COMMUNITY STRUCTURE OF ARBUSCULAR MYCORRHIZAL FUNGI IN TEMPERATE GRASSLAND AND TROPICAL LAND-USE SYSTEMS

Dissertation

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Zusammenfassung

Fast alle Pflanzen in den gemäßigten und tropischen Klimazonen gehen eine symbiontische Beziehung mit Mykorrhizapilzen ein. Studien über die Symbiose von Mykorrhizen konzentrieren sich vor allem auf die gemäßigten Klimazonen der Erde und nur wenige Informationen über tropische Landnutzungssysteme stehen zur Verfügung. Die Assoziation von Pflanzenwurzel und arbuskulären Mykorrhizapilzen (AMF) ist von wesentlicher Bedeutung, da der Pilz eine Schlüsselrolle für die Funktionsfähigkeit und Nachhaltigkeit von Ökosystemen einnimmt. Es wurden zwei Experimente durchgeführt, um die Gesellschaften von AMF zu untersuchen, eines in der gemäßigten Klimazone in einem Dauergrünland im Solling, Deutschland und das andere in tropischen Transformationssystemen auf Sumatra, Indonesien. Das Ziel des Experiments im Grünland war es festzustellen, ob es Unterschiede in der Zusammensetzung von AMF-Gesellschaften gibt, die durch verschiedene Flächenbewirtschaftungen und Herbivorie verursacht werden. Es wurde angenommen, dass (i) Düngung die AMF-Gesellschaftszusammensetzung verändert und der AMF-Artenreichtum herabgesenkt werden kann, (ii) regelmäßiges Mähen und Herbivorie potentiell zur Reduzierung von photosynthetischem Kohlenstoff in den Pflanzengeweben führt. Da die AMF auf die Kohlenstoffzufuhr durch ihre Wirtspflanzen angewiesen sind, gehen wir davon aus, dass Herbivorie und Mähen die AMF-Abundanz senkt, (iii) bisher ist der Zusammenhang zwischen AMF-Gesellschaften und pflanzlicher Diversität unklar. Wir erwarten, dass die Manipulation des Artenreichtums der Graslandvegetation negativen Einfluss auf die AMF-Abundanz hat. Im zweiten

Experiment sollte der Einfluss der Umwandlung von Tieflandregenwald in Kautschuk- und Ölpalmplantagen auf die Struktur von AMF-Gesellschaften untersucht werden. In dieser Studie wurde angenommen, dass die veränderte Landnutzung hingehend zu intensive bewirtschafteten Plantagen einhergeht mit einem Verlust des AMF-Artenreichtums und dass die AMF-Gesellschaftszusammensetzung verändert wird.

Die Graslandvegetation wurde durch die Anwendung von Herbiziden gegen entweder dikotyle oder monokotyle Pflanzen verändert um Unterschiede im Artenreichtum in den Flächen zu erlangen. Die so entstandenen unterschiedlichen Grünflächen wurden dann unterschiedlich durch Mähen, Düngung und dem Aussetzen von Fraßfeinden behandelt. Die Studie war ein Feldexperiment mit Latin Rectangle Design. Es beinhaltete drei Behandlungsfaktoren: Grünflächentyp, Anwendung und Nährstoffe. Der Faktor Grünflächentyp war durch drei Ausprägungen vertreten (unbehandelte Kontrollflächen [species richness], Flächen mit Reduktion von Dikotylen [monocots] und Flächen mit reduzierter Anzahl von Monokotylen [dicots]). Der Faktor Anwendung hatte zwei Level: einmalig gemäht und dreimal gemäht. Der Faktor Nährstoffe wurde ebenfalls durch zwei Level vertreten, zum einen durch die Düngung mit NPK und zum anderen durch den Verzicht auf Düngung. Um die AMF-Kolonisierung zu betrachten, wurde die relative Kolonisierungsrate durch Hyphen, die relative Abundanz von Vesikeln, sowie die relative Abundanz von Arbuskeln analysiert. Die Diversität von AMF OTUs (Operational Taxonomic Units) wurde anhand der Amplifikation der rDNA Region zum Sanger-Sequenzieren bestimmt. Die Ergebnisse zeigen, dass die

Hyphenkolonisierungsrate nicht durch die verschiedenen Flächenbewirtschaftungen beeinflusst wurde. Dünung als Einzelfaktor hat die relative Abundanz von Arbuskeln und Vesikeln sowie die AMF-Diversität signifikant herabgesetzt. Der Shannon Index für Diversität (*H'*) zeigt, dass ungedüngte Flächen diversere AMF OTUs aufweisen als gedüngte Flächen. Die relative AMF-Abundanz wurde nicht durch das Entfernen oberirdischer Pflanzenbiomasse, in Form von regelmäßigem Mähen und Herbivorie, beeinflusst. Auch die verschieden artenreichen Graslandvegetationen haben keinen Einfluss auf die relative AMF-Abundanz gezeigt. Dennoch hat die Interaktion von Grünflächentyp, Dünung und Schnittfrequenz zu einer signifikanten Veränderung der relativen Abundanz von Vesikeln und Arbuskeln geführt. Es wurden AMF OTUs innerhalb dreier Familien der Glomeromycota gefunden: *Glomeraceae, Claroideoglomeraceae,* und *Archaeosporaceae* wobei *Glomus* sp. am häufigsten gefunden wurde. Diese Ergebnisse weisen darauf hin, dass Dünung ein dominanter Faktor für die Veränderungen von AMF-Gesellschaften in Graslandvegetationen sein könnte.

Das zweite Experiment wurde in zwei Landschaften auf Sumatra, Indonesien durchgeführt, zum einen im Gebiet des Bukit Duabelas Nationalpark und zum anderem im Gebiet des Harapan Regenwalds. Die AMF-Artenabundanz in Wurzeln wurden untersucht, indem partielle rDNA-Fragmente amplifiziert wurde. Um die dazugehörigen Wirtspflanzenart zu identifizieren, wurde die pflanzliche DNA mit Hilfe der Marker *rbcL* und *matK* bestimmt. Es wurden insgesamt 112 Einzelwurzeln untersucht und 39 AMF OTUs gefunden. Die Rarefaction-Analysen zeigen, dass die Anzahl analysierter AMF Sequenzen

pro Probenahmestelle ausreichend waren, um eine Aussage über die AMF-Diversität in den Wurzeln der Landnutzungssysteme treffen zu können. Die pflanzliche DNA konnte mit dem *rbcL* Marker identifiziert werden, während der *matK* Marker keine zufriedenstellenden Ergebnisse lieferte. Es wurden 20 AMF Wirtspflanzen in Bukit Duabelas beziehungsweise 31 in Harapan gefunden. Die Diversitätsindizes zeigen, dass durch die Umwandlung von Regenwald in Ölpalm- und Kautschukplantagen der Artenreichtum von AMF signifikant verringert wird. Der Effekt der Landnutzung wurde mithilfe von PERMANOVA ermittelt und zeigte, dass unterschiedliche Landschaften und Plots die Zusammensetzung der AMF-Gesellschaften signifikant beeinflussen. Dieser Effekt kann durch Umweltfaktoren erklärt werden. Es konnte gezeigt werden, dass die Struktur der AMF-Gesellschaften in Verbindung stehen mit Kohlenstoff-, Stickstoff- und Aluminiumkonzentration der Wurzeln sowie mit dem pH-Wert der BÖden. Im Gegensatz dazu, hat der Phosphorgehalt der Wurzeln keinen signifikanten Einfluss auf die Struktur der AMF-Gesellschaften.

Die Ergebnisse dieser Studien zeigen klar den Einfluss von spezifischer Flächenbewirtschaftung in Grünflächen der gemäßigten Zone sowie den der Umwandlung des tropischen Regenwaldes zu intensiv bewirtschafteten Plantagen. Die Untersuchungsgebiete des Dauergrünlandes in der gemäßigten Klimazone und der Transformationssysteme des Regenwaldes in den Tropen enthalten genügend Umweltfaktoren, um viele Effekte von Flächenbewirtschaftung und Landnutzungsveränderungen auf die Zusammensetzung von AMF-Gesellschaften zu erklären.

Summary

Most plants in temperate and tropical regions form symbiotic association with mycorrhizal fungi. Studies on mutualistic association of mycorrhiza focus mainly on temperate regions of the world, whereas little information is available from the tropical land use systems. Associations between plant root and arbuscular mycorrhizal fungi (AMF) are essential components of ecosystems where the fungus plays a key role in the functioning and sustainability of the ecosystems. We conducted two experiments in a permanent temperate grassland in the Solling, Germany, and transformation systems in Sumatra, Indonesia, to examine the communities of AMF. The objective of the grassland experiment was to determine the changes within AMF community composition caused by management practices and herbivores. We hypostasized that: (i) fertilization will change the community composition the AMF and may reduce the species rich, (ii) frequent utilization by mowing and herbivore will potentially reduce the photosynthetic carbon in plant tissue. Since AMF required the carbon from their plant host, we expect that mowing and herbivore will decrease the AMF abundance (iii) AMF communities, so far, had unclear relation to the plant diversity. We expected that manipulating sward species richness will have negative impact on the AMF abundance. In the second experiment, we aimed to investigate the impact of transformation of tropical lowland rain forest into managed rubber tree and oil palm plantations on AMF community structure. The postulated hypothesis stated that land-use change into intensive agro

plantations will reduce the AMF species richness and alter its community composition.

We manipulated grassland vegetation by applying herbicides against either monocots or dicots to modify the swards species composition. The resulting swards were then treated with mowing and fertilizer, and herbivores were allowed to feed on the grass. The study was field experiment with Latin Rectangle design. It includes three treatment factors: sward type, utilization, and nutrients. Sward type consisted of three levels: untreated control sward (species-rich), dicots reduced (monocots-dominated), and monocots reduced (dicots- dominated). Utilization consisted of two levels: mowed once and mowed three times, whereas nutrients consisted of two levels: with and without nitrogen, phosphorus, and potassium. To assess the AMF colonization rate, the relative hyphal colonization rate, the relative abundance of arbuscules, and the relative abundance of vesicles were analyzed. The diversity of the AMF operational taxonomic units (OTUs) were analyzed by amplification of the rDNA region using Sanger sequencing method. Our results showed that hyphal colonization rate of arbuscular mycorrhizal fungi were not affected by management practices. Fertilization as a single factor significantly reduced the relative abundances of arbuscule and vesicle and decreased the AMF diversity. The Shannon diversity index (*H'*) indicated that unfertilized swards had more diverse AMF OTUs compared to fertilized plots. The relative abundance of AMF was not affected by removing plant aboveground biomass through mowing frequency and herbivores. We also found that different species rich sward did not impact the relative abundance of AMF. However, the interaction between

sward, fertilization, and cutting frequency significantly changed the relative abundance of arbuscule and vesicle. AMF OTUs belonged to three families of Glomeromycota: *Glomeraceae, Claroideoglomeraceae,* and *Archaeosporaceae* and *Glomus* sp. was the most abundant among the AMF. These results suggested that fertilization is a dominant factor in changing the AMF community in grassland vegetation.

The second set of experiments was carried out in two landscapes in the Bukit Duabelas National Park and Harapan Rainforest in Sumatra, Indonesia. The AMF species abundance in the roots was investigated by amplifying a partial rDNA fragment, and AMF host plant species were identified using DNA barcoding with markers *rbcL* and *matK*. A total of 112 single roots were analyzed and 39 AMF OTUs were detected. The rarefaction analysis indicated that the number of sequences analyzed per sampling site was sufficient to cover AMF diversity in the roots per land use system. Plant DNA barcoding was successful with *rbcL* marker, whereas *matK* had low species identification efficiency. We found 20 and 31 AMF host plant species in Bukit Duabelas and Harapan, respectively. Diversity indices showed that conversion of forest to oil palm and rubber tree plantations significantly decreased the AMF species richness. However, none of the AMF OTUs had strong host specificity. The effect of land use was determined by permutational multivariate analysis of variance, showing that different landscapes and plots significantly influenced the community composition of AMF, which effect was explained by environmental factors. We found that AMF community structures were related to

C, N, and Al concentrations in roots and soil pH. In contrast, P concentration in roots did not significantly affect the AMF community structure.

The results presented here clearly demonstrate the influence of management practices in temperate grassland and conversion of tropical forest into agro plantation on the AMF community structure. The study area in temperate grassland and transformation systems of tropical lowland rain forest covers sufficient environmental factors to explain multiple effects of management practice and land-use change on AMF community composition.

List of abbreviations

°C	Degree Celsius
AA	Amino acids
Al	Aluminum
AMF	Arbuscular mycorrhizal fungi,
BF	Forest in Bukit Duabelas transformation systems
BO	Oil palm plantation in Bukit Duabelas transformation systems
BR	Rubber plantation in Bukit Duabelas transformation systems
C	Carbon
c	Centi
Ca	Calcium
CBOL	Consortium for the Barcode of Life
cm	Centimeter
d	Day
E. coli	*Escherichia coli*
et al.	Et alii
Fe	Iron
g	Gramm
GLM	General linear model
h	Hour
ha	Hectar
HF	Forest in Harapan transformation systems
HO	Oil palm plantation in Harapan transformation systems
HR	Rubber plantation in Harapan transformation systems
K	Kilo
K	Potassium
KOSI	Kompetenzzentrum für Stabile Isotope; Center for Stable Isotope Research and Analysis
L	Litre
m	meter (length)
m	Milli
M	Molar
Mg	Magnesium
min	Minute
MP	Maximum parsimony
n	Amount of substances
N	Nitrogen
Na	Sodium
NCBI	National Center of Biotechnology Information
NMDS	Non-metric multidimensional scaling
OTU	Operational taxonomic units
P	Phosphorus
PCA	Principal Component analysis

PCR	Polymerase chain reaction
PERMANOVA	Permutational multivariate analysis of variance
REKI	Restorasi Ekosistem Indonesia
RFLP	Restriction fragment length polymorphisms
s	Second
SE	standard error
t	Time
U	Units (Enzyme activity)
UNFAO	United Nations Food and Agricultural Organization
µ	Micro

Chapter 1
General Introduction

1.1. Functional diversity of mycorrhiza

Majority of higher plants associate with mycorrhizal fungi (Smith and Read 2008). The term mycorrhiza was introduced by German scientist A. B. Frank more than 100 years ago. The term was defined as a mutualistic symbiosis between plant roots and fungi (Habte 2000). Mycorrhiza can improve nutrient uptake, water absorption, plant productivity, and protect the plant from soilborne pathogen (Smith and Read 2008). In exchange, the fungus receives nutrients from the plant host Among the different types of mycorrhizae, two common types of association are endomycorrhizal association of arbuscular mycorrhizal fungi (AMF, Figure 1.1) and ectomycorrhizal fungi (EMF, Figure 1.2) (Peterson et al. 2004, Smith and Read 2009, Bonfante and Genre 2010). AMF belong to the phylum Glomeromycota, and they develop intra-extra radical hyphae and produce highly branched nutrient-exchange structure in the roots called ‘arbuscule’. EMF, which belong to Ascomycota and Basidiomycota, produce hyphal web to cover the root tips (Bonfante and Genre 2010).

AMF form mutualistic associations with a broad spectrum of plant species. Studies have shown that the diversity of mycorrhizal fungi potentially influences to the ecosystem functioning by participating in phosphorous uptake (Cameron et al. 2007; Feddermann et al. 2010),

nitrogen metabolism (Cameron et al. 2006), and carbon storage (Moore et al. 2015). The role of plant community is also a determining factor that influences the AMF diversity (Burrows and Pfleger 2002). In ecosystems, AMF have been reported not only to assist the plant nutrient uptake but also contribute in plant diversity and ecosystem productivity (Klironomos et al. 2000; Lee et al. 2013).

AMF species diversity can be determined using molecular data (Lee et al. 2008; Stockinger et al. 2010; Krüger et al. 2012). In relation to the functionality, AMF species determine various functions within the symbiosis (Smith and Read 2008). Functional diversity governs the contribution of organisms in communities and ecosystems (Petchey and Gaston 2006). Functional diversity of AMF refers to the function associated with the host plant growth (Johnson et al. 1997).

Recent physiological and morphological study revealed that AMF play a major role in phosphorus uptake, which is required for plant growth (Smith et al. 2011). In return, AMF receive organic carbon (Smith and Smith 2012). Plant roots have the capacity to uptake inorganic phosphorus from soil (Gordon-Weeks 2003). Uptake of slowly diffused phosphorus in soil, is possible by plants associated with AMF since their hyphae increase the ability to explore soil pores (Smith and Read 2009; Schnepf et al. 2011).

Mutualistic interactions of AMF–host plant are based on the exchange of nutrients. Bonfante and Genre (2010) explained that specialized transporter in extra-radical mycelia of AMF translocates the

mineral organic nitrogen (ammonium, nitrate, and amino acids) and inorganic phosphorus (Pi) from soil into their host plant. As the feedback, plant delivers carbon to AMF via a hexose transporter (Figure 1.3).

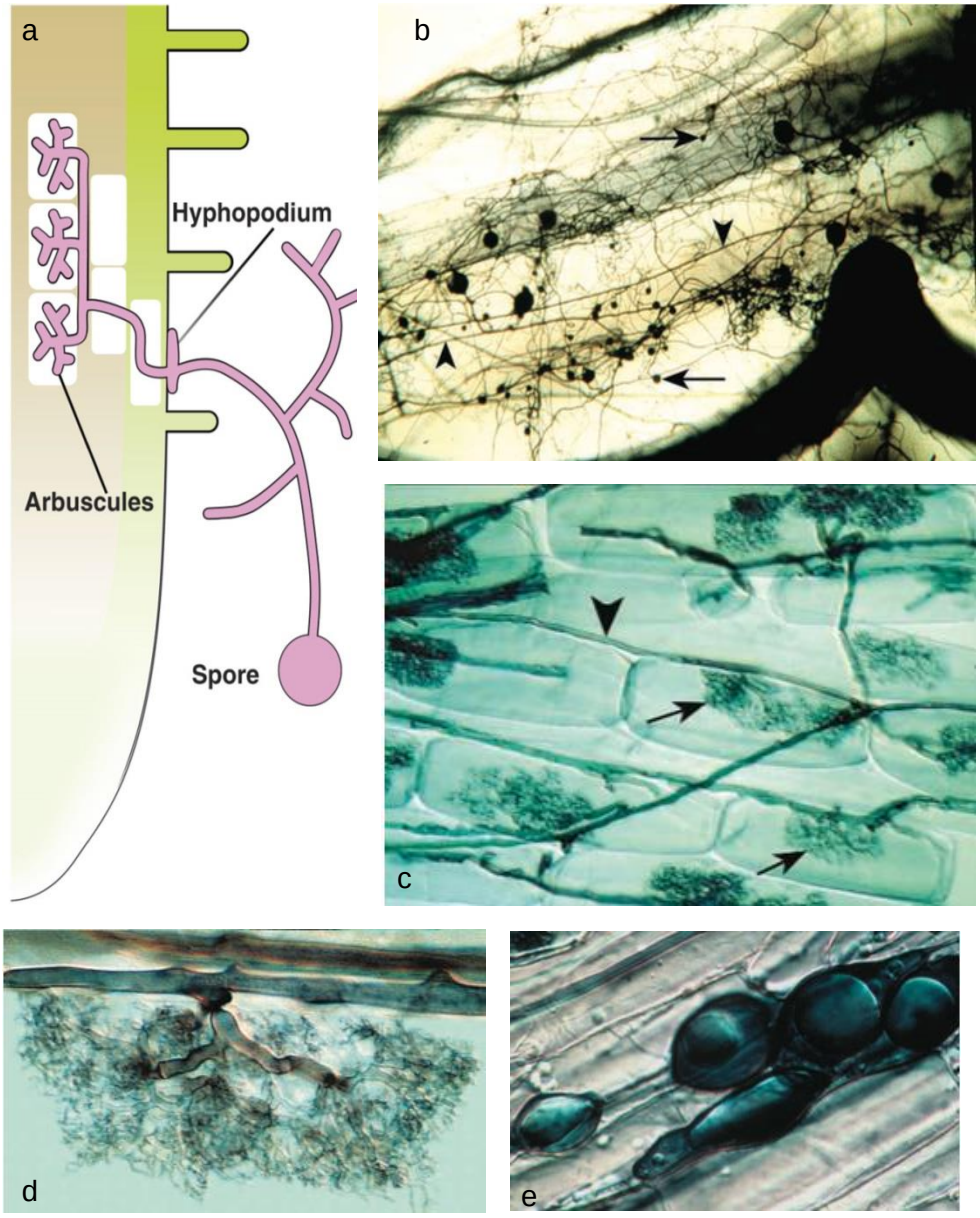

Figure1.1. a) Diagram showing root colonization structure in arbuscular mycorrhizal fungi (AMF) (Bonfante and Genre, 2010); b) extra-radical mycelia (arrowheads) and developing spores (arrows); c) intracellular hyphae (arrowhead) and arbuscules (arrows); d) fully developed arbuscules; e) vesicles of AMF (Peterson et al. 2004).

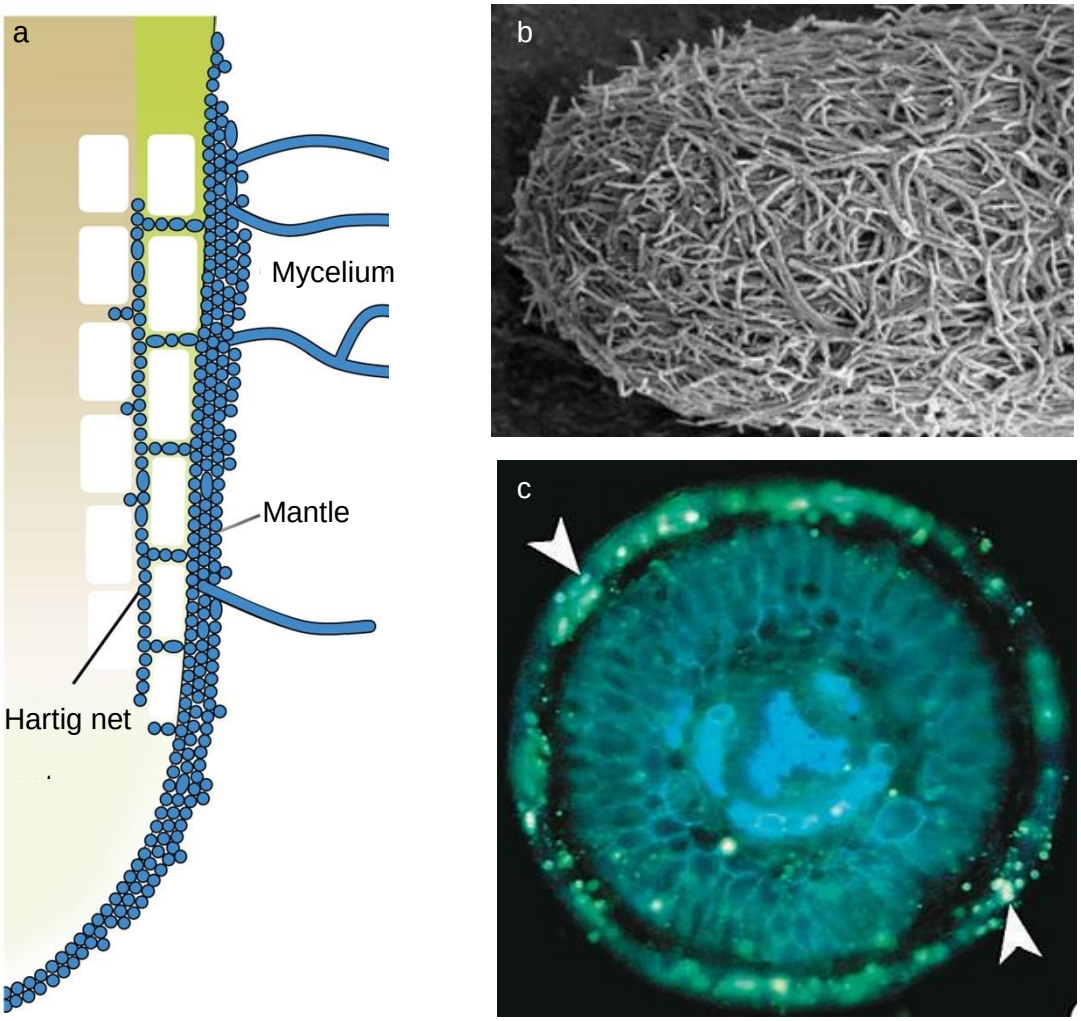

Figure 1.2. a) Diagram showing root colonization structure in ectomycorrhizal fungi (EMF) (Bonfante and Genre 2010); b) EMF mantle; c) Transverse section of EMF stained with fluorol yellow and examined by epifluorescence microscopy. Lipids (arrowheads) are present in the mantle hyphae (Peterson et al. 2004).

