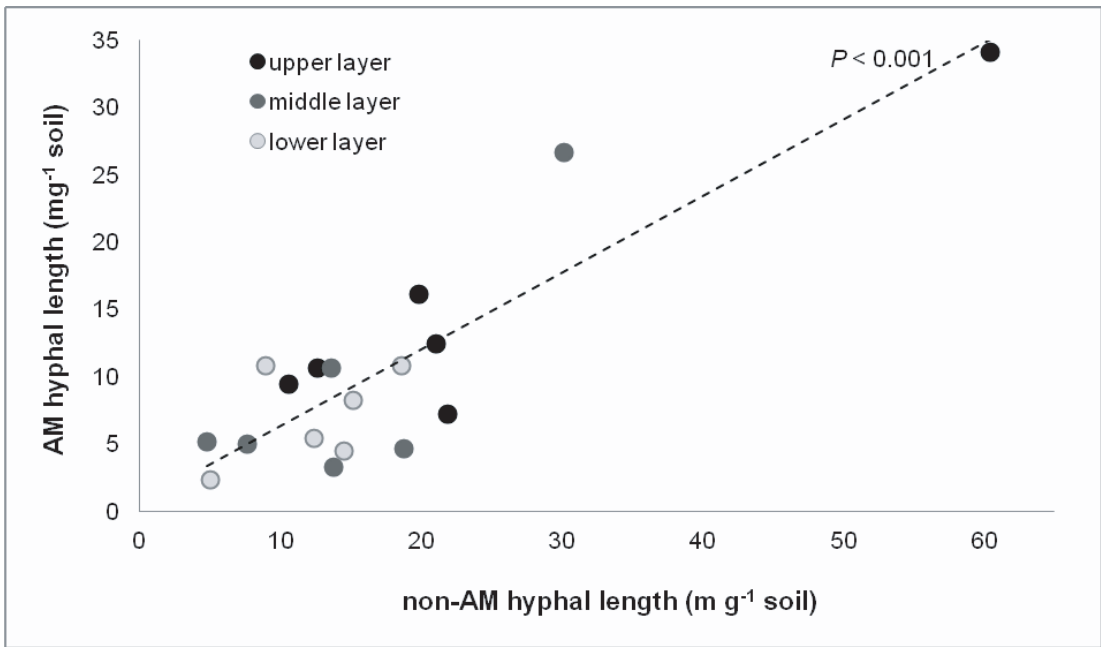

Fig. S5.3. Correlation of AM and non-AM hyphal abundance in the organic layer (Statistical model: ancova including „depth" as categorical independent variable and „sample" as random effect).