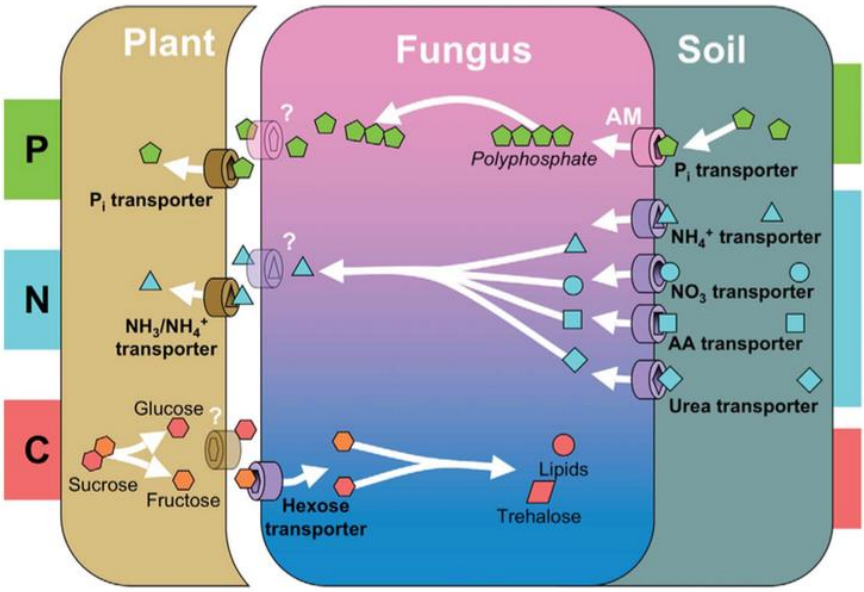

Figure 1.3. The diagram of the main nutrient exchange processes in mycorrhizal symbiosis (Bonfante and Genre 2010).

In addition, AMF species exhibit different ability to take up nitrogen because of accessibility of inorganic forms of nitrogen in soil (Read and Perez-Moreno 2003; Lambers et al. 2008). AMF have been reported to be able to take up and transfer significantly higher concentrations of nitrogen to their host plant than the amount of nitrogen non-mycorrhizal plants are able to absorb from the soil (He et al. 2003). Since AMF dominate the plant root association where nitrogen sources are abundant, this fungi are able to take up NO_3^- and NH_4^+ (Hodge and Storer 2014). With the extensive hyphal network in soil, AMF hyphae are better suited to uptake nitrogen in NH_4^+ form (López-Pedrosa et al. 2006). Using nanotechnology technique with quantum dots, (Whiteside et al. 2012) examined the organic nitrogen uptake by AMF *in situ* and found that AMF gained

recalcitrant and labile forms of organic nitrogen. Thus, plant productivity is supported by AMF when N availability is low.

1.2. Arbuscular mycorrhiza across temperate and tropical ecosystems

Arbuscular mycorrhizae are highly relevant in many ecosystems (Berruti et al. 2014). AMF generally occur in herbaceous species (Smith and Read, 2008), but they also present in trees (Wang and Qiu 2006). Moreover, it has been suggested that as many as 75% of plant species may form a symbiotic association with AMF (Tawaraya et al. 2003; Wang and Qiu 2006). AMF has been found in most of plant ecosytems, from meadows to woodland and agroplantation (Öpik et al. 2006). Mutualism between plant roots and mycorrhizal fungi is essential component in grassland communities. Moreover, Miller et al. (2012) indicated that most of the plants in grasslands form associations with AMF, but the symbiosis is dependent on plant taxa, soil fertility, and the season. Unlike the temperate region, many questions pertaining to mycorrhizal diversity and function in tropical forest remain unanswered. In tropical forests, majority of the trees are associated with AMF, whereas in temperate trees, the roots generally form ectomycorrhizal associations (Smith and Read 2008). However, little is known about basic biological information related to functional diversity of AMF particularly in Indonesian tropical rain forest.

Grass is a predominant and the most widespread vegetation type worldwide (Lieth 1975). Generally, most of the plants in grassland

ecosystems form symbioses with AMF (Miller et al. 2012). There is evidence that grassland vegetation structure can be affected by AMF (Zobel et al. 1997; Šmilauer and Šmilauerová 2000). The study of seasonal variation in grassland population conducted by Escudero and Mendoza (2004) in temperate grasslands of Argentina showed that two species of AMF, Glomus fasciculatum and Glomus intraradices, dominated the colonization of grassland. Field studies showed that AMF are able to colonize grassland vegetation in different soil conditions (Gai et al. 2006; Hempel et al. 2007; Yang et al. 2013). It has been reported that the species composition of AMF corresponds to nitrogen enrichment in grassland as reported by Egerton-Warburton et al. (2007) and the changes in AMF communities can be affected by fertilizers (Miller et al. 2012).

In tropical rain forests and agroforestry, most of the plants are associated with AM (Hopkins et al. 1996; Bakarr and Janos 1996; Alexander and Lee 2005). Ectomycorrhizae (EM)–plant association has also been observed in humid tropics (Torti et al. 2001; Henkel et al. 2002). Tedersoo et al. (2008) found that host preferences of AMF are mediated by host plant identity. However, other factors such as host phylogeny (Morris et al. 2008) and environmental conditions also play important role in AMF community composition (Aponte et al. 2010).

In tropical forest, AMF might exhibit a specific pattern of host association where AMF population shows a significant spatial heterogeneity and non-random association with different hosts and in

different environments (Klironomos 2000; Lovelock et al. 2003; Muthukumar et al. 2004).

1.3. Management practices affected AMF

In plant–fungal interaction, host plant and environmental factors are predicted to be the major driver of changes in AMF community. The AMF communities change when natural ecosystems are converted to managed vegetation by management practices (Oliveira and Sanders 1999; Mathimaran et al. 2007; Monreal et al. 2011), and nutrient and crop management can induce the diversity and change in function of AMF (Douds and Millner 1999; Plenchette et al. 2005).

Generally, adding fertilizer is expected to maintain soil fertility and plant productivity. However, continuous fertilizer application for a long period of time will impact soil rigidity through rapid loss of soil organic matter and decrease the diversity of soil microorganisms (Plenchette et al. 2005). The soil fertilizers, particularly phosphorous fertilizers, have a negative impact on the association between AMF and plants (Grant et al. 2005). It has been reported that a decline of AMF community is induced by application of high levels of inorganic fertilizers (Mäder et al. 2000; Kahiluoto et al. 2001). Increased phosphorus concentration in plant tissues reduces root exudation of strigolactones (a group of apocarotenoids), signal molecules for spore germination and recognition of AMF hyphal branching (Akiyama et al. 2005; García-Garrido et al. 2009). In addition, nitrogen fertilizers decline the AMF community composition

during their prolonged application (Van Diepen et al. 2007; Antoninka et al. 2011; Liu et al. 2014).

Field studies showed that conversion of natural grassland to managed grassland changes AMF community structure (Oehl et al. 2003; Jansa et al. 2009). The diversity of AMF was reduced after 10 years of contamination by phosphate residual in calcareous grassland in Thuringia, Germany (Renker et al. 2005). Enrichment of grassland vegetation with nitrogen fertilizer also decreased the AMF colonization (Corkidi et al. 2002).

Community structure of AMF may change across different land use systems due to environmental factors and the diversity of host plants (Bedini et al. 2007, Ndoye et al. 2012, Belay et al. 2013, Dai et al. 2013). Thus, forest conversion to managed agricultural plantation alters the AMF community composition (Lakshmipathy et al. 2012; Sharma et al. 2012).

1.4. Scope of the study

Ecosystem changes in temperate permanent grassland and tropical rain forest are predicted to alter the community composition of AMF. This study therefore aims to understand the impact of management practices and land use change on AMF communities in these two ecosystems.

The objectives of this study were to investigate: (i) the impact of land management and herbivory on AMF colonization and diversity in temperate grassland Solling, Germany as a model system; (ii) the community structure of AMF across a tropical land use gradient in lowland

rain forest transformation systems in Indonesia. The results of the study are presented in two corresponding chapters. This study, therefore, targets the following two questions:

1. Are the colonization rate and AMF diversity altered by fertilizer application, swards species richness, mowing, and herbivory in upland permanent grassland?
2. Does the conversion of rain forest into rubber tree and oil palm plantations change the community composition and reduce the species richness of AMF?

1.5. References

Akiyama K, Matsuzaki K, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435:824–827.

Alexander IJ, Lee SS (2005) Mycorrhizas and ecosystem processes in tropical rain forest: implications for diversity. In: Biotic Interactions in the Tropics. Cambridge University Press, pp 165-203.

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 Phytol 192:200–214.

Aponte C, García LV, Marañón T, Gardes M (2010) Indirect host effect on ectomycorrhizal fungi: Leaf fall and litter quality explain changes in fungal communities on the roots of co-occurring Mediterranean oaks. Soil Biol Biochem 42:788–796.

Bakarr MI, Janos DP (1996) Mycorrhizal associations of tropical legume trees in Sierra Leone, West Africa. For Ecol Manag 89:89–92.

Bedini S, Avio L, Argese E, Giovannetti M (2007) Effects of long-term land use on arbuscular mycorrhizal fungi and glomalin-related soil protein. Agric Ecosyst Environ 120:463–466.

Belay Z, Vestberg M, Assefa F, others (2013) Diversity and abundance of arbuscular mycorrhizal fungi associated with acacia trees from different land use systems in Ethiopia. Afr J Microbiol Res 7:5503–5515.

Berruti A, Borriello R, Orgiazzi A, Berbera AC, Lumini E, Bianciotto V (2014) Arbuscular Mycorrhizal Fungi and their Value for Ecosystem Management. In: Grillo O (ed) Biodiversity - The Dynamic Balance of the Planet. Intech, pp 159-191

Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. Nat Commun 1:1–11.

Burrows RL, Pfleger FL (2002) Arbuscular mycorrhizal fungi respond to increasing plant diversity. Can J Bot 80:120–130.

Cameron DD, Johnson I, Leake JR, Read DJ (2007) Mycorrhizal

Acquisition of Inorganic Phosphorus by the Green-leaved Terrestrial Orchid Goodyera repens. Ann Bot 99:831–834.

Cameron DD, Leake JR, Read DJ (2006) Mutualistic mycorrhiza in orchids: evidence from plant–fungus carbon and nitrogen transfers in the green-leaved terrestrial orchid Goodyera repens. New Phytol 171:405–416.

Corkidi L, Rowland DL, Johnson NC, Allen EB (2002) Nitrogen fertilization alters the functioning of arbuscular mycorrhizas at two semiarid grasslands. Plant Soil 240:299–310.

Dai M, Bainard LD, Hamel C, Gan Y, Lynch D (2013) Impact of Land Use on Arbuscular Mycorrhizal Fungal Communities in Rural Canada. Appl Environ Microbiol 79:6719–6729.

Douds DD, Millner PD (1999) Biodiversity of arbuscular mycorrhizal fungi in agroecosystems. Agric Ecosyst Environ 74:77–93.

Egerton-Warburton LM, Johnson NC, Allen EB (2007) Mycorrhizal community dynamics following nitrogen fertilization: a cross-site test in five grasslands. Ecol Monogr 77:527–544.

Escudero V, Mendoza R (2004) Seasonal variation of arbuscular mycorrhizal fungi in temperate grasslands along a wide hydrologic gradient. Mycorrhiza 15:291–299.

Feddermann N, Finlay R, Boller T, Elfstrand M (2010) Functional diversity in arbuscular mycorrhiza – the role of gene expression, phosphorous nutrition and symbiotic efficiency. Fungal Ecol 3:1–8.

Gai JP, Feng G, Cai XB, Christie P, Li XL (2006) A preliminary survey of the arbuscular mycorrhizal status of grassland plants in southern Tibet. Mycorrhiza 16:191–196.

García-Garrido JM, Lendzemo V, Castellanos-Morales V, Steinkellner S, Vierheilig H (2009) Strigolactones, signals for parasitic plants and arbuscular mycorrhizal fungi. Mycorrhiza 19:449–459.

Gordon-Weeks R (2003) Restricted spatial expression of a high-affinity phosphate transporter in potato roots. J Cell Sci 116:3135–3144.

Grant C, Bittman S, Montreal M, Plenchette C, Norel C (2005) Soil and fertilizer phosphorus: Effects on plant P supply and mycorrhizal development. Can J Plant Sci 85:3–14.

Habte M (2000) Mycorrhizal fungi and plant nutrition. In Plant Nutrient Management in Hawaii's Soils, Approaches for Tropical and Subtropical Agriculture. College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, pp 127–131.

Hempel S, Renker C, Buscot F (2007) Differences in the species composition of arbuscular mycorrhizal fungi in spore, root and soil communities in a grassland ecosystem. Environ Microbiol 9:1930–1938.

Henkel TW, Terborgh J, Vilgalys RJ (2002) Ectomycorrhizal fungi and their leguminous hosts in the Pakaraima Mountains of Guyana. Mycol Res 106:515–531.

He X-H, Critchley C, Bledsoe C (2003) Nitrogen Transfer Within and Between Plants Through Common Mycorrhizal Networks (CMNs). Crit Rev Plant Sci 22:531–567.

Hodge A, Storer K (2014) Arbuscular mycorrhiza and nitrogen: Implications for individual plants through to ecosystems. Plant Soil 386:1–19.

Hopkins MS, Reddell P, Hewett RK, Graham AW (1996) Comparison of root and mycorrhizal characteristics in primary and secondary rainforest on a metamorphic soil in North Queensland, Australia. J Trop Ecol 12:871–885.

Jansa J, Oberholzer H-R, Egli S (2009) Environmental determinants of the arbuscular mycorrhizal fungal infectivity of Swiss agricultural soils. Eur J Soil Biol 45:400–408.

Johnson NC, Graham J-H, Smith FA (1997) Functioning of mycorrhizal associations along the mutualism–parasitism continuum. New Phytol 135:575–585.

Kahiluoto H, Ketoja E, Vestberg M, Saarela I (2001) Promotion of AM utilization through reduced P fertilization 2. Field studies. Plant Soil 231:65–79.

Klironomos JN (2000) Host-specificity and functional diversity among arbuscular mycorrhizal fungi. Microb Biosyst New Front 845–851.

Klironomos JN, McCune J, Hart M, Neville J (2000) The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. Ecol Lett 3:137–141.

Krüger M, Krüger C, Walker C, Stockinger H, Schüßler (2012) Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. New Phytol 193:970–984.

Lakshmipathy R, Balakrishna AN, Bagyaraj DJ (2012) Abundance and diversity of AM fungi across a gradient of land use intensity and their seasonal variations in Niligiri Biosphere of the Western Ghats, India. J Agric Sci Technol 14:903–918.

Lambers H, Raven JA, Shaver GR, Smith SE (2008) Plant nutrient-acquisition strategies change with soil age. Trends Ecol Evol 23:95–103.

Lee EH, Eo JK, Ka KH, Eom AH (2013) Diversity of Arbuscular Mycorrhizal Fungi and Their Roles in Ecosystems. Mycobiology 41:121–125.

Lee J, Lee S, Young JPW (2008) Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi: PCR primers for arbuscular mycorrhizal fungi. FEMS Microbiol Ecol 65:339–349.

Lieth H (1975) Primary Production of the Major Vegetation Units of the World. In: Lieth H, Whittaker RH (eds) Primary Productivity of the Biosphere. Springer Berlin Heidelberg, pp 203–215

Liu W, Jiang S, Zhang Y, Yue S, Christie P, Murray PJ, Li X, Zhang J (2014) Spatiotemporal changes in arbuscular mycorrhizal fungal communities under different nitrogen inputs over a 5-year period in intensive agricultural ecosystems on the North China Plain. FEMS Microbiol Ecol 90:436–453.

López-Pedrosa A, González-Guerrero M, Valderas A, Azcón-Aguilar C, Ferrol N. (2006) GintAMT1 encodes a functional high-affinity ammonium transporter that is expressed in the extraradical mycelium of Glomus intraradices. Fungal Genet Biol FG B 43:102–110.

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.

Mäder P, Edenhofer S, Boller T, Wienken A, Niggli U (2000) Arbuscular mycorrhizae in a long-term field trial comparing low-input (organic,

biological) and high-input (conventional) farming systems in a crop rotation. Biol Fertil Soils 31:150–156.

Mathimaran N, Ruh R, Jama B, Verchotb L, Frossarda E, Jansa J (2007) Impact of agricultural management on arbuscular mycorrhizal fungal communities in Kenyan ferralsol. Agric Ecosyst Environ 119:22–32. doi: 10.1016/j.agee.2006.06.004

Miller RM, Wilson GWT, Johnson NC (2012) Arbuscular Mycorrhizae and Grassland Ecosystems. In: Southworth D (ed) Biocomplexity of Plant–Fungal Interactions. Wiley-Blackwell, pp 59–84

Monreal MA, Grant CA, Irvine RB, Mohr RM, McLaren DL, Khakbazan M (2011) Crop management effect on arbuscular mycorrhizae and root growth of flax. Can J Plant Sci 91:315–324.

Moore JAM, Jiang J, Post WM, Classen AT (2015) Decomposition by ectomycorrhizal fungi alters soil carbon storage in a simulation model. Ecosphere 6:art29.

Morris MH, Smith ME, Rizzo DM, Rejmánek M, Bledsoe CS (2008) Contrasting ectomycorrhizal fungal communities on the roots of co-occurring oaks (Quercus spp.) in a California woodland. New Phytol 178:167–176.

Muthukumar T, Udaiyan K, Shanmughavel P (2004) Mycorrhiza in sedges—an overview. Mycorrhiza 14:65–77.

Ndoye F, Kane A, Léonard A, Mangaptché N, Bakhoum N, Sanon A, Diouf D, Ourèye M, Baudoin E, Noba K, Prin Y (2012) Changes in Land Use System and Environmental Factors Affect Arbuscular Mycorrhizal Fungal Density and Diversity, and Enzyme Activities in Rhizospheric Soils of Acacia senegal (L.) Willd. Int Sch Res Not 2012:e563191.

Oehl F, Sieverding E, Ineichen K, Mäder P, Boller T, Wiemken A (2003) Impact of Land Use Intensity on the Species Diversity of Arbuscular Mycorrhizal Fungi in Agroecosystems of Central Europe. Appl Environ Microbiol 69:2816–2824.

Oliveira AAR, Sanders FE (1999) Effect of management practices on mycorrhizal infection, growth and dry matter partitioning in field-grown bean. Pesqui Agropecuária Bras 34:1247–1254.

Öpik M, Moora M, Liira J, Zobel M (2006) Composition of root-colonizing

arbuscular mycorrhizal fungal communities in different ecosystems around the globe. J Ecol 94:778–790.

Petchey OL, Gaston KJ (2006) Functional diversity: back to basics and looking forward. Ecol Lett 9:741–758.

Peterson RL, Massicotte HB, Melville LH (2004) Mycorrhizas anatomy and cell biology. NRC Research Press ; CABI Pub., Ottawa; Wallingford, Oxon, UK, pp 55-80.

Plenchette C, Clermont-Dauphin C, Meynard JM, Fortin JA (2005) Managing arbuscular mycorrhizal fungi in cropping systems. Can J Plant Sci 85:31–40.

Read DJ, Perez-Moreno J (2003) Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? New Phytol 157:475–492.

Renker C, Blanke V, Buscot F (2005) Diversity of arbuscular mycorrhizal fungi in grassland spontaneously developed on area polluted by a fertilizer plant. Environ Pollut 135:255–266.

Schnepf A, Leitner D, Klepsch S, Pellerin S, Mollier A (2011) Modelling Phosphorus Dynamics in the Soil–Plant System. In: Bünemann E, Oberson A, Frossard E (eds) Phosphorus in Action. Springer Berlin Heidelberg, pp 113–133.

Sharma NK, Singh RJ, Kumar K (2012) Dry Matter Accumulation and Nutrient Uptake by Wheat (*Triticum aestivum* L.) under Poplar (*Populus deltoides*) Based Agroforestry System. Int Sch Res Not 2012:e359673.

Šmilauer P, Šmilauerová M (2000) Effect of AM symbiosis exclusion on grassland community composition. Folia Geobot 35:13–25.

Smith SE, Jakobsen I, Grønlund M, Smith FA (2011) Roles of Arbuscular Mycorrhizas in Plant Phosphorus Nutrition: Interactions between Pathways of Phosphorus Uptake in Arbuscular Mycorrhizal Roots Have Important Implications for Understanding and Manipulating Plant Phosphorus Acquisition. Plant Physiol 156:1050–1057.

Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic Press, Amsterdam; Boston, pp 11-188.

Smith SE, Smith FA (2012) Fresh perspectives on the roles of arbuscular

mycorrhizal fungi in plant nutrition and growth. Mycologia 104:1–13.

Stockinger H, Krüger M, Schüßler A (2010) DNA barcoding of arbuscular mycorrhizal fungi. New Phytol 187:461–474.

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. For Ecol Manag 182:381–386.

Tedersoo L, Jairus T, Horton BM, Abarenkov K, Subi T, Saar I, Köljalg U (2008) Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. New Phytol 180:479–490.

Torti SD, Coley PD, Kursar TA (2001) Causes and consequences of monodominance in tropical lowland forests. Am Nat 157:141–153.

Van Diepen LTA, Lilleskov EA, Pregitzer KS, Miller RM (2007) Decline of arbuscular mycorrhizal fungi in northern hardwood forests exposed to chronic nitrogen additions. New Phytol 176:175–183.

Wang B, Qiu YL (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363.

Whiteside MD, Digman MA, Gratton E, Treseder KK (2012) Organic nitrogen uptake by arbuscular mycorrhizal fungi in a boreal forest. Soil Biol Biochem 55:7-13.

Yang W, Zheng Y, Gao C, He X, Ding Q, Kim Y, Rui Y, Wang S, Guo LD (2013) The Arbuscular Mycorrhizal Fungal Community Response to Warming and Grazing Differs between Soil and Roots on the Qinghai-Tibetan Plateau. PLoS ONE 8:e76447.

Zobel M, Moora M, Haukioja E (1997) Plant Coexistence in the Interactive Environment: Arbuscular Mycorrhiza Should Not Be out of Mind. Oikos 78:202.

Chapter 2

Impact of land management and herbivory on arbuscular mycorrhizae in upland permanent grassland

2.1. Introduction

Recently, there has been a growing interest in studying interactions between above and belowground organisms in grassland ecosystems, and especially in elucidating the role of each organism. One of the important groups of organisms are plant root associated mutualistic fungi, called arbuscular mycorrhizal fungi (AMF) (Klironomos 2003, Leake et al. 2004, Bonfante and Genre 2010). These fungi form mutualistic association with a majority of vascular plants in which the fungi help their host to take up nutrients from the soil and in return, obtain photosynthetically derived carbon compounds from the host (Smith and Read 2008).

AMF are present in a broad range of plant vegetation (Öpik et al. 2009) and can tolerate various ecological conditions (Klironomos et al. 2001; Entry et al. 2002; Finlay 2008). The AMF community composition is determined by the plant diversity (Lumini et al. 2010, van der Gast et al. 2011) and management practices (Titus and Leps 2000; Mathimaran et al. 2007). Nevertheless, identifying factors that regulate the community assemblages of AMF is challenging. To address the relationship between AMF community composition and management practices in permanent grassland vegetation, manipulations of plant species in a long-term experiment has been conducted in the Solling upland permanent grasslands.

Grassland, determined as an ecosystem covered with grass, legumes, and herbs, functions as a carbon sink, nitrogen fixation source, and a habitat for animals, and it prevents erosion (Carlier et al. 2009). Grassland also supports biodiversity, soil fertility, as well as environmental, economic, and social functions beyond the farm (Hopkins and Morris 2002). Generally, permanent grassland comprises 40% of the terrestrial area (White et al. 2000), provides livelihood for more than 800 million people (Reynolds et al. 2005), and a habitat for animals (WallisDeVries et al. 2002). The Solling upland grasslands have been used traditionally as an extensive pasture and meadow since the end of the 19th century. However, it was reported as a nutrient-poor ecosystem dominated by *Agrotis capillaris* and *Festuca rubra* (Petersen et al. 2012).

Management practices such as fertilization (Wu et al. 2011) and mowing (Titus and Leps 2000) can influence AMF abundance and diversity. It has been reported that changes in either type or amount of fertilizer can directly affect the AMF community (Bhadalung et al. 2005; Nijjer et al. 2010; Wu et al. 2011), although the effects of fertilizer on AMF communities is not well understood. Fertilizers decrease extraradical hyphae (Johnson 1993) and impact spore formation of certain AMF species (Thomson et al. 1992, Egerton-Warburton and Allen 2000, Kahiluoto et al. 2001). In contrast, Nijjer et al. (2010) found an increase in hyphal colonization of AMF after the fertilization.

Besides affecting plant performance, removal of above ground biomass by mowing, clipping, and grazing by herbivores are factors that

also alter the AMF colonization (Barto and Rillig 2010) by reducing the photosynthetic carbon stored in plant tissue as well as by triggering changes in plant physiology that arise after the removal of above ground biomass (Barto and Rillig 2010). A study on the effect of combination of mowing (simulated grazing) and fertilization in different seasons in a prairie significantly decreased the AMF abundance (Bentivenga and Hetrick 1992).

Herbivores such as insects and snails have also been shown to variously impact mycorrhizal colonization of plant roots. It has been reported that herbivores did not significantly change the AMF colonization (Gange et al. 2002; Wamberg et al. 2003). In contrast, other studies reported that herbivores significantly decreased the AMF communities (Kula et al. 2005; Mueller and Gehring 2006; Currie et al. 2011). Gehring and Whitham (1994) hypothesized that mycorrhizal colonization decreases in response to increasing intensity of aboveground herbivory. Variation in AMF responses to herbivores have been attributed to the age of the plant host (Wamberg et al. 2003), the level of defoliation (Gange et al. 2002), and the timing of AM colonization (Currie et al. 2011).

In the present study, manipulation of grassland vegetation was conducted using herbicides against either monocots or dicots. The resulting swards were then mowed and fertilized and herbivores were allowed to feed on the grass. This experiment can potentially assess the effect of land management practices and herbivory on AMF abundance and community composition in different swards. We hypothesized that: (1)

fertilization will change the community composition and may reduce the species rich of AMF, (2) frequent utilization by mowing and herbivore will potentially reduce the photosynthetic carbon in plant tissue. Since AMF required the carbon from their plant host, we expect that mowing and herbivore will decrease the AMF abundance, (3) AMF communities, so far, had unclear relation to the plant diversity. We expected that manipulating sward species richness will have negative impact on the AMF abundance.

2.2. Materials and Methods

2.2.1. Study site

The research was performed in a permanent grassland in the Solling uplands, located between Silberborn and Neuhaus, Central Germany (51°44'53"N, 9°32'42"E, 490 m a.s.l). There has been moderate fertilization (80 kg N ha^{-1} yr^{-1}), liming, and overseeding with high value forage species without plowing since 1966 (farm records Relliehausen). The fertilization was terminated 2 years prior to the experiment. According to the climate data from 1960 to 1991 (station Silberborn-Holzminden) the annual temperature and rainfall were 6.9°C and 1033 mm. The soil type is stony haplic Cambisol on middle Bunter sandstone with pH 5.2–5.6 (Keuter et al. 2012). The vegetation in the study area belongs to *Lolio-Cynosuretum* association with high abundance of *Festuca rubra* and *Agrostis capillaris* (Petersen et al. 2012). In 2008, before the start of the experiment, soil samples were collected throughout the grassland to analyze the nutrient contents (Petersen et al. 2012) for detail information).

2.2.2. Experimental design

The study was field experiment with Latin Rectangle design (Figure S2.1). It includes three treatment factors: sward type, utilization, and nutrients. Sward type consisted of three levels: untreated control sward (species-rich), dicots reduced (monocots-dominated), and monocots reduced (dicots-dominated); utilization consisted of two levels: mowed once or mowed three times; and nutrients consisted of two levels: with and without nitrogen, phosphorus, and potassium (Table 2.1; for details see Petersen et al. 2012). These treatments were replicated six times in two different plots, of which one was subjected to grazing by herbivores (herbivory plot) and another where grazing was not allowed (control plot).

Table 2.1. Experimental factors and treatment levels in this experiment.

Treatment	Level	Abbreviation
Plot	Control	
	Herbivory	
Sward	Untreated control sward (species-rich)	S
	Dicots reduced (monocots-dominated)	M
	Monocots reduced (dicots-dominated)	D
Utilization	Cut once (July)	1
	Cut three times (May, July, September)	3
Nutrients	No	-N
	fertilization180/30/100 kg NPK ha^{-1} yr^{-1}[b]	+N

[b]N fertilizer: calcium ammonium nitrate N27, P&K fertilizer: Thomaskali® (8% P_2O_5, 15% K_2O, 20% CaO; Carten-Haage, Erfurt, Germany).

2.2.3. Plot installation, sampling, and quantification of mycorrhizal colonization

In the herbivory plots, lysimeters were installed a year before herbivory experiment was carried out (August and September 2010). The lysimeter was constructed from transparent Plexiglass cylinders (height 30 cm, inside diameter 14.4 cm, wall thickness 0.3 cm). Two weeks before the grass harvested, four grasshoppers (*Chorthippus* sp.) and one snail (*Helix pomatia*) were placed into each lysimeter located in herbivory plots and were allowed to feed on plants. Once a week, dead herbivores were replaced. The plant roots were harvested for AMF analysis. The root samples with soil were washed in a 500 µm sieve (Retsch GmbH, Haan, Germany) to keep all of the fine roots. The fine roots were divided in two portions. One portion was stored in an Eppendorf reaction vial with 70% ethanol (Carl Roth GmbH, Karlsruhe, Germany) and the remaining plant root samples were stored at -80°C without ethanol for molecular analyses. AMF hyphae were stained using a root clearing method described by Phillips and Hayman (1970) with modifications. Plant roots were cleared with 2.5% KOH at 90°C for 30 min and then rinsed three times with water. Dark colored roots were re-cleared with 2.5% KOH at 90°C for 15 min and soaked in 3% HCl for 15 min at room temperature, washed with water and finally stained using lactophenol blue (1 g L^{-1}, pH 2.3). Excess dye was removed by soaking the root sample in acidic glycerol solution (50 mL glycerin, 45 mL H_2O, 5 mL 1% HCl) for 60 min. Finally, the stained roots were preserved in 50% glycerol. The AMF root colonization was

determined using the magnified intersection method (Mc Gonigle et al. 1990) under a compound light and fluorescence microscope (Axio Observer Z1, Carl Zeiss MicroImaging GmbH, Göttingen, Germany) at 200 × magnification. The AMF structures observed were hyphae, arbuscules, and vesicles (Figure 2.1). Five root tips were observed per sample and 100 intersections were examined for each root. Total hyphal colonization and arbuscule and vesicle abundance were calculated as follows:

$$\text{Hyphal colonization (\%)} = \frac{\text{total intersection with hyphae}}{\text{total intersection with roots}} \times 100$$

$$\text{Relative abundance of arbuscules (\%)} = \frac{\text{total intersection with arbuscules}}{\text{total intersection with roots}} \times 100$$

$$\text{Relative abundance of vesicles (\%)} = \frac{\text{total intersection with vesicles}}{\text{total intersection with roots}} \times 100$$

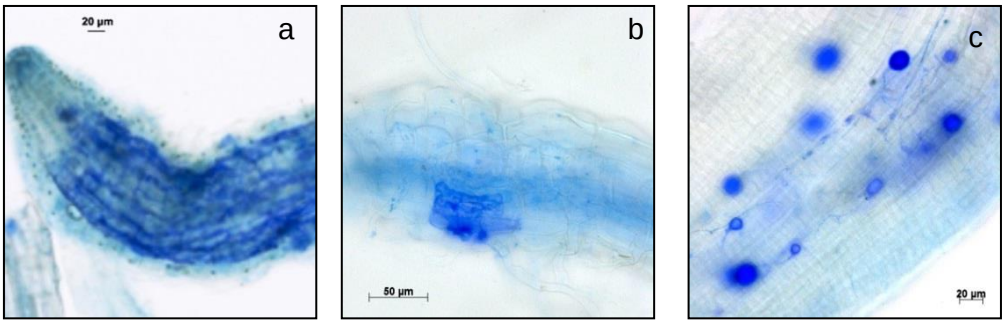

Figure 2.1. The arbuscular mycorrhizal structures in a root tip with hyphae (a), arbuscule (b), and vesicles (c).

2.2.4. Molecular identification of arbuscular mycorrhizal fungi

Each single root was ground individually in a ball mill (Retsch MM 2000, Haan, Germany). Total DNA was extracted using an innuPREP plant DNA kit (Analytik Jena, Jena, Germany) following the manufacturer's instructions. DNA concentration was measured in an Eppendorf Bio photometer (Eppendorf, Hamburg, Germany). Free nuclease water (Sigma-Aldrich Corp., St. Louis, MO, USA) was used as a solvent to suspend the DNA. The DNA samples were diluted to 10–fold for more accurate reading by the photometer. Each DNA sample (100 µL) was placed in a UV transparent cuvette (12.5 × 12.5 × 45 mm, Sarstedt, Nümbrecht, Germany) and measured in the photometer at optical density of 260 nm (OD_{260}). At 1 cm path length, OD_{260} equals 1.0 for a DNA concentration of 50 µg mL^{-1}. Two microliters of 50 ng DNA were used as a template for PCR reaction.

A nested PCR was carried out to amplify 25S rDNA of general fungi with LR1 and FLR2 primer pair (Table 2.2). Each PCR reaction (25 µL) contained: 2.5 µL 1x PCR buffer with $(NH_4)_2SO_4$ (Thermo Scientific Bio, Darmstadt, Germany), 2 µL 2 mM MgCl (Thermo Scientific Bio, Germany), 0.5 µL 0.2 mM dNTPs mix (Thermo Scientific Bio, Germany), 1.25 µL 0.5 mM of each primer, 0.125 µL 0.5 U Taq polymerase (Thermo Scientific Bio, Darmstadt, Germany), and 15.375 µL water (AppliChem GmbH, Darmstadt, Germany). The following PCR conditions were applied: initial denaturation at 94°C for 3 min, followed by 30 cycles at 94°C for 30 s, 58°C for 1 min, and 72°C for 1 min, and a final extension cycle at 72°C for

10 min. Five microliters of the resulting PCR product were used as DNA template for the second PCR using specific primers for AMF, 28G1 and 28G2 (Table 2.2). The volume and PCR mixture was the same as described above except that the volume of water was adjusted to 12.375 µL. The PCR conditions for the second run were as follows: initial denaturation at 94°C for 3 min, followed by 30 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, and a final extension cycle of 10 min at 72°C. Positive and negative controls using PCR-positive template and sterile water, respectively, were also included in all amplifications. All PCR reactions were run on a Mastercycler gradient (Eppendorf, Hamburg, Germany). To confirm successful PCR reactions, the PCR products (5 µL) were mixed with 1 µL 6x DNA loading buffer (3 mL glycerol, 25 mg bromophenol blue, and water to 10 mL) and electrophoresed on 1.2% agarose gel (Makovets 2013) (Biozym Scientific GmbH, Oldendorf, Germany) containing 2% GelRed (Biotium, Hayward, USA) with 1x TBE electrophoresis buffer (10x TBE: 108 g Tris base [$C_4H_{11}NO_3$.], 55 g boric acid in 900 mL deionized water, 40 mL of 0.5M ethylenediaminetetraacetic acid [EDTA, pH 8.0], adjusted to a final volume of 1 L with deionized water) at 120 V for 60 min. Those PCR products that showed a band were subsequently purified with an innuPREP PCRpure Kit (Analytik Jena, Germany) following manufacturer's protocol.

PCR products were cloned into pGEM-T Easy vector (Promega, Madison, USA) following manufacturer's instruction and transformed into electrocompetent *E. coli* top10F' cells made in-house (Department of

Forest Botany and Tree Physiology, Göttingen, Germany) using Bio-Rad *E. coli* pulser (Bio-RAD, Hercules, CA, USA). Eight positive transformants were analyzed from each sample used for cloning. Colony-PCR was performed using primer pair M13-20/M13RP (Table 2.2). The PCR mix and amplification conditions were the same as described above for the second PCR run, except that the annealing temperature was set to 55°C. The single clone-PCR product confirmed by the presence of single band on the gel was subsequently purified by adding 35 µL 99.8% isopropanol (Roth GmbH, Karsruhe, Germany), incubating at room temperature for 60 min, and centrifuging for 30 min at 10,000× *g*. The supernatant was then removed and the pellet was resuspended in free nuclease water.

Table 2.2. Details of primers used in this study

Primer	Sequence (5'–3')	Target group	Source
LR1	GCATATCAATAAGCGGAGGA	Fungi	Trouvelot et al. 1999
FLR2	GTCGTTTAAAGCCATTACGTC		
28G1	CATGGAGGGTGAGAATCCCG	LSU rRNA gene of AMF	Silva et al. 2006
28G2	CCATTACGTCAACATCCTTAACG		
M13-20	CGACGTTGTAAAACGACGGCCAGT	General primer for AMF sequencing	pGEM-T Easy vector primers
M13 RP	TTTCACACAGGAAACAGCTATGAC		

LSU: large subunit; AMF: arbuscular mycorrhizal fungi.

In order to estimate DNA polymorphisms in the clones, restriction fragment length polymorphisms (RFLP) analysis was conducted with HinfI or BsuRI (HaeIII) following the manufacturer protocol (Life Technologies

GmbH, Darmstadt, Germany). The RFLP mixtures were incubated overnight at 4°C. To observe the different DNA band pattern, all RFLP products were separated on a 3% agarose gel at 90 V for 20 min followed by 120 V for 90 min as described above. Three samples for each different DNA band pattern were sequenced by a company (SEQLAB Sequence Laboratories Göttingen GmbH, Göttingen, Germany).

2.2.5. Sequence analysis

Sequences were edited with the open access program BioEdit (Hall 1999) and aligned in MEGA 6 (Tamura et al. 2013). BLAST searches for AM fungal species were performed against the MaarjAM data base (Öpik et al. 2010) and NCBI Reference Sequence Database (Robbertse and Tatusova 2011). Phylogenetic trees were constructed using maximum parsimony method implemented in MEGA 6 and the close-neighbor-interchange algorithm. The bootstrap values were estimated with 1000 replicates. The deletion option in MEGA 6 was used for eliminating gaps and missing data.

Arbuscular mycorrhizal operational taxonomic units (OTUs) were defined on the basis of sequence similarities as surrogates for species. A threshold of 97% similarity was selected as the minimum value to assign a sequence to the same OTU since this value has been commonly used by various authors (Haug et al. 2013; Toju et al. 2014). The AMF sequences have been submitted to NCBI (accession numbers: KT223123-KT223132).

2.2.6. Data analysis

General linear model (GLM) was conducted using the software R 3.0.2 (The R Foundation for Statistical Computing) with lme4 package (Bates et al. 2015). GLM was used to determine the significance of herbivore, sward types, utilization, and nutrient on AMF abundances. The hyphal colonization rate, relative abundances of arbuscule and vesicle were used as dependent variable while herbivore, sward types, and utilization were used as explanatory variables. The interactions of herbivore, sward types, and utilization were also analyzed. In addition, row and column of the Latin Square were included as fixed effect to incorporate the spatial heterogeneity of the study site. Tukey-Kramer, a multiple comparison test, was used to determine whether three or more means differ significantly. The AMF diversity index was calculated as Shannon index (*H'*) using the equation:

$H' = -\sum p_i(\ln p_i)$

Where p_i is the proportion of individuals in the *i*th species.

A multivariate principal component analysis (PCA) was performed to examine whether the sward type, mowing frequencies, and fertilizer could differentiate AMF diversity. We used AMF raw species richness matrices to analyze the ordination (Table S2.1). The PCA was done using R 3.0.2.

2.3. Results

AMF hyphae colonized > 50% of grass roots in all treatments in both control and herbivory plots (Figure 2.2). The factor nutrient significantly decreased the relative abundance of arbuscules and vesicles (Figures 2.3 and 2.4). In contrast, hyphal colonization rate and the relative abundances of arbuscules and vesicles were not affected by both herbivory and sward species richness, nor were they affected by utilization (Table 2.3). Combination of sward type and utilization significantly influenced the relative abundance of arbuscules and vesicles. Combination of nutrient and herbivore significantly changed the hyphal colonization rate (P = 0.02), and combination of all treatments affected the relative abundance of arbuscules (P = 0.04) but not vesicles (P = 0.66). The row and column that were taken as fixed effects to for potential inhomogeneity of the study site had significant effect on the hyphal colonization rate (P = 0.05 and 0.003 respectively).

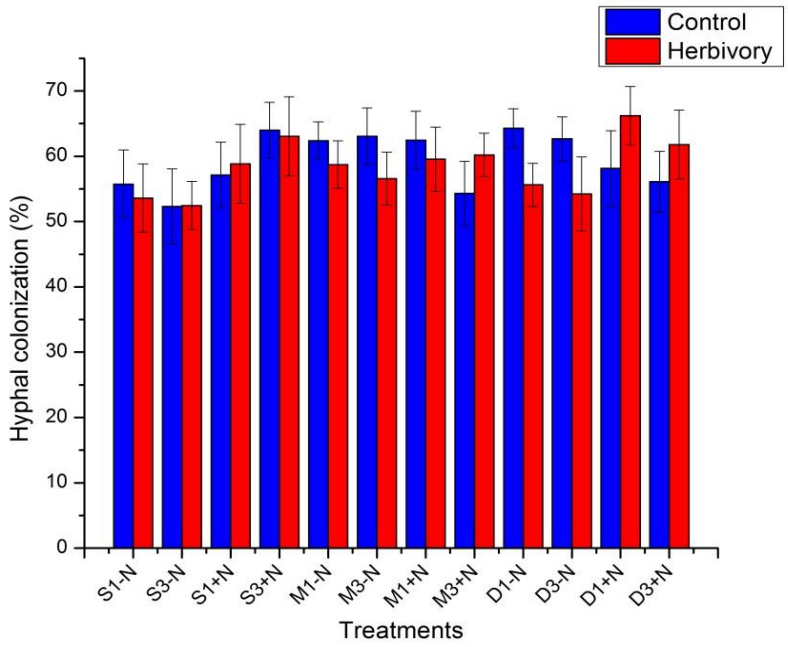

Figure 2.2. Effect of sward type (S: species-rich, M: monocot-dominated, D: dicot-dominated), utilization (1: mowing once, 3: mowing three times), and nutrients (-N: unfertilized, +N: fertilized) on hyphal root colonization in control and herbivory plots. Data are mean ± SE (n = 6). For statistical results, see Table 2.3.

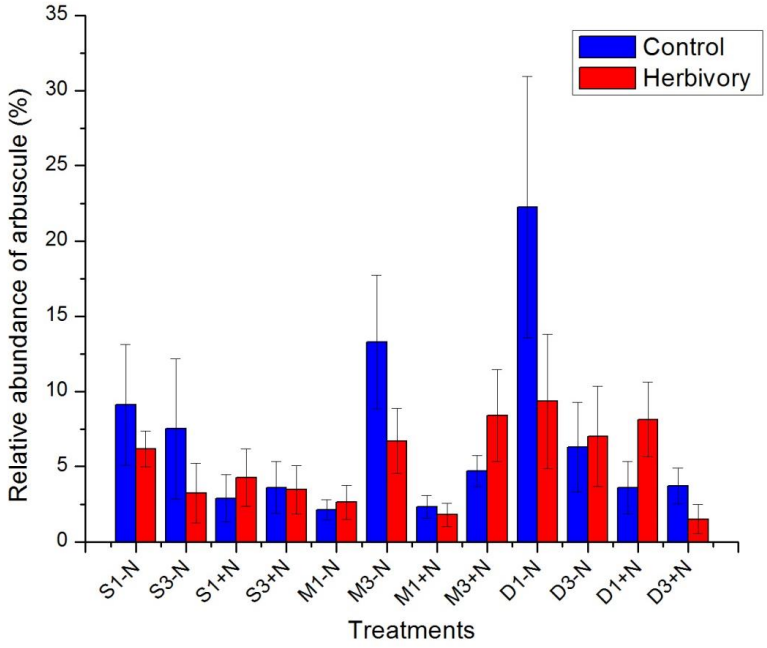

Figure 2.3. Effect of sward type (S: species-rich, M: monocot-dominated, D: dicot-dominated), utilization (1: mowing once, 3: mowing three times), and nutrients (-N: unfertilized, +N: fertilized) on arbuscules relative abundance in control and herbivory plots. Data show means ± SE (n = 6). For statistical results, see Table 2.3.

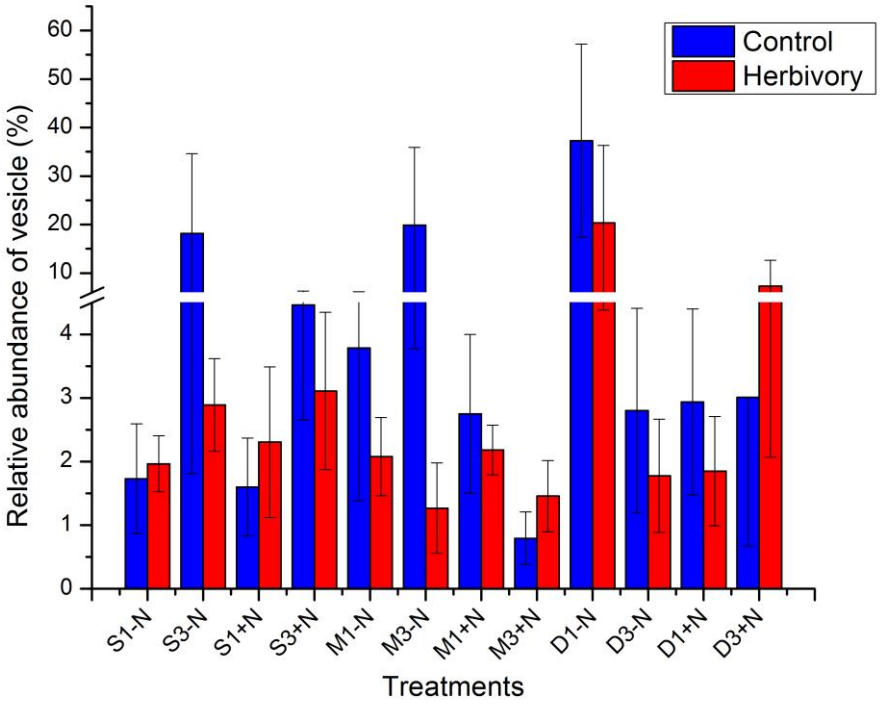

Figure 2.4. Effect of sward type (S: species-rich, M: monocot-dominated, D: dicot-dominated), utilization (1: mowing once, 3: mowing three times), and nutrients (-N: unfertilized, +N: fertilized) on vesicles relative abundance in control and herbivory plots. Data show means ± SE (n = 6). For statistical results, see Table 2.3.

Table 2.3. ANOVA of the effects of treatments on relative abundance of hyphae, arbuscules, and vesicles. Statistics was performed with general linear model (GLM). Numbers in bold indicate significant differences at $P \leq 0.05$ (n = 6).

Source	Hyphae			Arbuscule			Vesicle		
	MS	F	*P*	MS	F	*P*	MS	F	*P*
Row	1.093	2.270	**0.052**	3.091	1.810	0.117	2.081	0.580	0.713
Column	1.888	3.920	**0.003**	5.630	3.300	**0.008**	2.074	0.580	0.714
Sward	0.548	1.140	0.324	1.608	0.940	0.393	4.460	1.250	0.291
Utilization	0.151	0.310	0.577	0.000	0.000	0.987	1.703	0.480	0.491
Sward * Utilization	0.269	0.560	0.574	17.443	10.220	**<0.001**	13.089	3.670	**0.029**
Nutrient	0.992	2.060	0.154	16.806	9.850	**0.002**	15.915	4.460	**0.037**
Sward * Nutrient	0.984	2.050	0.134	1.505	0.880	0.417	2.888	0.810	0.448
Utilization * Nutrient	0.051	0.110	0.746	0.389	0.230	0.634	3.129	0.880	0.351
Sward * Utilization * Nutrient	0.452	0.940	0.394	1.386	0.810	0.447	14.510	4.070	**0.020**
Herbivore	0.187	0.390	0.534	2.031	1.190	0.278	3.331	0.930	0.336
Sward * Herbivore	0.022	0.050	0.955	0.090	0.050	0.949	0.288	0.080	0.923
Utilization * Herbivore	0.025	0.050	0.821	0.413	0.240	0.624	0.248	0.070	0.793
Sward * Utilization * Herbivore	0.063	0.130	0.877	0.173	0.100	0.904	4.065	1.140	0.324
Nutrient * Herbivore	2.417	5.020	**0.027**	4.172	2.440	0.121	6.934	1.940	0.166
Sward * Nutrient * Herbivore	0.755	1.570	0.213	0.035	0.020	0.980	1.227	0.340	0.710
Utilization * Nutrient * Herbivore	0.012	0.030	0.874	1.148	0.670	0.414	2.420	0.680	0.412
Sward * Utilization * Nutrient * Herbivore	0.293	0.610	0.546	6.446	3.780	**0.026**	1.378	0.390	0.681

The community composition of AMF was analyzed only in the control plot because there was no significant difference between the effect of control and herbivory plots on hyphal colonization rate and the relative abundances of arbuscules and vesicles. AMF OTUs were assigned to three families of Glomeromycota: *Glomeraceae, Claroideoglomeraceae,* and *Archaeosporaceae* (Figure 5). Of all identified AMF OTUs, five OTUs belonged to *Glomeraceae*, three OTUs belonged to *Claroideoglomeraceae*, and two OTUs belonged to *Archaeosporaceae* (Table 2.4). The Shannon diversity index was calculated to compare the diversity of AMF in fertilized and unfertilized grasslands. Paired *t*-test indicated that unfertilized grasslands had more diverse AMF OTUs than the fertilized plots ($H_{\text{unfertilized}}$ = 1.54, $H_{\text{fertilized}}$ = 0.61, p = 0.003). The number of OTUs in unfertilized and fertilized plots is illustrated in Figure 2.6. The most abundant family was *Glomeraceae* with *Glomus* sp. (36.5%), *Glomus* OTU26 (18.8%), and *Glomus* Li14 Glo7 (16.7%). Interestingly, all detected AMF OTUs were found in unfertilized grassland vegetation, whereas *Claroideoglomus* OTU52, *Claroideoglomus claroideum*, and *Archaeospora* Li14 Arc2 were absent in fertilized grassland vegetation.

PCA was used as a linear ordination method to illustrate the community composition of AMF in this experiment (Figure 2.7). The cumulative percentage of variance of AMF OTUs data showed that the first two PCA axes explain 57.76% of the variability of AMF OTUs. This result indicated that Glomeraceae family was found in most of the treatments.

Claroideoglomeraceae family was associated only with swards rich in species and monocot-dominated that were mowed once and were not amended with fertilizers. AMF family of Archaeosporaceae was detected mostly in unfertilized treatment in different sward types and mowing treatment

Table 2.4. Molecular identification of arbuscular mycorrhizal fungi based on BLASTN queries against NCBI database.

OTUs ID	GenBank accession No.	Closest blast match	Query Length	E-value	Max identity
Glo-Ire	KT223123	Glomeraceae *Glomus irregulare*	594	0	97%
Glo26	KT223124	Glomeraceae *Glomus* OTU26	514	0	97%
Glo	KT223125	Glomeraceae *Glomus* sp.	522	0	98%
GloGL05	KT223126	Glomeraceae *Glomus* An08 GLO5	582	0	97%
Glo07	KT223127	Glomeraceae *Glomus* Li14 Glo7	480	0	97%
Arch1	KT223128	Archaeosporaceae *Archaeospora* Li14 Arc1	395	0	98%
Arch2	KT223129	Archaeosporaceae *Archaeospora* Li14 Arc2	537	0	97%
Cla52	KT223130	Claroideoglomeraceae *Claroideoglomus* OTU52	548	0	97%
Cla	KT223131	Claroideoglomeraceae *Claroideoglomus claroideum*	578	0	98%
Cla16	KT223132	Claroideoglomeraceae *Claroideoglomus* OTU16	538	0	97%

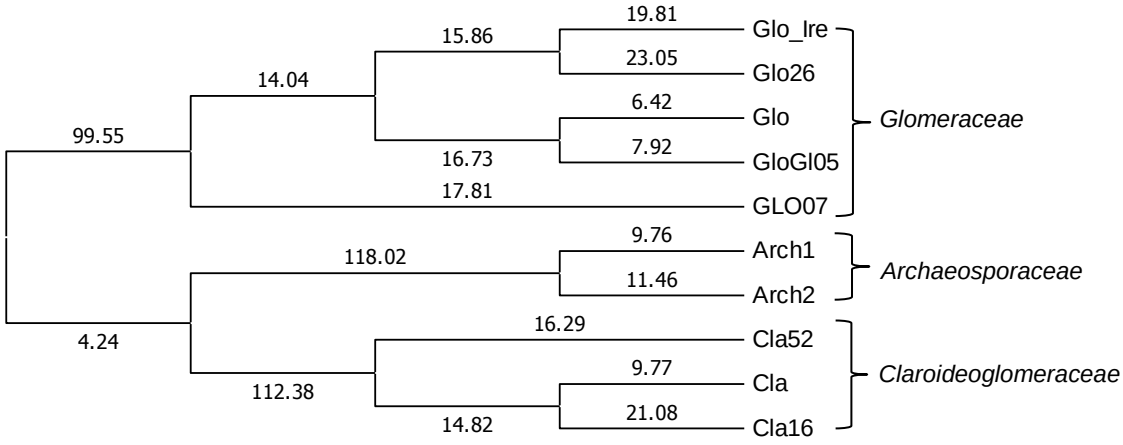

Figure 2.5. The most parsimonious tree inferring phylogenetic relationship between arbuscular mycorrhizal OTUs in grassland vegetation. Numbers above branches indicate bootstrap values (1,000 replicates).

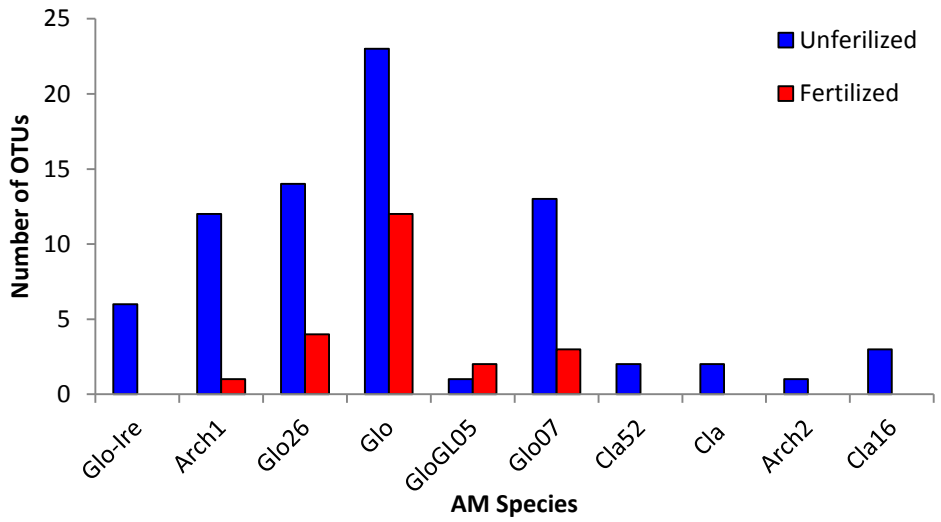

Figure 2.6. Species richness of arbuscular mycorrhizal OTUs in fertilized and unfertilized plots. For OTU abbreviations refer to Table 2.4.

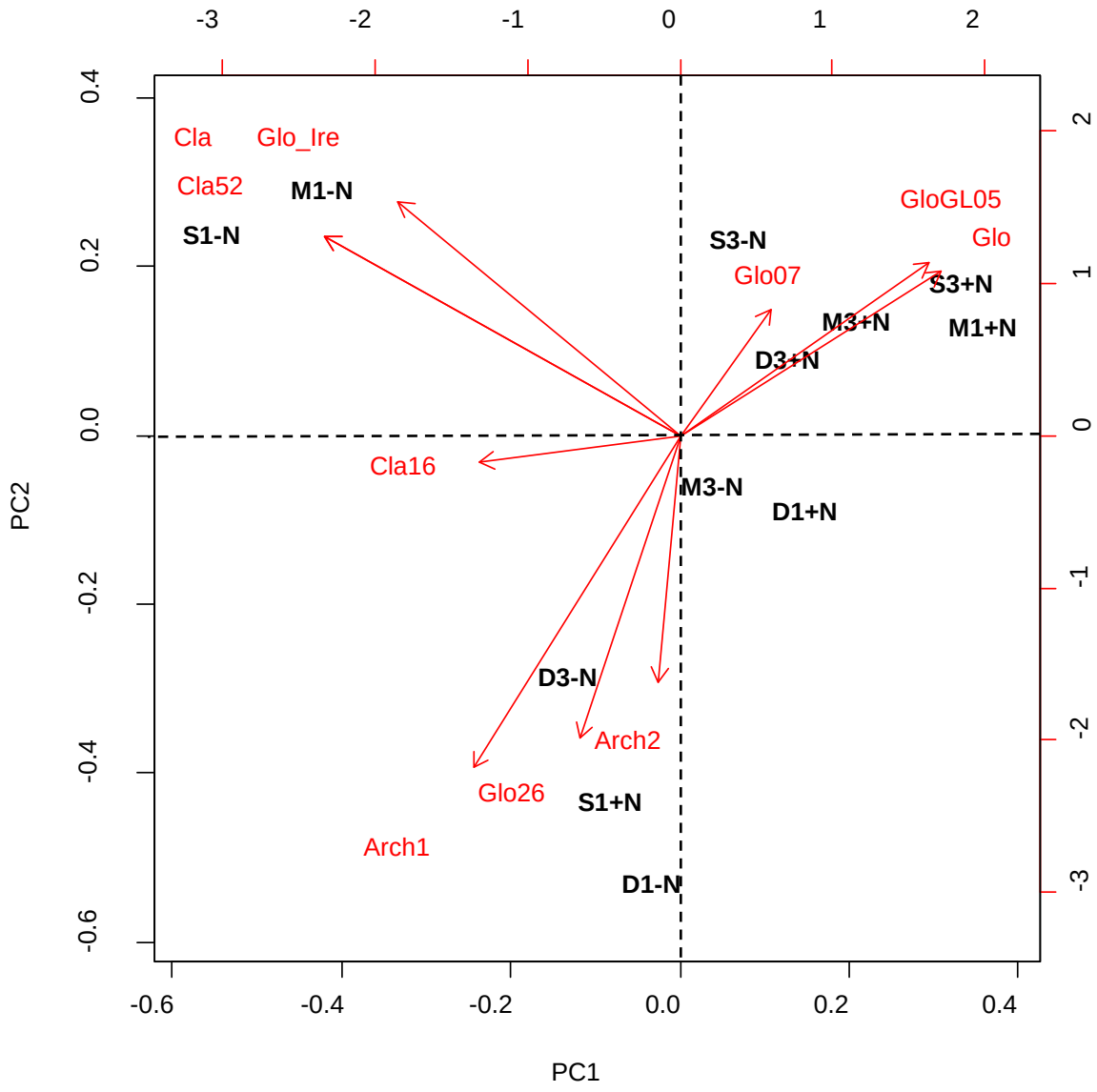

Figure 2.7. Principal component analysis of arbuscular mycorrhizal OTUs (red letters) in treatment plots (black letters). For OTUs code refer to Table 2.4.

2.4. Discussion

This study showed that species-rich swards and monocots- and dicots-dominated plots did not significantly influence the AMF hyphal root colonization. These results were expected since most of the vascular plants can be colonized by AMF (Schüβler et al. 2001). Arbuscules and vesicles, the key structures for plant-fungal nutrient exchange and storage, respectively, were more responsive to the applied treatments than the hyphae. Increasing the nutrient input by applying nitrogen, phosphorus, and potassium fertilizer reduced the number of arbuscules and vesicles and thus affected the AMF symbiosis. In this study, the relative abundances of arbuscules and vesicles in unfertilized sites were the highest when the site was not mowed. Previous studies have shown that AMF support plant growth and reproduction without fertilizer application (Johnson 1993; Titus and Leps 2000) and fertilization suppressed the development of extramatrical hyphae of AMF (Eom 2009) and spore abundance (Mårtensson and Carlgren, 1994).

Utilization as an independent factor had a negative effect on AMF abundance. However, interaction between the sward type and utilization positively affected arbuscules abundance in both control and herbivory plots. Management practices may affect AM abundance (Sieverding 1990; Miller et al. 1995). In relation to this, we found no significant effect on AMF colonization through mowing treatments.

Removing aboveground biomass can also be caused by herbivores. It has been reported that *Helix pomatia* (Ledergerber et al. 1998) and grasshoppers (Branson and Sword 2009) are potential herbivores in grassland. Both of these herbivores have been used in this experiment. The herbivory had no effect on AMF hyphal root colonization and the relative abundances of arbuscules and vesicles. Many studies reported the effects of insect herbivores on AMF–plant association (Gehring and Whitham 2003; Gange 2007; Gehring and Bennett 2009). However, they did not report the variation in the response of AMF colonization affected by insect herbivores. Recent meta-analysis found that the effect of insect herbivore on AMF status depends on the feeding mode and diet breadth of the insect (Koricheva et al. 2009). Additionally, removing the above ground biomass by herbivores leads to carbon limitation that influence alters the AMF status (Barto and Rillig 2010).

The combination between the sward type and utilization affected the relative abundance of arbuscules and vesicles. The interaction among the nutrients, sward type, and utilization also influenced the vesicle abundance, whereas interactions between all treatments affected arbuscule abundance. These interactions may be due to species richness of swards and fertilization. The interaction of treatments in this study is difficult to interpret as AMF arbuscule abundance interacted with all factors, whereas vesicle abundance was not affected. All of the treatments alter the arbuscular mycorrhizalmutualism. AMF are affected by

management practices such as fertilization, mowing, and plant diversity (Titus and Leps 2000; Mathimaran et al. 2007).

In the present study, changes in the AMF community composition were detected among fertilization treatments. It is noteworthy that most of the AMF OTUs were present in unfertilized grassland and their number was higher than that in fertilized grassland, as shown in Figure 2.6. It has been reported that limited content of nitrogen and phosphorous in soil is the key determinant factor to AMF diversity in montane grasslands (Karanika et al. 2008). Long-term nitrogen and phosphorus fertilization also decreased the AMF species diversity and relative frequency and abundance (Bhadalung et al. 2005; Šmilauerová et al. 2012; Liu et al. 2012).

The results presented herein support the hypothesis that fertilization alters the community composition of AMF. The sward type, utilization, and grazing by herbivores as single factor did not influence the AMF hyphal colonization. However, combination of all these treatments influeced the arbuscule abundance.

2.5. References

Barto EK, Rillig MC (2010) Does herbivory really suppress mycorrhiza? A meta-analysis. J Ecol 98:745–753.

Bates D, Maechler M, Bolker BM, Walker SC (2015) Fitting Linear Mixed-Effects Models using lme4. ArXiv e-print; in press, Journal of Statistical Software, http://arxiv.org/abs/1406.5823.

Bentivenga SP, Hetrick BAD (1992) The effect of prairie management practices on mycorrhizal symbiosis. Mycologia 84:522–527.

Bhadalung NN, Suwanarit A, Dell B, Nopamornbodi O, Thamchaipenet A, Rungchuang J (2005) Effects of long-term NP-fertilization on abundance and diversity of arbuscular mycorrhizal fungi under a maize cropping system. Plant Soil 270:371–382.

Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. Nat Commun 1:1–11.

Branson DH, Sword GA (2009) Grasshopper herbivory affects native plant diversity and abundance in a grassland dominated by the exotic grass agropyron cristatum. Restor Ecol 17:89–96.

Carlier L, Rotar I, Vlahova M, Vidican R (2009) Importance and functions of grasslands. Not Bot Horti Agrobot Cluj-Napoca 37:25–30.

Currie AF, Murray PJ, Gange AC (2011) Is a specialist root-feeding insect affected by arbuscular mycorrhizal fungi? Appl Soil Ecol 47:77–83.

Eom DCH (2009) The effect of fire, mowing and fertilizer amendment on arbuscular mycorrhizas in tallgrass prairie. Am Midl Nat 55–70.

Egerton-Warburton LM, Allen EB (2000) Shifts in arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient. Ecol Appl 10:484–496.

Entry JA, Rygiewicz PT, Watrud LS, Donnelly PK (2002) Arbuscular mycorrhizal response to adverse soil conditions. In: Arbuscular mycorrhizae interactions in plants rhizosphere and soil. Science Pulisher, Inc. pp 135-158.

Finlay RD (2008) Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extraradical mycelium. J Exp Bot 59:1115–1126.

Gange AC (2007) Onsect-mycorrhizal interactions: patterns, processes, and consequences. In: Ecological Communities. Cambridge University Press, pp 124-143.

Gange AC, Bower E, Brown VK (2002) Differential effects of insect herbivory on arbuscular mycorrhizal colonization. Oecologia 131:103–112.

Gehring CA, Whitham TG (1994) Interactions between aboveground herbivores and the mycorrhizal mutualists of plants. Trends Ecol Evol 9:251–255.

Gehring CA, Whitham TG (2003) Mycorrhizae-herbivore interactions: population and community consequences. In: Heijden DMGA Van Der, Sanders DIR (Eds) Mycorrhizal Ecology. Springer Berlin Heidelberg, Pp 295–320

Gehring C, Bennett A (2009) Mycorrhizal fungal–plant–insect interactions: The importance of a community approach. Environ Entomol 38:93–102.

Hall T (1999) Bioedit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98.

Haug I, Setaro S, Suárez 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.

Hopkins A, Morris C (2002) Multi-functional roles of grassland in organic farming systems. In: Proceedings of The UK Organic Research 2002 Conference. Organic Centre Wales, Institute Of Rural Studies, University Of Wales Aberystwyth, pp 75–80

Johnson NC (1993) Can fertilization of soil select less mutualistic mycorrhizae? Ecol Appl 3:749–757.

Kahiluoto H, Ketoja E, Vestberg M, Saarela I (2001) Promotion of am utilization through reduced p fertilization. 2. Field studies. Plant Soil 231:65–79.

Karanika ED, Mamolos AP, Alifragis DA, Kalburtji KL, Veresoglou DS (2008) Arbuscular mycorrhizas contribution to nutrition, productivity, structure and diversity of plant community in mountainous herbaceous grassland of Northern Greece. Plant Ecol 199:225–234.

Keuter A, Hoeft I, Veldkamp E, Corre MD (2012) Nitrogen response efficiency of a managed and phytodiverse temperate grassland. Plant Soil 364:193–206.

Klironomos JN (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology 84:2292–2301.

Klironomos JN, Hart MM, Gurney JE, Moutoglis P (2001) Interspecific differences in the tolerance of arbuscular mycorrhizal fungi to freezing and drying. Can J Bot 79:1161–1166.

Koricheva J, Gange AC, Jones T (2009) Effects of mycorrhizal fungi on insect herbivores: A meta-analysis. Ecology 90:2088–2097.

Kula AAR, Hartnett DC, Wilson GWT (2005) Effects of mycorrhizal symbiosis on tallgrass prairie plant–herbivore interactions. Ecol Lett 8:61–69.

Leake J, Johnson D, Donnelly D, Muckle G, Boddy L, Read D (2004) Networks of power and influence: the role of mycorrhizal mycelium in controlling plant communities and agroecosystem functioning. Can J Bot 82:1016–1045.

Ledergerber S, Leadley PW, Stöcklin J, Baur B (1998) Feeding behaviour of juvenile snails (helix pomatia) to four plant species grown at elevated atmospheric CO_2. Acta Oecologica 19:89–95.

Liu Y, Shi G, Mao L, Cheng G, Jiang S, Ma X An L, Du G, Collins JN, Feng H (2012) Direct and indirect influences of 8 years of nitrogen and phosphorus fertilization on Glomeromycota in an Alpine meadow ecosystem. New Phytol 194:523–535.

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. Environ Microbiol 12:2165–2179.

Makovets S (Ed) (2013) DNA electrophoresis: Methods and protocols. Humana Press, New York, pp.63-82.

Mårtensson AM, Carlgren K (1994) Impact of phosphorus fertilization on vam diaspores in two swedish long-term field experiment. Agric Ecosyst Environ 47:327–334.

Mathimaran N, Ruh R, Jama B, Verchot L, Frossard E, Jansa J (2007) Impact of agricultural management on arbuscular mycorrhizal fungal communities in Kenyan ferralsol. Agric Ecosyst Environ 119:22–32.

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 Phytol 115:495–501.

Miller MH, Mcgonigle TP, Addy HD (1995) Functional ecology of vesicular arbuscular mycorrhizas as influenced by phosphate fertilization and tillage in an agricultural ecosystem. Crit Rev Biotechnol 15:241–255.

Mueller RC, Gehring CA (2006) Interactions between an above-ground plant parasite and below-ground ectomycorrhizal fungal communities on Pinyon pine. J Ecol 94:276–284.

Nijjer S, Rogers WE, Siemann E (2010) The impacts of fertilization on mycorrhizal production and investment in Western Gulf Coast grasslands. Am Midl Nat 163:124–133.

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 Phytol 184:424–437.

Öpik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij JM, Reller Ü, Zobel M (2010) The online database maarjam reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). New Phytol 188:223–241.

Petersen U, Wrage N, Köhler L, Leuschner C, Isselstein J (2012) Manipulating the species composition of permanent grasslands—a new approach to biodiversity experiments. Basic Appl Ecol 13:1–9.

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–IN18.

Robbertse B, Tatusova T (2011) Fungal genome resources at NCBI. Mycology 2:142–160.

Schüßler A, Schwarzott D, Walker C (2001) A New fungal phylum, the Glomeromycota: Phylogeny and evolution. Mycol Res 105:1413–1421.

Sieverding E (1990) Ecology of VAM fungi in tropical agrosystems. Agric Ecosyst Environ 29:369–390.

Silva GA Da, Lumini E, Maia LC, Bonfante P, Bianciotto V (2006) Phylogenetic analysis of Glomeromycota by partial LSU rDNA sequences. Mycorrhiza 16:183–189.

Šmilauerová M, Lokvencová M, Šmilauer P (2012) Fertilization and forb:Graminoid Ratio affect arbuscular mycorrhiza in seedlings but not adult plants of *Plantago lanceolata*. Plant Soil 351:309–324.

Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic Press, Amsterdam; Boston, pp 11-145.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol 30:2725–2729.

Thomson B, Robson A, Abbott L (1992) The effect of long-term applications of phosphorus fertilizer on populations of vesicular-arbuscular mycorrhizal fungi in pastures. Aust J Agric Res 43:1131–1142.

Titus JH, Leps J (2000) The response of arbuscular mycorrhizae to fertilization, mowing, and removal of dominant species in a diverse oligotrophic wet meadow. Am J Bot 87:392–401.

Toju H, Sato H, Tanabe AS (2014) Diversity and spatial structure of belowground plant–fungal symbiosis in a mixed subtropical forest of ectomycorrhizal and arbuscular mycorrhizal plants. Plos ONE.

Trouvelot S, Tuinen D Van, Hijri M, Gianinazzi-Pearson V (1999) Visualization of ribosomal dna loci in spore interphasic nuclei of glomalean fungi by fluorescence in situ hybridization. Mycorrhiza 8:203–206.

Van Der Gast CJ, Gosling P, Tiwari B, Bending GD (2011) Spatial scaling of arbuscular mycorrhizal fungal diversity is affected by farming practice. Environ Microbiol 13:241–249.

Wallisdevries MF, Poschlod P, Willems JH (2002) Challenges for the conservation of calcareous grasslands in northwestern europe: integrating the requirements of flora and fauna. Biol Conserv 104:265–273.

Wamberg C, Christensen S, Jakobsen I (2003) Interaction between foliar-feeding insects, mycorrhizal fungi, and rhizosphere protozoa on pea plants. Pedobiologia 47:281–287.

White RP, Murray S, Rohweder M (2000) Pilot analysis of global ecosystems: Grassland ecosystems. World Resources Institute, Washington, DC, pp. 1-65.

Wu F, Dong M, Liu Y, Ma X, An L, Peter J, Young W, Feng H (2011) Effects of long-term fertilization on am fungal community structure and glomalin-related soil protein in the loess plateau of China. Plant Soil 342:233–247.

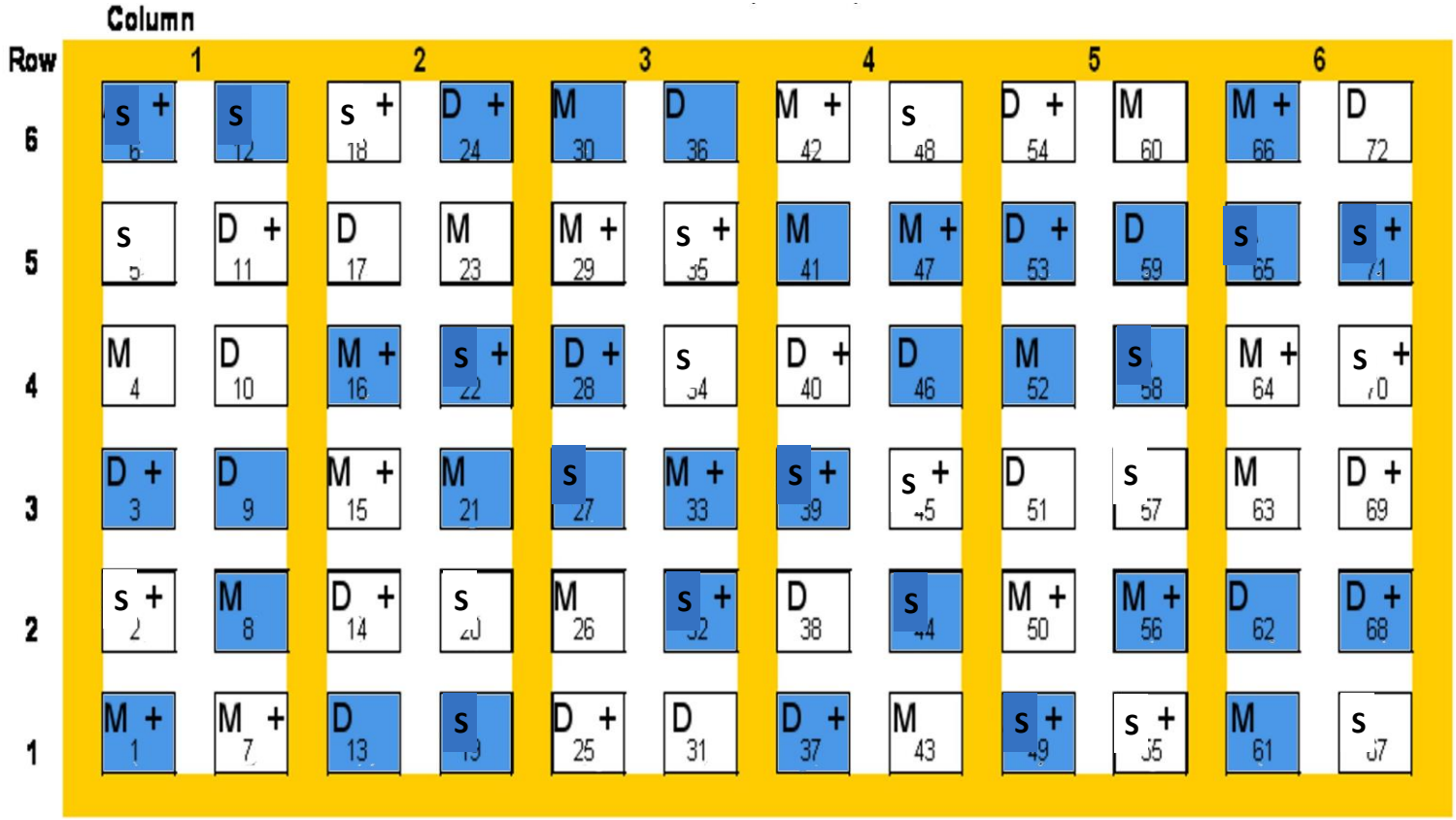

Figure S2.1. Experimental design of Latin rectangle with 12 treatments and 6 replications. M: monocot-dominated, D: dicot-dominated, S: sward species richness, +: fertilized, blue box: 3 times cutting, and white box is one time cutting.

Table S2.1. AMF abundance in grassland vegetation of different swards, utilization and nutrient treatments. For AMF OTUs abbreviations refer to Table 2.4.

Treatment				AMF OTUs									
Sward	Utilization	Nutient	Abreviation	Glo_Ire	Arch1	Glo26	Glo	GloGL05	Glo07	Cla52	Cla	Arch2	Cla16
Speciesrichness	1 × cut	Unfertilized	S1-N	1	1	1	1	0	1	1	1	0	1
Speciesrichness	3 × cut	Unfertilized	S3-N	1	0	1	3	1	2	0	0	0	0
Monocots	1 × cut	Unfertilized	M1-N	1	1	1	2	0	1	1	1	0	0
Monocots	3 × cut	Unfertilized	M3-N	0	1	1	3	0	2	0	0	0	1
Dicots	1 × cut	Unfertilized	D1-N	0	3	2	2	0	0	0	0	1	0
Dicots	3 × cut	Unfertilized	D3-N	0	2	3	1	0	1	0	0	0	1
Speciesrichness	1 × cut	NPK	S1+N	0	2	6	0	0	0	0	0	0	0
Speciesrichness	3 × cut	NPK	S3+N	0	0	0	4	2	2	0	0	0	0
Monocots	1 × cut	NPK	M1+N	0	0	0	5	3	0	0	0	0	0
Monocots	3 × cut	NPK	M3+N	0	0	0	4	0	4	0	0	0	0
Dicots	1 × cut	NPK	D1+N	0	0	4	4	0	0	0	0	0	0
Dicots	3 × cut	NPK	D3+N	0	0	0	0	0	8	0	0	0	0

Chapter 3

Arbuscular mycorrhiza affected by land use in lowland rain forest transformation systems in Sumatra Indonesia

3.1. Introduction

Deforestation by replacing natural forest to non-forestry-related land uses (Margono et al. 2012) is one of the main causes of changes in ecosystem's functions (Costa and Pires 2010; Butt et al. 2011). Currently, in Indonesia, expanding oil palm and rubber tree plantations have changed the rainforest and decrease the biodiversity (Koh and Wilcove 2008; Margono et al. 2014). Generally, rainforests are replaced by monoculture vegetation (Carnus et al. 2006; Stephens and Wagner 2007), oil palm plantations (Koh and Wilcove 2008), rubber tree plantations or they are modified for agroforestry (Ketterings et al. 1999). According to the United Nations Food and Agricultural Organization (UNFAO), tropical rain forests in Indonesia are destructed at a tremendous speed compared to that in the rest of the world (UNFAO, 2010). In total, over 6.02 million ha of Indonesian primary forest cover was lost from 2000 to 2012, and the loss continues to dramatically increase on average by 47,600 ha per year (Margono et al. 2014). Global production of palm oil in 2009 was 43.4 million tons, of which, Indonesia contributed 47% (USDA-FAS 2010). Moreover, Indonesia is the second largest rubber producing country after Thailand, contributing 27.3% (UNFAO 2013) of the total rubber produced in the world. Currently, rubber tree plantations occupy 3.5 million ha in

Indonesia (Indonesian Minister of Agriculture 2015). A number of studies have reported on the impact of land use changes in responses to ecosystem services (Lambin and Meyfroidt 2010; Polasky et al. 2010) and above-ground biodiversity (Chemini and Rizzoli 2003; Butler et al. 2010). Investigating land use change is of a continuous concern in biodiversity including the changes in soil microbial communities.

Common beneficial soil fungi are arbuscular mycorrhizal fungi (AMF). They form a mutualistic symbiotic association with the root systems of a majority of higher plants. The intra-extra radical hyphal networks formed by the fungi help the absorption of mineral nutrients from soil and deliver them to the host plant (Cheng and Baumgartner 2006; Camenzind and Rillig 2013), particularly phosphorus (Smith and Smith 2012) and nitrogen (Veresoglou et al. 2012). In return, AMF receives photosynthetically derived carbon compounds from the host plant (Smith and Read 2008). Besides their role in promoting plant growth, AMF are also reported to play an important role in protecting the host plant against the soil borne pathogens (Wehner et al. 2010) and environmental stress (Smith et al. 2009, Zhu et al. 2009, Grover et al. 2010). They may also increase organic carbon decomposition (Cheng and Baumgartner 2006).

Because of their various beneficial impacts on ecosystems, AMF have attracted continuous attention of the researchers. Land use change may also drive a change in the AMF communities. Here, we study the impact of forest conversion into rubber tree and oil palm plantations on the AMF communities. Investigation of the AMF affected by land use

transformation from natural forest to managed agro-plantation, particularly in the tropical region, is still lacking. Therefore, our objective was to study the AMF communities in different transformation systems and to observe if the environmental factors affect the AMF communities.

Our previous study was conducted in two landscapes, Bukit Duabelas National Park (hereafter referred as Bukit Duabelas) and Harapan rain forest, in Sumatra, Indonesia. Each landscape comprises three different systems (rain forest, rubber tree plantation, and oil palm plantation). We found that arbuscular mycorrhizal root colonization was abundant and generally unaffected by the land use system. However, root vitality was lower and spore abundance higher in oil palm plantations than in the forest and rubber tree plantation (Sahner et al. in press). In the present study, we continue the research in the same sites to observe the community composition in different land use systems. We hypothesized that transformation of rainforest into managed rubber tree and oil palm plantations will reduce species richness of AMF communities. To test this hypothesis, we conducted molecular identification of plant hosts and associated AMF in a single root.

3.2. Materials and Methods

3.2.1. Study sites

The study was carried out in two land use systems of Bukit Duabelas and Harapan rainforest landscapes located in Jambi province, Sumatra, Indonesia. Both landscapes have tropical climate with > 2000 mm rain fall annually. The annual mean temperature and precipitation in Bukit Duabelas are 26.8°C and 2860 mm, respectively (location: Lubuk Kepayang, http://en.climate-data.org/location/587840/), whereas in Harapan, they are 26.9°C and 2332 mm, respectively (location: Dusun Baru, http://en.climate-data.org/location/ 595657/). Three different land use systems were selected in each landscape: rain forest, oil palm plantation, and rubber tree plantation (detailed plot information is provided in Table 3.1) and three sub plots were selected in each land use systems (designated as a, b, and c).

3.2.2. Sampling and export permission

The entry permit to conservation area was issued by the National Park of Bukit Duabelas Office (Balai Taman Nasional Bukit Duabelas: Surat Izin Memasuki Kawasan Konservasi [SIMAKSI], number: SI.71/BTNBD-1/2013). The entry to Harapan rain forest was approved by the PT. Restorasi Ekosistem Indonesia (REKI) via email communication between Collaborative Research Center (CRC) office in Universitas Jambi and PT. REKI. The Research Center for Biology of the Indonesian Institute of Science (LIPI: Lembaga Ilmu Pengetahuan Indonesia, Jakarta,

Indonesia) issued a sample collection permit (Rekomendasi Ijin Pengamblian dan Angkut [SAT-DN] Sampel Tanah dan Akar, number: 2696/IPH.1/KS:02/XI/2012) for domestic transportation. Recommendation for export permit (number: S.16/KKH-2/2013, Rekomendasi Ijin Membawa/ Mengirim Sampel Tanah dan Akar ke Jerman [SAT-LN], number: 2538/IPH.I/KS.01/XII/2013) was also issued by LIPI. Export permit (reference number: 48/KKH-5/TRP/2014) for all samples from the plot (Table 3.1) was issued by the Directorate General of Forest Protection and Nature Conservation PHKA (Perlindungan Hutan dan Konservasi Alam, Jakarta, Indonesia) under the Ministry of Forestry of the Republic of Indonesia. The agency for Agricultural Quarantine under the Ministry of Forestry of the Republic of Indonesia certified the samples with "phytosanitary certificate" (reference number: 2013.2.10.03. K10.E000014). The Chamber of Agriculture of Lower Saxony (Plant Protection Office, Hannover, Germany) issued the import permits (Letter of Authority, numbers: DE-NI-12- 69 -2008-61-EC, DE-NI-14- 08 -2008-61-EC).

Table 3.1. Plot location in Bukit Duabelas and Harapan land use systems.

Transformation systems	Plot code	Latitude	Longitude	Altitude (m)
Landscape: Bukit Duabelas				
Rain forest	BF1	S 01°59'42.5"	E 102°45'08.1"	83
Rain forest	BF2	S 01° 58'55.1''	E 102°45'02.7"	77
Rain forest	BF3	S 01°56'33.9"'	E 102°34'52.7"'	87
Rain forest	BF4	S 01°56'31.0"'	E 102°34'50.3"	87
Oil palm plantation	BO1	S 02°04'26.1"	E 102°48'55.1"	75
Oil palm plantation	BO2	S 02°04'32.0"	E 102°47'30.7"	84
Oil palm plantation	BO3	S 02°04'15.2"	E 102°47'30.6"	71
Oil palm plantation	BO4	S 02°03'01.5"	E 102°45'12.1"	34
Rubber tree plantation	BR1	S 02°05'30.7"	E 102°48'30.7"	71
Rubber tree plantation	BR2	S 02°05'06.8"	E 102°47'20.7"	95
Rubber tree plantation	BR3	S 02°05'43.0"	E 102°46'59.6"	90
Rubber tree plantation	BR4	S 02°04'36.1"	E 102°46'22.3"	51
Landscape: Harapan				
Rain forest	HF1	S 02°09'09.9"	E 103°21'43.2"	76
Rain forest	HF2	S 02°09'29.4"	E 103°20'01.5"	75
Rain forest	HF3	S 02°10'30.1"	E 103°19'57.8"	58
Rain forest	HF4	S 02°11'15.2"	E 103°20'33.4"	77
Oil palm plantation	HO1	S 01°54'35.6"	E 103°15'58.3"	81
Oil palm plantation	HO2	S 01°53'00.7"	E 103°16'03.6"	55
Oil palm plantation	HO3	S 01°51'28.4"	E 103°18'27.4"	64
Oil palm plantation	HO4	S 01°47'12.7"	E 103°16'14.0"	48
Rubber tree plantation	HR1	S 01°54'39.5"	E 103°16'00.1"	77
Rubber tree plantation	HR2	S 01°52'44.5"	E 103°16'28.4"	59
Rubber tree plantation	HR3	S 01°51'34.8"	E 103°18'02.1"	90
Rubber tree plantation	HR4	S 01°48'18.2"	E103°15'52.0"	71

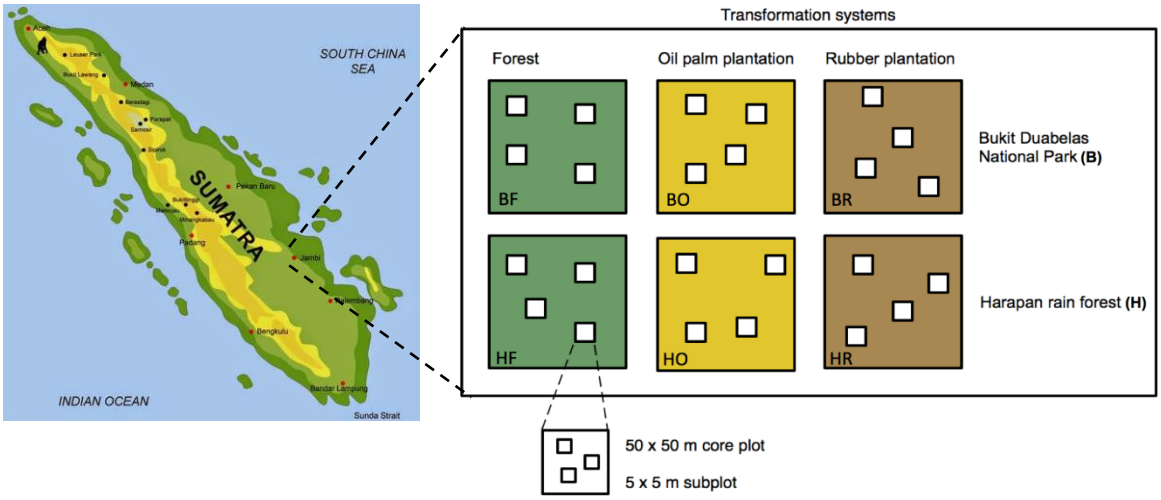

Figure 3.1. Overview map of research area in two landscapes: Bukit Duabelas National Park (B) and Harapan rain forest (H) in Sumatra Indonesia (modified after Kreft et al. 2011).

3.2.3. Sampling and root selection

Three soil cores (0.04 m diameter and 0.20 m depth) were collected per sub-plot at a distance of 1 m from the tree. Each soil core was placed in a zip plastic bag and stored in a cooling box (Sarstedt, Nümbrecht, Germany) to be transported to laboratory of University of Jambi where they were stored at 4°C. From each soil core, one single root was collected (three single roots per sub-plot = nine roots per plot). Each single root was washed with tap water, placed in a water-filled Petri dish (92 × 16 mm, Sarstedt, Germany) and observed under the stereo microscope (Leica EZ4HD, Wetzlar, Germany) at 35 × magnification. Root colonized by ectomycorrhiza (EM) was distinguished from non-EM root tips by the presence of typical mantle of the fungus that covers the roots (Peterson et al. 2004; Smith and Read 2009). An example of EM roots is show in Figure 3.2A, non-EM roots in Figure 3.2B, and dead root in Figure 3.2C.

Roots were documented with the camera attached to the microscope. The root samples were preserved by freeze drying (Benchhtop K, VirTis, SP Industries, Gardiner, NY, USA): the samples were frozen overnight at -80°C and then dried at -72°C for 30 hours. Subsequently, each single root was stored in a 2-mL reaction tube (Sarstedt, Nümbrecht, Germany) and shipped to University of Göttingen, Germany, in a reaction tube box (Sarstedt, Germany) filled with silica gel (Sarstedt, Germany).

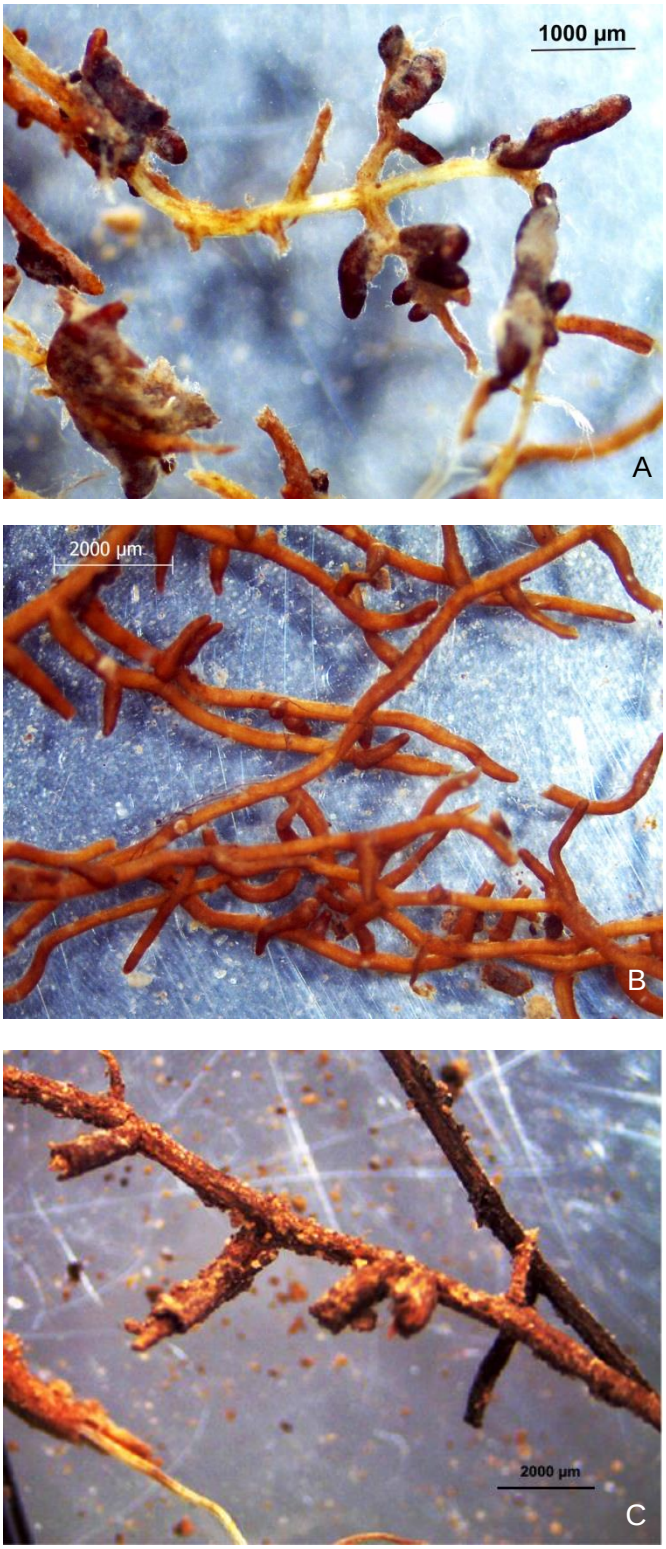

Figure 3.2. Ectomycorrhizal root tips (A), non-ectomycorrhizal root tips (B) and dead root tips(C).

3.2.4. Molecular identification of arbuscular mycorrhizal fungi

DNA was extracted using the same method described in Chapter 2. A nested PCR was carried out to amplify the partial small subunit rDNA with NS1 and NS4 general fungi primers and the amplicons were used as DNA templates in the amplification with specific primers for AMF, AML1, and AML2 (Table 3.2). The PCR reactions and conditions, cloning, restriction fragment length polymorphism (RFLP), gel electrophoresis, and sequencing were conducted as described in Chapter 2. For PCR conditions, the first and second annealing temperature were adjusted to 40°C and 50°C respectively.

Table 3.2. Details of primers used in this study.

Primer	Sequence (5'–3')	Target group	Source
NS1	GTAGTCATATGCTTGTCTC	Fungal (18S rRNA gene)	White et al. (1990)
NS4	CTTCCGTCAATTCCTTTAAG		
AML1	ATCAACTTTCGATGGTAGGATAGA	All AMF groups	Lee et al. (2008)
AML2	GAACCCAAACACTTTGGTTTCC		
M13-20	CGACGTTGTAAAACGACGGCCAGT	General primer for AMF sequencing	pGEM-T Easy vector primers
M13 RP	TTTCACACAGGAAACAGCTATGAC		
rbcLaf	ATGTCACCACAAACAGAGACTAAAGC	Land plants (ribulose-bisphosphate carboxylase gene)	Kress et al. (2009)
rbcLar2	GAAACGGTCTCTCCAACGCAT		
MatKnewF	GTTCAAACTCTTCGCTACTGG	Land plants (chloroplast maturase K gene)	Kress et al. (2009), Yu et al. (2011)
MatKnewR	GAGGATCCACTGTAATAATGAG		
3FKim(MatK)	CGTACAGTACTTTTGTGTTTACGAG		
1RKim(MatK)	ACCCAGTCCATCTGGAAATCTTGGTTC		

AMF: arbuscular mycorrhizal fungi

3.2.5. Molecular identification of mycorrhizal host plants

In total, 112 single roots were analyzed including 72 single roots from the forest plots (36 single roots from each), 20 single roots from oil palm plantations in two landscapes (10 single roots from each), and 20 single roots from rubber tree plantations in two landscapes (10 single roots from each).

All PCR-sequencing and chemicals needed were conducted and provided by the Department of Forest Genetics and Tree Breeding, University of Göttingen. The DNA extracted from the single roots as described above was used for plant identification with markers *rbcL* and *matK.* These two markers were recommended by the Consortium for the Barcode of Life (CBOL) plant working group because of the straightforward recovery of the *rbcL* region and the discriminatory power of *matK* (CBOL Plant Working Group 2009).

The PCR mixture kit (HOT FIREPol®, Tartu, Estonia) contained 1.5 µL 1x PCR buffer with B2 (Mg^{2+} free), 1.5 µL 2 mM $MgCl_2$, 1 µL 0.2 mM dNTPs mix, 0.2 µL 0.5 U DNA polymerase, 1 µL 0.5 mM of each primer, 6.8 µL water (Roth GmbH, Germany), and 1 µL 10-fold diluted DNA. The PCR conditions were as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles at 94°C for 1 min, 50°C for 1 min, and 72°C for 1.5 min, and a final extension cycle at 72°C for 10 min. One set of *rbcL* primers and three sets of *matK* primers were tested for plant identification. The three sets of *matK* primer amplified the same region at different

binding sites. This three primer sets were tested individually for amplification efficiency.

PCR products were cleaned using innuPREP Gel Extraction Kit (Analytik Jena, Germany). Two microliter of the resulting PCR products were used for sequencing in a total volume of 10 µL of the reaction mix with BrightDye® Terminator Cycle Sequencing Kit (Nimagen, Carlsbad, CA, USA) following manufacturer's instructions. Sequencing conditions were the same as for the plant DNA amplification described above. PCR products were purified with DyeEx Kit (Qiagen, Hilden, Germany) following manufacturer's protocol. The sequence reactions were read on an Applied Biosystems 3130xl Genetic Analyzer (Life Technologies GmbH, Darmstadt, Germany) in the Department of Forest Genetics and Tree Breeding, University of Göttingen).

3.2.6. Sequence analysis

AMF and plant sequences were edited following the protocols described Chapter 2. BLAST searches of AM fungal species were performed against the MaarjAM data base (Öpik et al. 2010) and NCBI (Sequeira 2013). Plant sequences were BLAST searched against NCBI database and confirmed with BOLD Systems (Ratnasingham and Hebert 2007). Phylogenetic trees were constructed using maximum parsimony method implemented in MEGA 6 and the close-neighbor-interchange algorithm. The bootstrap values were estimated with 1000 replicates. The

deletion option in MEGA 6 was used for eliminating gaps and missing data.

Arbuscular mycorrhizal operational taxonomic units (OTUs) were defined on the basis of sequence similarities as surrogates for species. A threshold of 97% similarity was selected as the minimum value to assign a sequence to the same OTU since this value has been commonly used by various authors (Haug et al. 2013; Toju et al. 2014). Phylogenetic trees of AM fungi and their host plants are provided in supplementary Figures S3.1 and S3.2, respectively. The AM fungal sequences have been deposited in NCBI (accession numbers: KR822761 to KR822799).

3.2.7. Statistical analyses

A data set of matrix representing symbiosis of AMF and host plant with environmental variables was used for the analyses (Table S3.3). The environmental variables used were carbon in the root, phosphorus in the root, nitrogen in the root, aluminum in the root, and soil pH (Sahner et al. 2015 in press; Allen et al. 2015).

To obtain an overview on the AMF species richness from the results of sampling, the AMF OTUs abundance in each host plant was used to develop a rarefaction curve (Gotelli and Colwell 2011). This analysis was conducted in ’vegan’ package of R version 3.0.3 (Oksanen et al. 2013).

Species richness (S) was calculated as the number of AMF OTUs per land use system. Diversity indices were calculated using the following equations (Buzas and Hayek 2005; Keylock 2005).

- Simpson index (D) (equation 3.1)

$$D = \sum \frac{n_i(n_i - 1)}{N(N-1)}$$

- Shannon index (*H'*) (equation 3.2)

$$H' = -\sum p_i \ln p_i$$

- Buzas and Gibson's evenness (equation 3.3)

$$Buzas\ and\ Gibson's\ evenness = \frac{e^H}{S}$$

where n_i is the number of individuals of a taxon *i*, p_i is the proportion of the *i*-th species, *N* is the total number of species in the dataset, *e* is evenness, *H* is Shannon's *H*, and *S* is the number of species in the community. Simpson index ranges from 0 (all taxa are equally present) to 1 (one taxon completely dominates the community). In the Shannon index, *H'* varies from 0 for communities with a single taxon to high values for communities with many taxa.

To calculate the number of AM OTUs shared among different types of land uses, Venn diagrams were created using "Venny 2.0" (Oliveros, 2007-2015). The distribution of AM OTUs among distinct host plant

species was visualized using the network analysis of the AMF abundance data (Table S3.3). The analysis was conducted in R (R Development Core Team, 2014) using “bipartite” v2.05 package with “plotweb” function in R (Dormann et al. 2009). The specificity of AMF OTUs to the plant host was calculated with the *d*’ index of specialization (Blüthgen et al. 2007)

Two-way permutational multivariate analysis of variance (PERMANOVA; Anderson et al. 2011) was used to analyze the variance in AMF community composition in correlation to the landscape and plot. Bray-Curtis dissimilarity was used to calculate the distance between pairs of AMF communities using the following equation:

$$\mathrm{BC} = \sum_{k=1}^{n} \left| \left| x_i k - x_j k \right| \right| / \sum_{k=1}^{1} \left(x_i k - x_j k \right)$$

where *i* and *j* are different samples, *k* indicates different virtual taxa, and *x* is the proportional composition for a given sample and taxon. The significance of factors was estimated with 999 permutations. PERMANOVA was conducted in PAST 2.17c (Hammer et al. 2001). A non-metric multidimensional scaling (NMDS) was used to elucidate dissimilarities in AMF community composition in different land use systems. To compare the AMF community between rubber tree, oil palm, and forest roots, we only selected tree roots from each system. Because each of rubber tree and oil palm plantations had 10 samples, only 10 trees were also selected from each forest system of Bukit Duabelas and Harapan. AMF OTUs abundances in each host plant were used for NMDS

analysis (Table S3.3). Environmental variables were also included in the analysis. Each plant and their associated AMF were treated as one replication. The NMDS plot was created in PAST using the Bray-Curtis similarity index. To calculate the influence of environmental factors on the AMF OTU community, the plant hosts and environmental factors were fitted onto NMDS and subjected to goodness-of-fit statistics (R^2) using 'envfit' function in the vegan package 2.2-1 in R (Oksanen et al. 2013). *P* values were based on 999 permutations (Oksanen et al. 2013).

3.3. Results

3.3.1. AMF and host plant species

From 112 single roots (clone library), we screened 896 clones for AMF OTUs identification. On average, eight clones were analyzed per sample. A total of 39 different AMF OTUs were detected. Rarefaction curves were calculated in order to estimate AMF species richness in the sampling results. The rarefaction curve showed that most of the curves reached saturation point at the chosen sequencing depth (Figure 3.3). The number of sequences analyzed per sampling site was sufficient to cover the AMF diversity in a single root per land use system.

BLAST search revealed that 25% of OTUs had a high degree of similarity (100%), 10.7% of OTUs had 99% similarity, 21.4% OTUs had 98% similarity, and 42.9% OTUs had 97% similarity to taxa belonging to AMF. The AMF OTUs that were abundant both in Harapan rain forest and

Bukit Duabelas belonged to the families of *Acaulosporaceae*, *Ambisporaceae*, *Archaeosporaceae*, *Claroideoglomeraceae*, *Diversisporaceae*, *Gigasporaceae*, and *Glomeraceae*. The AMF OTUs detected in Bukit Duabelas and Harapan transformation systems are provided in Tables S3.1 and 3.2. The most abundant genus of AMF OTUs was *Glomus*. Sequences of 11 OTUs were presented for the first time, and phylogenetic analysis revealed that these unknown OTUs were related to *Archaeosporaceae* (Figure 3.4). We referred to them as unknown Glomeromycota in our further analysis.

The identity of the plant host of the AMF was assessed using two markers, *rbcL* and *matK*. The *rbcL* marker successfully amplified all plant DNA samples (Tables S3.4 and S3.5), whereas *matK* could amplify only a very low number of samples. Therefore, only the data from *rbcL* marker was used in this study. The 36 roots analyzed from the forest in Bukit Duabelas belonged to 20 plant species in 16 families. The 36 roots from the forest in Harapan belonged to 31 plant species in 17 families. All single roots obtained from rubber tree and oil palm plantations belonged to rubber and oil palm trees, respectively. Plant functional groups included 5 herbs, 1 shrub, and 50 trees as inferred from all 56 single roots obtained from the Bukit Duabelas land use systems. In Harapan systems, 4 shrubs, and 52 trees were identified from 56 single roots (Table 3.3).

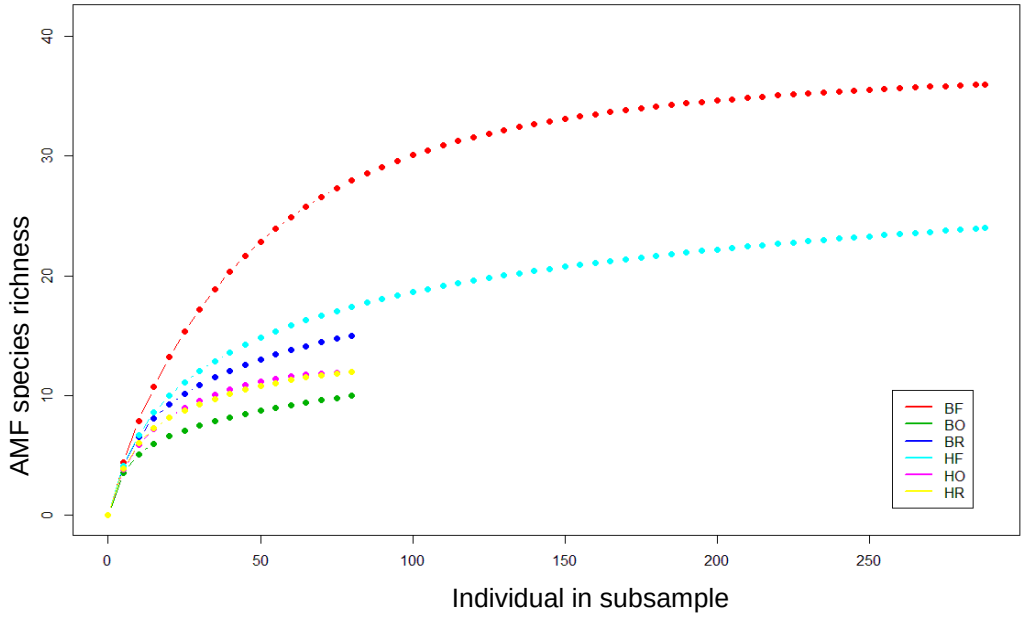

Figure 3.3. Sampling effort for the arbuscular mycorrhizal (AM) community in a single plant root from the forest in Bukit Duabelas (BF, n = 36), oil palm plantation in Bukit Duabelas (BO, n = 10), rubber tree plantation in Bukit Duabelas (BR, n = 10), forest in Harapan (HF, n = 36), oil palm plantation in Harapan (HO, n = 10), and rubber tree plantation in Harapan (HR, n = 10). Eight AM fungal clones were analyzed from each single root.

The diversity indices were calculated to compare the diversity of AMF in different land use systems. The species richness of AM was significantly higher in the rain forest than in the oil palm and rubber tree plantations, which was further corroborated by the diversity indices (Simpson, Shannon, and evenness, Table 3.4).

Table 3.3. Plant functional groups in Bukit Duabelas National Park and Harapan inferred from *rbcL* sequencing marker.

	Family	Species	Grass	Herb	Shrub	Tree
Bukit Duabelas	16	20	0	5	1	50
Harapan	17	31	0	0	4	52

Table 3.4. Arbuscular mycorrhizal diversity across land use systems in Bukit Duabelas National Park and Harapan transformation systems.

Diversity indices	BF		BO		BR		HF		HO		HR		*P*
Species richness	31	b	10	a	12	ab	21	b	10	ab	9	ab	0.007**
Simpson	0.844	b	0.738	a	0.803	ab	0.850	b	0.791	ab	0.806	ab	0.005**
Shannon	1.917	b	1.486	a	1.761	ab	1.951	b	1.679	ab	1.741	ab	0.005**
Evenness	0.970	b	0.900	a	0.940	ab	0.971	b	0.927	ab	0.954	ab	0.019*

BF: forest in Bukit Duabelas, BO: oil palm plantation in Bukit, BR: rubber tree plantation in Bukit Duabelas, HF: forest in Harapan, HO: oil palm plantation in Harapan, and HR: rubber tree plantation in Harapan. Significance levels: *$P < 0.05$, ** $P < 0.01$, ***$P < 0.001$.

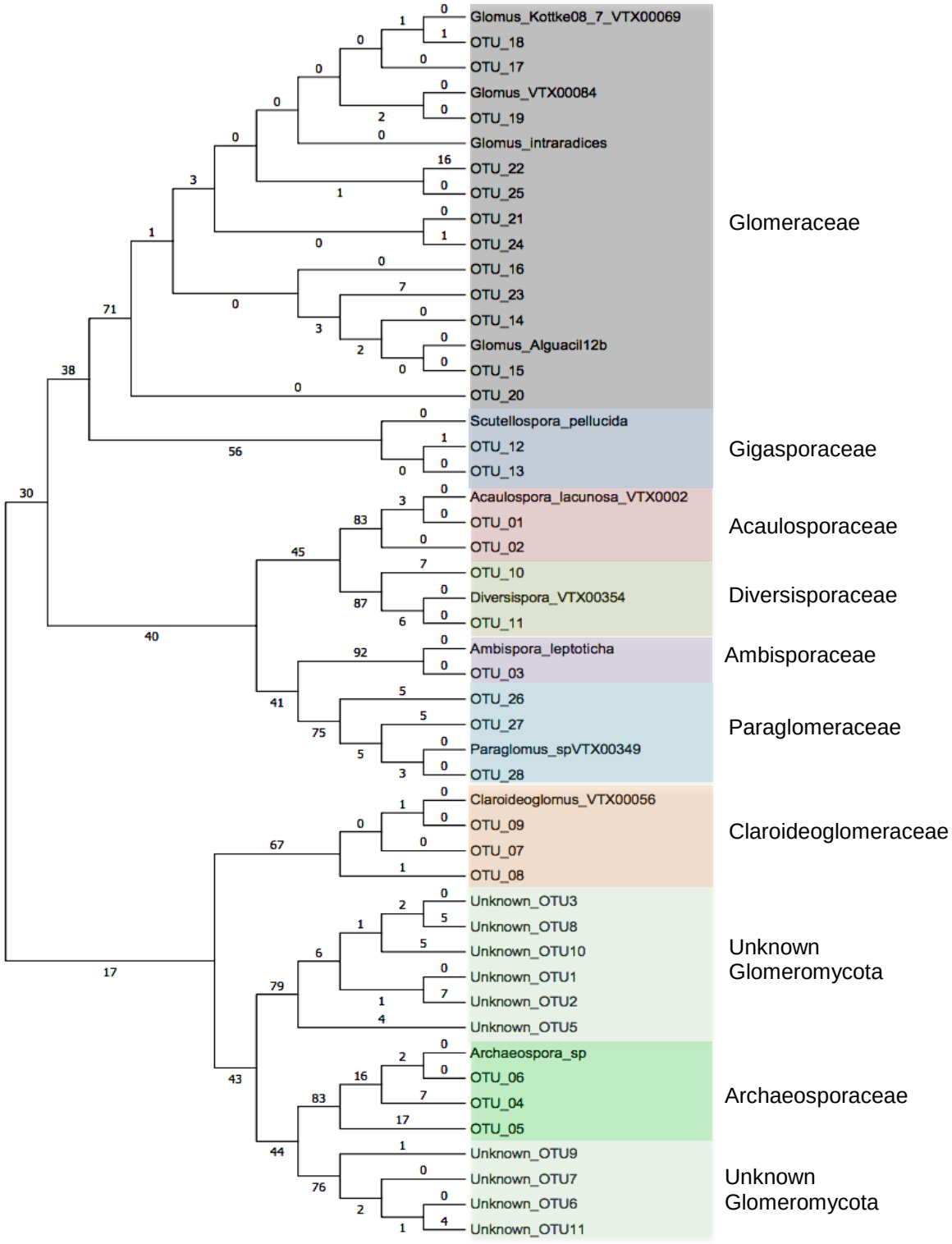

Figure 3.4. Phylogenetic tree of arbuscular mycorrhizal fungi isolated from the roots in Bukit Duabelas and Harapan transformation systems based on maximum parsimony. Numbers above branches indicate bootstrap values (1,000 replicates).

3.3.2. AMF communities across land use systems

To characterize the AMF community composition, we determined the number of unique and shared AMF OTUs in different land use systems. Venn diagrams showed eight AMF OTUs shared across the land use system in Bukit Duabelas and there were 24 and two unique AMF OTUs in forest and rubber tree plantation respectively, but no unique AMF OTU in oil palm plantation. Similar to the Bukit Duabelas, oil palm and rubber tree plantations in Harapan also showed lower number of unique AMF OTUs, two in oil palm and one in rubber tree plantation. There were five AMF OTUs shared across the Harapan transformation systems (Figures 3.5A and 3.5B). The forest in the Bukit Duabelas showed a higher number of AMF OTUs compared to Harapan forest (Figure 3.5C). In oil palm plantation, two unique AMF OTUs originated from the Bukit Duabelas system and five from the Harapan system and both shared eight AMF OTUs (Figure 3.5D). Rubber tree plantation shared 10 AMF OTUs whereas five unique OTUs were in Bukit Duabelas and two AMF OTUs belonged to Harapan rubber tree plantation (Figure 3.5E). Rubber tree and oil palm shared only few AMF species from the genera *Glomus*, *Acaulospora*, and *Gigaspora* (Tables S3.3 and S3.4). The most abundant OTUs were *Glomus* sp. VTX00363 (10.6%), *Glomus* VTX00149 (10.3%), *Glomus* VTX00126 (7.5%), and *Glomus* intraradices (6.8%) (Table S3.6).

In both transformation systems, Glomeraceae was the most dominant family of AMF OTUs. The abundance and distribution of AMF in their host plants was visualized in a bipartite network plot (Figure 3.6).).

Plant–AMF association indicated that higher percentage of plant roots in forest communities associated with AMF. In contrast, oil palm and rubber tree roots were less associated to the AMF. Blüthgen (*d*‘) index (Blüthgen et al., 2006) indicates the host specificity of AMF OTUs and it ranges from 0 (generalization) to 1 (specialization). Association specificity analysis found that none of the AMF OTUs were host-specific (*d*‘ index ranged from 0.019 to 0.509, Table S3.6).

Multivariate analysis (PERMANOVA) revealed that AMF communities significantly differed between the landscapes (Bukit Duabelas and Harapan) and among land use systems (forest, rubber tree, and oil palm; Table 3.5). This finding was further corroborated by NMDS. Carbon, nitrogen, phosphorus, and aluminum in roots, soil pH, and available phosphorus in soil were used as environmental variables to assess the AM community structure along a land use gradients and between plant hosts (Figure 3.7). The NMDS stress (0.21) was within the accepted values since lower number implies low error of the distance between sample and low similarity between the groups that were compared. These results indicate that AMF community structures were related to different environmental factors such as C, N, and Al in roots and soil pH (P = 0.001). In contrast, P in roots was not significantly related to the AMF community structure (P = 0.173, Table 3.6).

Table 3.5. Permutational multivariate analysis of variance of arbuscular mycorrhiza in plant hosts along a transformation systems and land use gradient.

Source	df	Sum of square	Mean square	F	*P*	
Landscape	1	0.645	0.645	6.214	0.001	***
Plot	2	4.804	2.402	23.142	0.001	***
Interaction	2	1.817	0.909	8.754	0.001	***
Residual	54	5.605	0.104			
Total	59	12.872				

Significance levels: *$P < 0.05$, ** $P < 0.01$, ***$P < 0.001$.

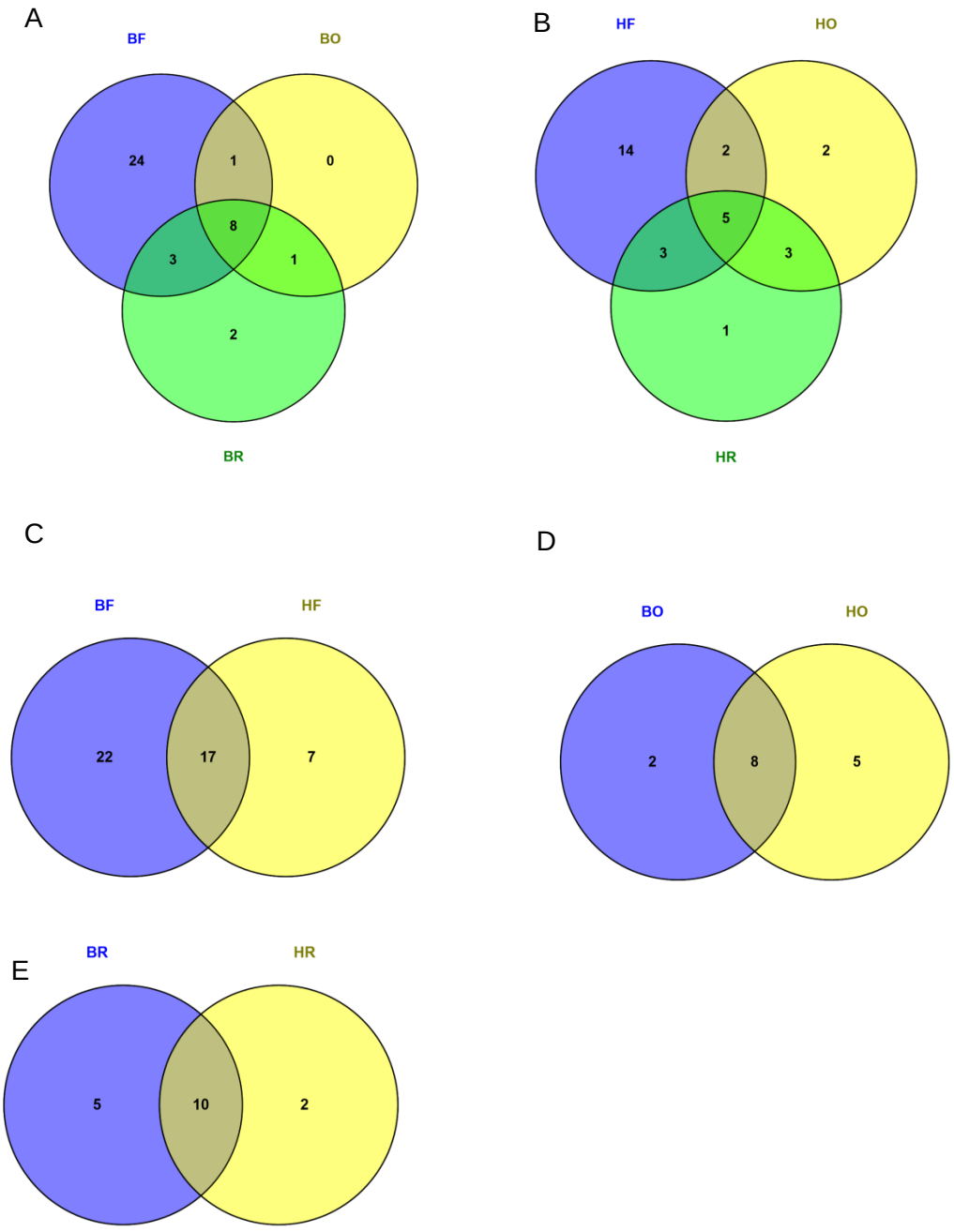

Figure 3.5. Venn diagrams showing unique and shared arbuscular mycorrhizal OTUs in A) Bukit Duabelas National Park transformation systems (BF: Forest, BO: Oil palm, BR: Rubber tree); B) Harapan transformation systems (HF: Forest, HO: Oil palm, HR: Rubber tree); C) Forest in Bukit Duabelas (BF) and Harapan (HF); D) Oil palm in Bukit Duabelas (BO) and Harapan (HO); Rubber tree in Bukit Duabelas (BR) and Harapan (HR). Numbers indicate unique and shared AM OTUs.

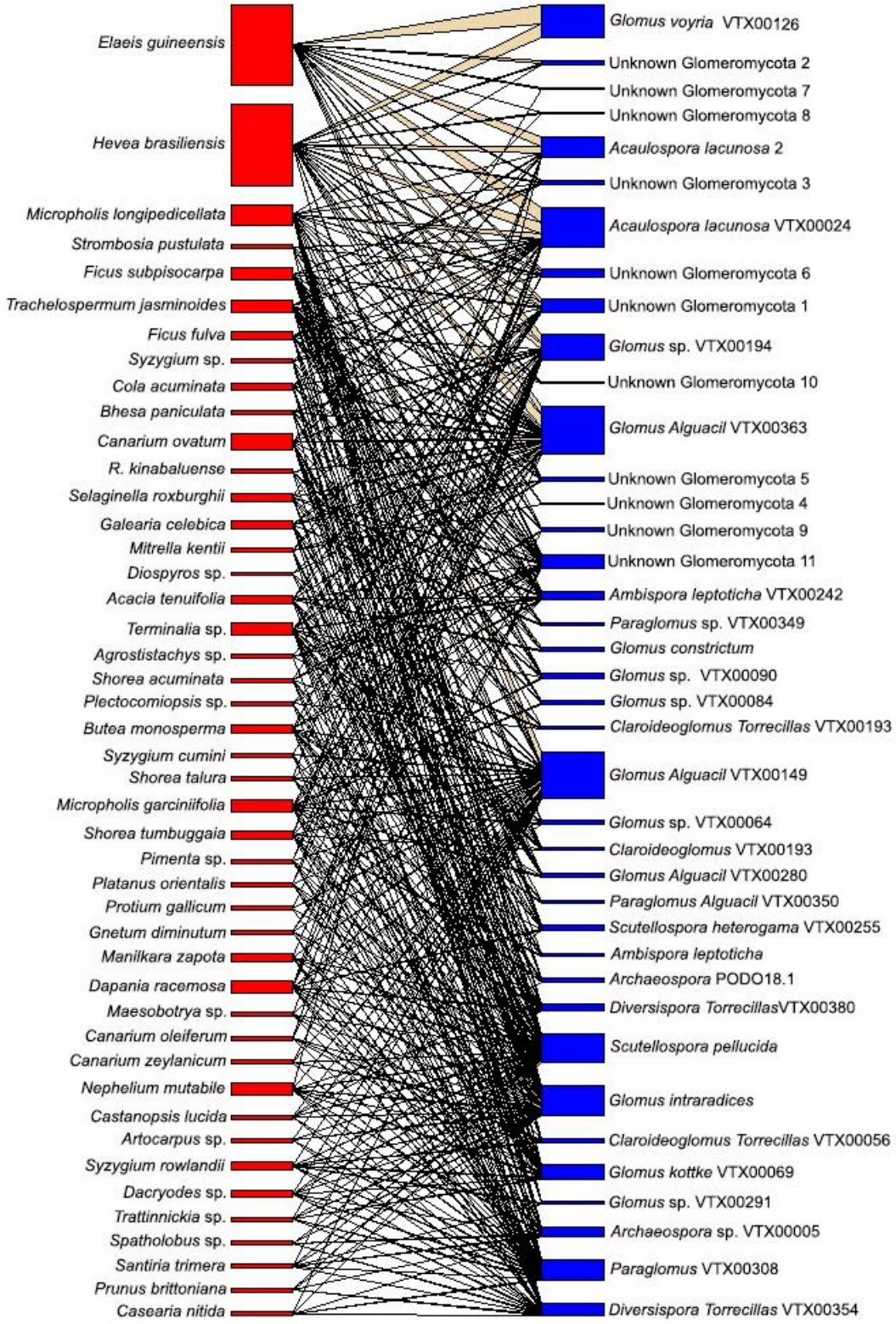

Figure 3.6. Network structure of plant hosts and arbuscular mycorrhizal (AM) OTUs in all transformation systems. Red bars represent host plants and blue bars represent AM OTUs. The bar thickness indicate generalist (thick bars) to specialist (thin bars) of AMF-host plants association.

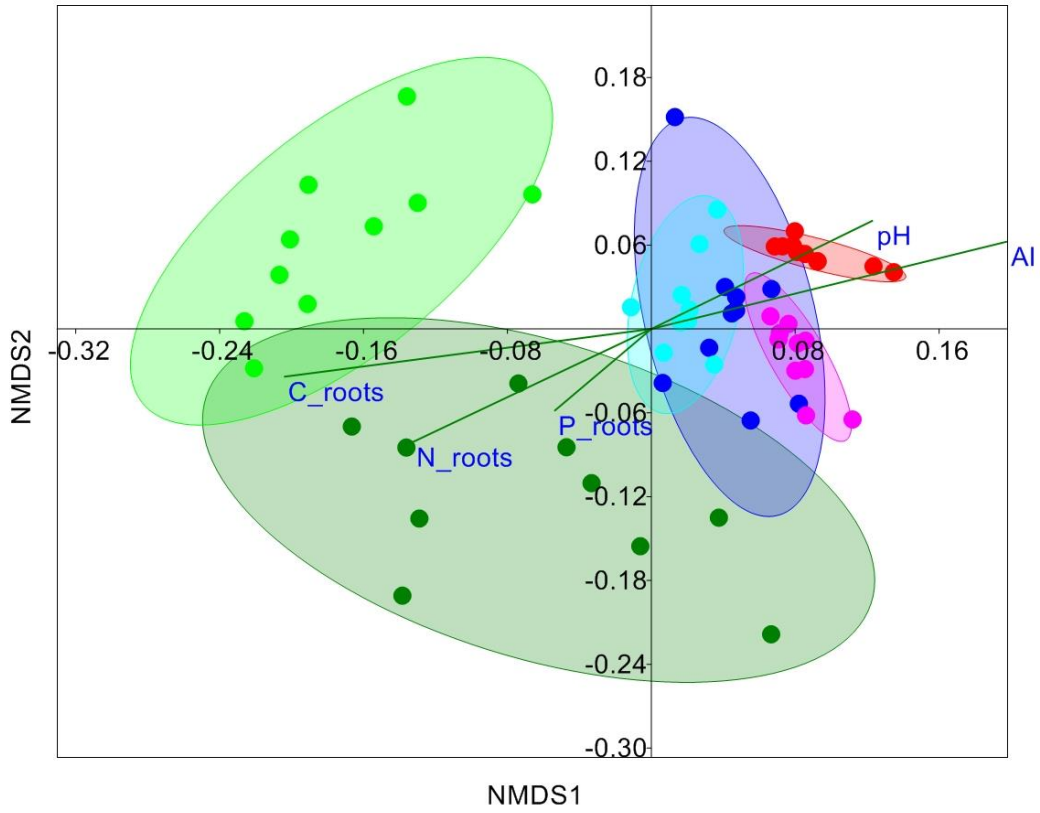

Figure 3.7. Differences in land use systems (in Bukit Duabelas: forest is in dark green, rubber tree in dark blue, oil palm in red; in Harapan: forest in light green, rubber tree in light blue, oil palm in pink) and environmental factors (carbon concentration in roots [C_roots], nitrogen concentration in roots [N_roots], phosphorus concentration in roots [P_roots], aluminum concentration in roots [Al], and soil pH [pH]) affected community composition of arbuscular mycorrhizal fungi.

Table 3.6. Goodness of fit statistics (R^2) of host plants and environmental factors fitted to the nonmetric multidimensional scaling (NMDS) ordination of arbuscular mycorrhizal community structure. The significance was based on 999 permutations.

Variables	R^2	P	
Carbon in root	0.374	0.001	***
Nitrogen in root	0.103	0.001	***
Phosphorous in root	0.059	0.173	
Aluminum in root	0.349	0.001	***
Soil pH	0.250	0.001	***

Significance levels: *P < 0.05, ** P < 0.01, ***P < 0.001.

3.4. Discussion

The present study examined the effect of transformation systems on AMF communities in lowland tropical rain forest in Sumatra, Indonesia. Our study in three different land use systems showed that transformation of rain forest into rubber tree and oil palm plantations altered the community composition and decreased species richness of AMF. The AMF species richness in rain forests was significantly higher than that found in managed plantations. Consequently, very low AMF species richness was found in managed rubber tree and oil palm plantations. Interestingly, 11 AMF OTUs were not assigned to any of the previously sequenced taxa and thus remain unknown. Of all AMF OTUs found, *Glomus* showed higher dispersal potential compared to other genera found in all land use systems since they are associated to the majority of identified plant species.

Assessing AMF community structure in relation to the land use changes is one of the objectives of this study. The results of this study support our hypothesis that transformation of rain forest into managed rubber tree and oil palm plantations reduces species richness of AMF communities. AMF community structure is related to the host plant diversity and environmental resources (Carrenho et al. 2002; Hart et al. 2003; Yang et al. 2015). A number of studies have reported that selectivity of AMF for host plants may affect the AMF community structure (Grime et al. 1987; Miller and Kling 2000; Carrenho et al. 2002; Bever 2002; Zhang et al. 2010). Specificity of AM is also related to environmental factors such

as soil nutrients and water availability (Martínez-García and Pugnaire 2011).

In tropical lowland rain forest of Bukit Duabelas and Harapan, most of identified AMF OTUs formed symbioses with detected plant species. This finding suggests that a host preference is not likely to be a prime determinant of the AMF assemblage in forest plant communities. Host preference is used to describe the intricate relationship between AMF and their specific host plant (Gadkar et al. 2001) and may play important role in relation to plant community (Klironomos 2000; Kernaghan 2005). Previous studies have shown that AMF lack host specificity (Clapp et al. 1995; Santos et al. 2006; Torrecillas et al. 2012), but meta-analysis suggest that AMF may have a preference for certain plant communities (Hoeksema et al. 2010; Yang et al. 2012).

The preferences of AMF do not depend on a single factor such as host plant. Various environmental factors including nitrogen, phosphorus, and soil properties potentially influence the AMF preference (Bohrer et al. 2001; Wang et al. 2006; Carrenho et al. 2007; Hoeksema et al. 2010). Environmental heterogeneity, land management practices, and geographic distance were found to be the determinant factors affecting AM species richness at the landscape scale of farming practices (van der Gast et al. 2011). Low input in agricultural management was related to high diversity of AMF through a land use gradient (Lumini et al. 2010). Soil type and land use intensity were also important in predicting AMF community composition (Oehl et al. 2010; Stürmer and Siqueira 2010). Low AMF

species richness was found in managed land systems (Lumini et al. 2010), and limited resources, particularly nitrogen and phosphorus, are a factor for adaptation in symbioses (Johnson et al. 2010). In the present study, nitrogen, carbon, and aluminum in fine roots and soil pH were factors with a significant effect on the AM community structures. Unexpectedly, phosphorus in roots was not significantly related to the AMF community, which may be due to many factors that contribute in the uptake of inorganic phosphorus (Smith and Read 2008). In mycorrhizal roots, demands for phosphorus is regulated by the activity of the transporter for phosphorus in fungus (Schachtman et al. 1998; Bonfante and Genre 2010). However, increasing polyphosphate levels in mycorrhizal roots resulted in similar levels of vacuolar inorganic phosphorus in mycorrhizal and non mycorrhizal plants (MacFall et al. 1992), indicating that the role of phosphorus in regulation of AMF–plant symbiosis is still poorly understood.

Nitrogen and phosphorus are two essential elements for plant growth (Whiting et al. 2004; Smith and Read 2008). AMF can enhance plant acquisition of phosphorus and take up nitrogen from soil (Treseder 2004, Smith and Smith 2012). The availability of nitrogen, phosphorus, and carbon is required for mycorrhizal fungi and it controls their abundance (Treseder 2004). In contrast, AMF are involved by plant in nutrient balance during the nutrient uptake from soil (Clarkson 1985; Marschner 1995). For example, the amelioration of aluminum by mycorrhizal fungi in *Liriodendron tulipifera* was associated with acquisition

of inorganic phosphorus (Lux and Cumming 2001), and AMF may suppress aluminum when present at toxic levels in the soil (Cumming and Ning 2003).

Changes in the AMF community structures are also triggered by the land use change (Dai et al. 2013). In the present paper, we have found that the structure of AMF communities differs between the forest and managed oil palm and rubber tree plantations. In Borneo, Indonesia, forest clearance for oil palm plantations can reduced fungal community composition (Kerfahi et al. 2014). In response to land use change, conversion of tropical forest to oil palm plantation in Malaysia altered fungal community composition (McGuire et al. 2015) and similarly, conversion of tropical rain forest to rubber tree and oil palm plantation modified the AMF communities in Jambi, Indonesia (Krashevska et al. 2015). The differences in plant vegetation and environmental factors across land use systems likely explain the differences observed in AMF community in this study. AMF species richness is higher in diverse vegetation compared to that in conventional mono-cropping vegetation (Bainard et al. 2012). (Krashevska et al. 2015) found that AMF community in the forest was more pronounced compared to other land uses. Additionally, the effects of ecosystem, biogeographical, and climatic factors might be mediated by host plants (Yang et al. 2012). It can therefore be assumed that the plant diversity drives the species richness of AMF communities. This finding supports our hypothesis that conversion

of forest to managed oil palm and rubber plantations resulted in altered community structure and decreased species richness of AMF.

3.5. References

Allen K, Corre MD, Tjoa A, Veldkamp E (2015) Soil Nitrogen-Cycling Responses to Conversion of Lowland Forests to Oil Palm and Rubber Plantations in Sumatra, Indonesia. PLoS ONE 10(7): e0133325.

Anderson MJ, Crist TO, Chase JM (2011) Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist: Roadmap for beta diversity. Ecol Lett 14:19–28.

Bainard LD, Koch AM, Gordon AM, Klironomos JN (2012) Temporal and compositional differences of arbuscular mycorrhizal fungal communities in conventional monocropping and tree-based intercropping systems. Soil Biol Biochem 45:172–180.

Bever JD (2002) Host-specificity of AM fungal population growth rates can generate feedback on plant growth. Plant Soil 244:281–290.

Blüthgen N, Menzel F, Hovestadt T, Fiala B, Blüthgen N (2007) Specialization, constraints, and conflicting interests in mutualistic networks. Curr Biol 17:341–346.

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*. J Veg Sci 12:279–288.

Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. Nat Commun 1:1–11.

Butler SJ, Boccaccio L, Gregory RD, Vorisek P, Norris K (2010) Quantifying the impact of land-use change to European farmland bird populations. Agric Ecosyst Environ 137:348–357.

Butt N, de Oliveira PA, Costa MH (2011) Evidence that deforestation affects the onset of the rainy season in Rondonia, Brazil. J Geophys Res Atmospheres 116:D11120.

Buzas MA, Hayek L-AC (2005) On richness and evenness within and between communities. Paleobiology 31:199–220.

Camenzind T, Rillig MC (2013) Extraradical arbuscular mycorrhizal fungal hyphae in an organic tropical montane forest soil. Soil Biol Biochem 64:96–102.

Carnus J-M, Parrotta J, Brockerhoff E, Arbez M, jactel H, Kremer A, Lamb D, O'Hara K, Walters B (2006) Planted forests and biodiversity. J For 104:65–77.

Carrenho R, Trufem SFB, Bononi VLR (2002) Effects of using different host plants on the detected biodiversity of arbuscular mycorrhizal fungi from an agroecosystem. Braz J Bot 25:93–101.

Carrenho R, Trufem SFB, Bononi VLR, Silva ES (2007) The effect of different soil properties on arbuscular mycorrhizal colonization of peanuts, sorghum and maize. Acta Bot Bras 21:723–730.

Chemini C, Rizzoli A (2003) Land use change and biodiversity conservation in the Alps. J Mt Ecol 7:1-7

Cheng X, Baumgartner K (2006) Effects of mycorrhizal roots and extraradical hyphae on 15N uptake from vineyard cover crop litter and the soil microbial community. Soil Biol Biochem 38:2665–2675.

Clapp JP, Young JPW, Merryweather JW, Fitter AH (1995) Diversity of fungal symbionts in arbuscular mycorrhizas from a natural community. New Phytol 130:259–265.

Clarkson DT (1985) Factors Affecting Mineral Nutrient Acquisition by Plants. Annu Rev Plant Physiol 36:77–115.

Costa MH, Pires GF (2010) Effects of Amazon and Central Brazil deforestation scenarios on the duration of the dry season in the arc of deforestation. Int J Climatol 30:1970–1979.

Cumming JR, Ning J (2003) Arbuscular mycorrhizal fungi enhance aluminium resistance of broomsedge (*Andropogon virginicus* L.). J Exp Bot 54:1447–1459.

Dai M, Bainard LD, Hamel C, Gan Y, Lynch D (2013) Impact of Land Use on Arbuscular Mycorrhizal Fungal Communities in Rural Canada. Appl Environ Microbiol 79:6719–6729.

Dormann CF, Fründ J, Blüthgen N, Gruber B (2009) Indices, graphs and null models: analyzing bipartite ecological networks. Ecol J 2:7-24.

Gadkar V, David-Schwartz R, Kunik T, Kapulnik Y (2001) Arbuscular mycorrhizal fungal colonization. factors involved in host recognition. Plant Physiol 127:1493–1499.

Gotelli NJ, Colwell RK (2011) Estimating species richness. Biol Divers Front Meas Assess 12:39–54.

Grime JP, Mackey JML, Hillier SH, Read DJ (1987) Floristic diversity in a model system using experimental microcosms. Publ Online 30 July 1987 Doi101038328420a0 328:420–422.

CBOL Plant Working Group (2009) A DNA barcode for land plants. Proc Natl Acad Sci 106:12794–12797.

Grover M, Ali SZ, Sandhya V, Rasul A, Venkateswarlu B (2010) Role of microorganisms in adaptation of agriculture crops to abiotic stresses. World J Microbiol Biotechnol 27:1231–1240.

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 4(1): pp 9.

Hart MM, Reader RJ, Klironomos JN (2003) Plant coexistence mediated by arbuscular mycorrhizal fungi. Trends Ecol Evol 18:418–423.

Haug I, Setaro S, Suárez 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.

Hoeksema JD, Chaudhary VB, Gehring CA, Johnson NC, Karst J, Koide RT, Pringle A, Zabinski C, Bever JD, Moore JC, Wilso GWT, Klironomos JN, Umbanhowar J (2010) A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. Ecol Lett 13:394–407.

Johnson NC, Wilson GWT, Bowker MA, Wilson JA, Miller RM (2010) Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proc Natl Acad Sci 107:2093–2098.

Kerfahi D, Tripathi BM, Lee J, Edwards DP, Adams JM (2014) The Impact of Selective-Logging and Forest Clearance for Oil Palm on Fungal Communities in Borneo. PLoS ONE 9:e111525.

Kernaghan G (2005) Mycorrhizal diversity: Cause and effect? Pedobiologia 49:511–520.

Ketterings QM, Tri Wibowo T, van Noordwijk M, Penot E (1999) Farmers' perspectives on slash-and-burn as a land clearing method for small-scale rubber producers in Sepunggur, Jambi Province, Sumatra, Indonesia. For Ecol Manag 120:157–169.

Keylock CJ (2005) Simpson diversity and the Shannon–Wiener index as special cases of a generalized entropy. Oikos 109:203–207.

Klironomos JN (2000) Host-specificity and functional diversity among arbuscular mycorrhizal fungi. Microb Biosyst New Front 845–851.

Koh LP, Wilcove DS (2008) Is oil palm agriculture really destroying tropical biodiversity? Conserv Lett 1:60–64.

Krashevska V, Klarner B, Widyastuti R, Maraun N, Scheu S (2015) Impact of tropical lowland rainforest conversion into rubber and oil palm plantations on soil microbial communities. Biol Fertil Soils. Accepted: 28 April 2015 DOI 10.1007/s00374-015-1021-4

Kress WJ, Erickson DL, Jones FA, Swenson NG, Perez R, Sanjur O, Bermingham E (2009) Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. Proc Natl Acad Sci 106:18621–18626.

Lambin EF, Meyfroidt P (2010) Land use transitions: Socio-ecological feedback versus socio-economic change. Land Use Policy 27:108–118.

Lee J, Lee S, Young JPW (2008) Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi: PCR primers for arbuscular mycorrhizal fungi. FEMS Microbiol Ecol 65:339–349.

Lux HB, Cumming JR (2001) Mycorrhizae confer aluminum resistance to tulip-poplar seedlings. Can J For Res 31:694–702.

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. Environ Microbiol 12:2165–2179.

MacFall JS, Slack SA, Wehrli S (1992) Phosphorus Distribution in Red Pine Roots and the Ectomycorrhizal Fungus Hebeloma arenosa1. Plant Physiol 100:713–717.

Margono BA, Potapov PV, Turubanova S, Stolle F, Hansen MC (2014) Primary forest cover loss in Indonesia over 2000-2012. Nat Clim Change 4:730–735. doi: 10.1038/nclimate2277

Margono BA, Turubanova S, Zhuravleva I, Potapov P, Alexandra Tyukaniva, Baccini A, Goetz S, Hansen MC (2012) Mapping and monitoring deforestation and forest degradation in Sumatra (Indonesia) using Landsat time series data sets from 1990 to 2010. Environ Res Lett 7:034010.

Marschner H (1995) 2 - Ion Uptake Mechanisms of Individual Cells and Roots: Short-Distance Transport. In: Marschner H (ed) Mineral

Nutrition of Higher Plants (Second Edition). Academic Press, London, pp 6–78

Martínez-García LB, Pugnaire FI (2011) Arbuscular mycorrhizal fungi host preference and site effects in two plant species in a semiarid environment. Appl Soil Ecol 48:313–317.

McGuire KL, D'Angelo H, Brearley FQ, Gedallovich SM, Babar N, Yang N, Gillikin CM, Gradoville R, Bateman C, Turner BL, Mansor P, Leff JW, Fierer N. (2015) Responses of Soil Fungi to Logging and Oil Palm Agriculture in Southeast Asian Tropical Forests. Microb Ecol 69:733–747.

Ministry of Agriculture (2010) Area and Production by Category of Producers: Oil Palm, 1967-2010 (Indonesian Ministry of Agriculture, Jakarta, Indonesia).

Miller RM, Kling M (2000) The importance of integration and scale in the arbuscular mycorrhizal symbiosis. Plant Soil 226:295–309.

Oehl F, Laczko E, Bogenrieder A, Stahr K, Bösch R, van der Heijden M, Sieverding E (2010) Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. Soil Biol Biochem 42:724–738. d

Oksanen J, Blanchet FG, Kindt R, Legendre P, MichinPR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2013) Package "vegan." R Packag Ver 254:20–8.

Oliveros, J.C. (2007-2015) Venny. An interactive tool for comparing lists with Venn's diagrams. http://bioinfogp.cnb.csic.es/tools/venny/index.html

Öpik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij JM, Reier Ü, Zobel M (2010) The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). New Phytol 188:223–241.

Peterson RL, Massicotte HB, Melville LH (2004) Mycorrhizas anatomy and cell biology. NRC Research Press ; CABI Pub., Ottawa; Wallingford, Oxon, UK pp 55-77.

Polasky S, Nelson E, Pennington D, Johnson KA (2010) The Impact of land-use change on ecosystem services, biodiversity and returns to landowners: A case study in the State of Minnesota. Environ Resour Econ 48:219–242.

Ratnasingham S, Hebert PD (2007) BOLD: The Barcode of life data system (http://www. barcodinglife. org). Mol Ecol Notes 7:355–364.

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

Schachtman DP, Reid RJ, Ayling SM (1998) Phosphorus Uptake by Plants: From Soil to Cell. Plant Physiol 116:447–453.

Sequeira E (2013) The NCBI handbook 2nd edition. National Center for Biotechnology Information Bethesda, US, online handbook in http://www.ncbi.nlm.nih.gov/books/NBK143764/. Accessed in 11 May 2015.

Smith SE, Facelli E, Pope S, Smith FA (2009) Plant performance in stressful environments: interpreting new and established knowledge of the roles of arbuscular mycorrhizas. Plant Soil 326:3–20.

Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic Press, Amsterdam; Boston, pp 11-145.

Smith SE, Smith FA (2012) Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth. Mycologia 104:1–13.

Stephens SS, Wagner MR (2007) Forest Plantations and Biodiversity: A Fresh Perspective. J For 105:307–313.

Stürmer SL, Siqueira JO (2010) Species richness and spore abundance of arbuscular mycorrhizal fungi across distinct land uses in Western Brazilian Amazon. Mycorrhiza 21:255–267.

Toju H, Sato H, Tanabe AS (2014) Diversity and Spatial Structure of Belowground Plant–Fungal Symbiosis in a Mixed Subtropical Forest of Ectomycorrhizal and Arbuscular Mycorrhizal Plants. PLoS ONE.

Torrecillas E, Alguacil MM, Roldán A (2012) Host Preferences of Arbuscular Mycorrhizal Fungi Colonizing Annual Herbaceous Plant Species in Semiarid Mediterranean Prairies. Appl Environ Microbiol 78:6180–6186.

Treseder KK (2004) A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO2 in field studies. New Phytol 164:347–355.

UNFAO (United Nations of Food and Agriculture Organization). 2010. Global Forest Resource Assessment 2010 Country Report: Indonesia Forestry Department. FRA 2010/095.

UNFAO (United Nations of Food and Agriculture Organization). 2013. FAO statistical yearbook 2013: World food and agriculture. Food

and Agriculture Organization of the United Nations, Rome, pp 123-200.

USDA-FAS. 2010. Oilseeds: World Markets and Trade (US Department of Agriculture–Foreign Agricultural Service, Washington, DC) Circular Series FOP 3-10

van der Gast CJ, Gosling P, Tiwari B, Bending GD (2011) Spatial scaling of arbuscular mycorrhizal fungal diversity is affected by farming practice. Environ Microbiol 13:241–249.

Veresoglou SD, Chen B, Rillig MC (2012) Arbuscular mycorrhiza and soil nitrogen cycling. Soil Biol Biochem 46:53–62.

Wang S, Feng Z, Wang X (2006) Effects of environmental pollutants on arbuscular mycorrhiza formation and function. PubMed 17:1321–1325.

Wehner J, Antunes PM, Powell JR, Mazukatow J, Rillig MC (2010) Plant pathogen protection by arbuscular mycorrhizas: A role for fungal diversity? Pedobiologia 53:197–201.

Whiting DE, Vickerman LG, Wilson CR, Card AB (2004) Plant nutrition. Colorado State University Cooperative Extension. CMG GardenNotes, 231: pp 1-6.

Yang G, Yang X, Zhang W, Wei Y, Ge G, Lu W, Sun J, Liu N, Kan H, Shen Y, Zhang Y (2015) Arbuscular mycorrhizal fungi affect plant community structure under various nutrient conditions and stabilize the community productivity. Oikos J, accepted 15 June 2015, doi: 10.1111/oik.02351.

Yang H, Zang Y, Yuan Y, Tang J, Chen X (2012) Selectivity by host plants affects the distribution of arbuscular mycorrhizal fungi: evidence from ITS rDNA sequence metadata. BMC Evol Biol 12:50.

Yu J, Xue J-H, Zhou S-L (2011) New universal matK primers for DNA barcoding angiosperms. J Syst Evol 49:176–181.

Zhang Q, Yang R, Tang J,Yang H, Hu S, Chen X (2010) Positive Feedback between Mycorrhizal Fungi and Plants Influences Plant Invasion Success and Resistance to Invasion. PLoS ONE 5:e12380.

Zhu X-C, Song F-B, Xu H-W (2009) Arbuscular mycorrhizae improves low temperature stress in maize via alterations in host water status and photosynthesis. Plant Soil 331:129–137.

Table S3.1. Molecular identification of arbuscular mycorrhizal fungal OTUs in Bukit Duabelas National Park transformation systems.

OTU ID	Query length	Based on MarJam database					Based on NCBI database				
		Accession	Closest blast match	Query coverage	E-value	Max identity	Accession	Closest blast match	Query cover	E value	Max identity
Forest											
			Acaulosporaceae					**Acaulosporaceae**			
OTU_1	558	FR719957	*Acaulospora lacunosa* VTX00024	99%	0	99%	KR822761.2	*Acaulospora lacunosa*	100%	0.0	99%
OTU_2	562	HE610427	*Acaulospora lacunose* 2 Ambisporaceae	99%	0	99%	HE610426.1	*Acaulospora lacunosa* Ambisporaceae	100%	0.0	99%
OTU_3	536	AB015712	*Ambispora leptoticha*	100%	0	100%	KR822763.	*Archaeospora leptoticha* clone A2	100%	0.0	100%
OTU_4	635	AJ301861	*Ambispora leptoticha* VTX00242	100%	0	100%	KR822764.1	*Archaeospora leptoticha* clone pWD147-1-1	100%	0.0	100%
			Archaeosporaceae					**Archaeosporaceae**			
OTU_5	487	AF452635	*Archaeospora* PODO18.1	97%	0%	97%	AB047306.1	*Archaeospora leptoticha* MAFF520057	100%	0.0	97%
OTU_6	618	JF414172	*Archaeospora* sp. VTX00005	95%	0	97%	JF414182.1	*Glomeromycota* sp. MIB 8442	100%	0.0	94%
			Claroideoglomeraceae					**Claroideoglomeraceae**			
OTU_7	577	EU340321	*Claroideoglomus* NF25 VTX00193	97%	0	97%	KR822767.1	Uncultured *Glomus* clone PAF376	100%	0.0	97%
OTU_8	601	HE614986	*Claroideoglomus* Torrecillas12b Glo G1 VTX00193	99%	0	99%	HE614989.1	Uncultured *Glomus* partial isolate CIR, clone 1-10	100%	0.0	99%
OTU_9	634	HE615004	*Claroideoglomus* Torrecillas12b Glo G3 VTX00056	97%	0	97%	KR822769.1	Uncultured *Glomus* isolate ANA, clone 4-4	100%	0.0	97%
			Diversisporaceae					Diversisporaceae			
OTU_10	581	HE615041	*Diversispora* Torrecillas12b Div2 VTX00380	98%	0	98%	KR822770.1	Uncultured *Diversispora* isolate STI, clone 1-28	100%	0.0	99%
OTU_11	588	HE615058	*Diversispora* Torrecillas12b Div3 VTX00354	99%	0	99%	KR822771.1	Uncultured *Diversispora* isolate BRA, clone 1-4	100%	0.0	100%
			Gigasporaceae					**Gigasporaceae**			
OTU_12	609	FR774917	*Scutellospora heterogama* VTX00255	97%	0	97%	AB041344.1	*Scutellospora cerradensis* clone:SC21	100%	0.0	97%

Table S3.1. Continued

OTU ID	Query length	Based on MarJam database					Based on NCBI database				
		Accession	Closest blast match	Query coverage	E-value	Max identity	Accession	Closest blast match	Query cover	E value	Max identity
OTU_13	527	FR750215	*Scutellospora pellucida*	100%	0	100%	JN644450.1	Uncultured Gigasporaceae clone 251AM1_7	100%	0.0	99%
			Glomeraceae								
OTU_14	592	FR821564	*Glomus* Alguacil12a Glo G9 VTX00280	100%	0	100%	KR822774.1	Uncultured *Glomus* isolate C4-3	100%	0.0	100%
OTU_15	523	HE576928	*Glomus* Alguacil12b GLO G11 VTX00149	100%	0	100%	KR822775.1	Uncultured *Glomus* clone S10.28	100%	0.0	100%
OTU_16	674	FR750212	*Glomus constrictum* VTX00064	97%	0	97%	Y17635.3	*Glomus caledonium* isolate BEG20, clone pWD135-1	100%	0.0	95%
OTU_17	591	FR750209	*Glomus intraradices* VTX00100	100%	0	100%	GU140042.1	*Glomus* intraradices strain GA5	100%	0.0	99%
OTU_18	589	DQ336493	*Glomus* Kottke08-7 VTX00069	98%	0	98%	KR822778.1	Uncultured *Glomus* clone K171c6	100%	0.0	100%
OTU_19	651	AB546401	*Glomus* sp. VTX00084	100%	0	100%	KR822779.1	Uncultured *Glomus* clone: C1-6	100%	0.0	100%
OTU_20	656	AB546133	*Glomus* sp. VTX00194	100%	0	100%	KR822780.1	Uncultured *Glomus* clone: P2-1	100%	0.0	100%
OTU_21	584	AB220173	*Glomus* sp. RF1 VTX00090	100%	0	100%	JF414187.1	*Glomeromycota* sp. MIB 8366	99%	0.0	98%
OTU_22	651	EU169414	*Glomus* sp. VTX00064	100%	0	100%	KR822782.1	Uncultured *Glomus* ID28	100%	0.0	100%
OTU_23	591	FR821538	*Glomus* Alguacil12a Glo G8 VTX00363	98%	0	98	KR822783.1	Uncultured *Glomus* isolate R2-24	100%	0.0	98%
OTU_24	593	AB555664	*Glomus* sp. VTX00291	98%	0	98%	KR822784.1	Uncultured *Glomus* clone: K2H1-2	100%	0.0	99%
OTU_25	561	AJ430853	*Glomus* Voyria symbiont type 2 VTX00126	100%	0	97%	KJ952239.1	*Glomeromycota* sp. AI6n-3	100%	0.0	100%
			Paraglomeraceae					**Paraglomeraceae**			
OTU_26	594	HE576915	*Paraglomus* Alguacil12b PARA2 VTX00350	98%	0	98%	KR822786.1	Uncultured clone S12.29	100%	0.0	99%
OTU_27	592	FR693458	*Paraglomus* Para2 VTX00308	98%	0	98%	KR822787.1	Uncultured *Paraglomus* clone C2-19	100%	0.0	99%

Table S3.1. Continued

OTU ID	Query length	Based on MarJam database					Based on NCBI database				
		Accession	Closest blast match	Query coverage	E-value	Max identity	Accession	Closest blast match	Query cover	E value	Max identity
OTU_28	594	FR848081	*Paraglomus* sp. VTX00349	98%	0	98%	KR822788.1	Uncultured *Paraglomus* clone 1-5	100%	0.0	99%
OTU_29	506		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_30	437		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_31	488		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_32	695		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_33	472		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_34	497		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_35	665		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_36	514		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_37	684		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_38	571		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_39	515		Unknown Glomeromycota					Unknown Glomeromycota			
Oil palm											
			Acaulosporaceae					Acaulosporaceae			
OTU_1	558	FR719957	*Acaulospora lacunosa* VTX00024	99%	0	99%	KR822761.2	*Acaulospora lacunosa*	100%	0.0	99%
OTU_2	562	HE610427	*Acaulospora lacunosa* 2	99%	0	99%	HE610426.1	*Acaulospora lacunosa*	100%	0.0	99%
			Ambisporaceae					Ambisporaceae			
OTU_4	635	AJ301861	*Ambispora leptoticha* VTX00242	100%	0	100%	KR822764.1	*Archaeospora leptoticha* clone pWD147-1-1	100%	0.0	100%
			Glomeraceae								
OTU_20	656	AB546133	*Glomus* sp. VTX00194	100%	0	100%	KR822780.1	Uncultured *Glomus* clone: P2-1	100%	0.0	100%
OTU_25	561	AJ430853	*Glomus* Voyria symbiont type 2 VTX00126	100%	0	97%	KJ952239.1	*Glomeromycota* sp. AI6n-3	100%	0.0	100%
OTU_29	506		Unknown Glomeromycota					Unknown Glomeromycota			

Table S3.1. Continued

OTU ID	Query length	Based on MarJam database					Based on NCBI database				
		Accession	Closest blast match	Query coverage	E-value	Max identity	Accession	Closest blast match	Query cover	E value	Max identity
OTU_30			Unknown Glomeromycota					Unknown Glomeromycota			
OTU_34	437		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_37	684		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_39	515		Unknown Glomeromycota					Unknown Glomeromycota			
Rubber											
			Acaulosporaceae					**Acaulosporaceae**			
OTU_1	558	FR719957	*Acaulospora lacunosa* VTX00024	99%	0	99%	KR822761.2	*Acaulospora lacunosa*	100%	0.0	99%
OTU_2	562	HE610427	*Acaulospora lacunosa* 2	99%	0	99%	HE610426.1	*Acaulospora lacunosa*	100%	0.0	99%
			Glomeraceae								
OTU_15	523	HE576928	*Glomus* Alguacil12b GLO G11 VTX00149	100%	0	100%	KR822775.1	Uncultured *Glomus* clone S10.28	100%	0.0	100%
OTU_20	656	AB546133	*Glomus* sp. VTX00194	100%	0	100%	KR822780.1	Uncultured *Glomus* clone: P2-1	100%	0.0	100%
OTU_23	591	FR821538	*Glomus* Alguacil12a Glo G8 VTX00363	98%	0	98	KR822783.1	Uncultured *Glomus* isolate R2-24	100%	0.0	98%
OTU_25	561	AJ430853	*Glomus* Voyria symbiont type 2 VTX00126	100%	0	97%	KJ952239.1	*Glomeromycota* sp. AI6n-3	100%	0.0	100%
OTU_29	506		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_30	437		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_31	488		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_32	695		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_33	472		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_34	497		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_36	514		Unknown Glomeromycota					Unknown Glomeromycota			

Table S3.1. Continued

OTU ID	Query length	Based on MarJam database					Based on NCBI database				
		Accession	Closest blast match	Query coverage	E-value	Max identity	Accession	Closest blast match	Query cover	E value	Max identity
OTU_37	684		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_39	515		Unknown Glomeromycota					Unknown Glomeromycota			

Table S3.2. Molecular identification of arbuscular mycorrhizal fungal OTUs in Harapan transformation systems.

OTU ID	Query length	Based on MarJam database					Based on NCBI database				
		Accession	Closest blast match	Query coverage	E-value	Max identity	Accession	Closest blast match	Query cover	E value	Max identity
Forest											
			Acaulosporaceae					**Acaulosporaceae**			
OTU_1	558	FR719957	*Acaulospora lacunosa* VTX00024	99%	0	99%	KR822761.2	*Acaulospora lacunosa*	100%	0.0	99%
OTU_2	562	HE610427	*Acaulospora lacunosa* 2	99%	0	99%	HE610426.1	*Acaulospora lacunosa*	100%	0.0	99%
			Ambisporaceae					Ambisporaceae			
								Archaeospora leptoticha clone			
OTU_4	635	AJ301861	*Ambispora leptoticha* VTX00242	100%	0	100%	KR822764.1	pWD147-1-1	100%	0.0	100%
			Archaeosporaceae					**Archaeosporaceae**			
								Archaeospora leptoticha			
OTU_5	487	AF452635	*Archaeospora* PODO18.1	97%	0%	97%	AB047306.1	MAFF520057	100%	0.0	97%
OTU_6	618	JF414172	*Archaeospora* sp. VTX00005	95%	0	97%	JF414182.1	*Glomeromycota* sp. MIB 8442	100%	0.0	94%
			Claroideoglomeraceae					**Claroideoglomeraceae**			
								Uncultured *Glomus* clone			
OTU_7	577	EU340321	*Claroideoglomus* NF25 VTX00193	97%	0	97%	KR822767.1	PAF376	100%	0.0	97%
			Claroideoglomus Torrecillas12b					Uncultured *Glomus* isolate			
OTU_9	634	HE615004	Glo G3 VTX00056	97%	0	97%	KR822769.1	ANA, clone 4-4	100%	0.0	97%
			Diversisporaceae					Diversisporaceae			

Table S3.2. Continued

OTU ID	Query length	Based on MarJam database					Based on NCBI database				
		Accession	Closest blast match	Query coverage	E-value	Max identity	Accession	Closest blast match	Query cover	E value	Max identity
OTU_10	581	HE615041	*Diversispora* Torrecillas12b Div2 VTX00380	98%	0	98%	KR822770.1	Uncultured *Diversispora* isolate STI, clone 1-28	100%	0.0	99%
OTU_11	588	HE615058	*Diversispora* Torrecillas12b Div3 VTX00354	99%	0	99%	KR822771.1	Uncultured *Diversispora* isolate BRA, clone 1-4	100%	0.0	100%
			Gigasporaceae					**Gigasporaceae**			
OTU_12	609	FR774917	*Scutellospora heterogama* VTX00255	97%	0	97%	AB041344.1	*Scutellospora cerradensis* clone:SC21	100%	0.0	97%
OTU_13	527	FR750215	*Scutellospora pellucida*	100%	0	100%	JN644450.1	Uncultured Gigasporaceae clone 251AM1_7	100%	0.0	99%
			Glomeraceae								
OTU_15	523	HE576928	*Glomus* Alguacil12b GLO G11 VTX00149	100%	0	100%	KR822775.1	Uncultured *Glomus* clone S10.28	100%	0.0	100%
OTU_17	591	FR750209	*Glomus intraradices* VTX00100	100%	0	100%	GU140042.1	*Glomus intraradices* strain GA5	100%	0.0	99%
OTU_18	589	DQ336493	*Glomus* Kottke08-7 VTX00069	98%	0	98%	KR822778.1	Uncultured *Glomus* clone K171c6	100%	0.0	100%
OTU_20	656	AB546133	*Glomus* sp. VTX00194	100%	0	100%	KR822780.1	Uncultured *Glomus* clone: P2-1	100%	0.0	100%
OTU_24	593	AB555664	*Glomus* sp. VTX00291	98%	0	98%	KR822784.1	Uncultured *Glomus* clone: K2H1-2	100%	0.0	99%
			Paraglomeraceae					**Paraglomeraceae**			
OTU_27	592	FR693458	*Paraglomus* Para2 VTX00308	98%	0	98%	KR822787.1	Uncultured *Paraglomus* clone C2-19	100%	0.0	99%
OTU_29	506		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_32	695		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_33	472		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_34	497		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_36	514		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_37	684		Unknown Glomeromycota					Unknown Glomeromycota			

Table S3.2. Continued

OTU ID	Query length	Based on MarJam database					Based on NCBI database				
		Accession	Closest blast match	Query coverage	E-value	Max identity	Accession	Closest blast match	Query cover	E value	Max identity
OTU_39	515	KR822799	Unknown Glomeromycota					Unknown Glomeromycota			
Oil palm											
			Acaulosporaceae					**Acaulosporaceae**			
OTU_1	558	FR719957	*Acaulospora lacunosa* VTX00024	99%	0	99%	KR822761.2	*Acaulospora lacunosa*	100%	0.0	99%
OTU_2	562	HE610427	*Acaulospora lacunosa* 2	99%	0	99%	HE610426.1	*Acaulospora lacunosa*	100%	0.0	99%
			Glomeraceae					**Glomeraceae**			
OTU_23	591	FR821538	*Glomus* Alguacil12a Glo G8 VTX00363	98%	0	98	KR822783.1	Uncultured *Glomus* isolate R2-24	100%	0.0	98%
OTU_25	561	AJ430853	*Glomus* Voyria symbiont type 2 VTX00126	100%	0	97%	KJ952239.1	*Glomeromycota* sp. AI6n-3	100%	0.0	100%
			Unknown Glomeromycota					**Unknown Glomeromycota**			
OTU_29	506		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_30	437		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_31	488		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_33	472		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_34	497		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_35	665		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_36	514		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_37	684		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_39	515		Unknown Glomeromycota					Unknown Glomeromycota			
Rubber											
			Acaulosporaceae					Acaulosporaceae			
OTU_1	558	FR719957	*Acaulospora lacunosa* VTX00024	99%	0	99%	KR822761.2	Acaulospora lacunosa	100%	0.0	99%
OTU_2	562	HE610427	*Acaulospora lacunosa* 2	99%	0	99%	HE610426.1	Acaulospora lacunosa	100%	0.0	99%
			Gigasporaceae					Gigasporaceae			
OTU_13	527	FR750215	Scutellospora pellucida	100%	0	100%	JN644450.1	Uncultured Gigasporaceae clone 251AM1_7	100%	0.0	99%

Table S3.2. Continued

OTU ID	Query length	Based on MarJam database					Based on NCBI database				
		Accession	Closest blast match	Query coverage	E-value	Max identity	Accession	Closest blast match	Query cover	E value	Max identity
			Glomeraceae								
OTU_15	523	HE576928	*Glomus* Alguacil12b GLO G11 VTX00149 Glomeraceae	100%	0	100%	KR822775.1	Uncultured Glomus clone S10.28 Glomeraceae	100%	0.0	100%
OTU_23	591	FR821538	*Glomus* Alguacil12a Glo G8 VTX00363	98%	0	98	KR822783.1	Uncultured Glomus isolate R2-24	100%	0.0	98%
OTU_25	561	AJ430853	*Glomus* Voyria symbiont type 2 VTX00126	100%	0	97%	KJ952239.1	Glomeromycota sp. AI6n-3	100%	0.0	100%
			Unknown Glomeromycota					**Unknown Glomeromycota**			
OTU_29	506		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_30	437		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_34	497		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_37	684		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_38	571		Unknown Glomeromycota					Unknown Glomeromycota			
OTU_39	515		Unknown Glomeromycota					Unknown Glomeromycota			

Table S3.3. Matrix of symbiosis of AMF and host plants in Bukit Duabelas and Harapan transformation systems.

No	Plot ID	Phost plants	Environmental variables					AMF Abundance																																		
			C roots (mg/g)	N roots (mg/g)	Al roots (mg/g)	P roots (mg/g)	Soil pH	*Acaulospora lacunosa* VTX00024	*Acaulospora lacunosa 2*	*Ambisporaceae leptoticha*	*Ambispora leptoticha VTX00242*	*Archaeospora* PODO18.1	*Archaeospora* sp. VTX00005	*Claroideoglomus* VTX00193	*Claroideoglomus Torrecillas* VTX00193	*Claroideoglomus Torrecillas* VTX00056	*Diversispora Torrecillas* VTX00380	*Diversispora Torrecillas* VTX00354	*Scutellospora heterogama* VTX00255	*Scutellospora pellucida*	*Glomus* Alguacil VTX00280	*Glomus* Alguacil VTX00149	*Glomus constrictum* VTX00064	*Glomus intraradices*	*Glomus* Kottke08-7 VTX00069	*Glomus* sp. VTX00084	*Glomus* sp. VTX00194	*Glomus* sp. VTX00090	*Glomus* sp. VTX00064	*Glomus* sp. VTX0036	*Glomus* sp. VTX00291	*Glomus* sp.Voyria VTX00126	*Paraglomus* Alguacil12b VTX00350	*Paraglomus* Para2 VTX00308	*Paraglomus* sp. VTX00349	*Unknown Glomeromycota* 1	*Unknown Glomeromycota* 2	*Unknown Glomeromycota* 3	*Unknown Glomeromycota* 4	*Unknown Glomeromycota* 5	*Unknown Glomeromycota* 6	*Unknown Glomeromycota* 7	*Unknown Glomeromycota* 8	*Unknown Glomeromycota* 9	*Unknown Glomeromycota* 10	*Unknown Glomeromycota* 11
1	BF1 a-1	*Trachelospermum jasminoides*	450.59	14.61	7.67	0.67	4.3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	2	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	1
2	BF1 a-2	*Trachelospermum jasminoides*	450.59	14.61	7.67	0.67	4.3	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	3	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	BF1 a-3	*Galearia celebica*	450.59	14.61	7.67	0.67	4.3	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	2	0	0	0	0	0	2	0	0	0	0	0	1	0	0	0	1	0	0	0	0
4	BF1 b-1	*Galearia celebica*	482.33	11.52	7.67	0.53	4.3	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0
5	BF1 b-2	*Terminalia guyanensis*	482.33	11.52	7.67	0.53	4.3	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	2	0	0	0	0	0	1	0	0	0	1	0	0	0	0
6	BF1 b-3	*Micropholis longipedicellata*	482.33	11.52	7.67	0.53	4.3	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	2	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	BF1 c-1	*Ficus fulva*	469.21	13.83	7.67	0.73	4.3	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	2	0	2	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	BF1 c-2	*Ficus fulva*	469.21	13.83	7.67	0.73	4.3	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	0	0	1
9	BF1 c-3	*Terminalia guyanensis*	469.21	13.83	7.67	0.73	4.3	0	0	1	2	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
10	BF2 a-1	*Ficus subpisocarpa*	465.92	10.91	6.24	0.41	4.2	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0	0	1
11	BF2 a-2	*Ficus subpisocarpa*	465.92	10.91	6.24	0.41	4.2	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	1	0	0	0	0	1	0
12	BF2 a-3	*Selaginella roxburghii*	465.92	10.91	6.24	0.41	4.2	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
13	BF2 b-1	*Butea monosperma*	480.98	10.78	6.24	0.40	4.2	0	0	0	0	1	1	0	0	0	0	0	1	0	1	1	0	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
14	BF2 b-2	*Acacia tenuifolia*	480.98	10.78	6.24	0.40	4.2	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1
15	BF2 b-3	*Butea monosperma*	480.98	10.78	6.24	0.40	4.2	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	1	0	0	0	1
16	BF2 c-1	*Plectocomiopsis geminiflora*	464.42	15.66	6.24	0.64	4.2	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	2	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1
17	BF2 c-2	*Acacia tenuifolia*	464.42	15.66	6.24	0.64	4.2	0	0	0	0	1	0	1	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0
18	BF2 c-3	*Ficus subpisocarpa*	464.42	15.66	6.24	0.64	4.2	0	0	0	2	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
19	BF3 a-1	*Canarium ovatum*	469.81	11.04	5.26	0.52	4.2	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	2	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
20	BF3 a-2	*Cola acuminata*	469.81	11.04	5.26	0.52	4.2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	2	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	1
21	BF3 a-3	*Cola acuminata*	469.81	11.04	5.26	0.52	4.2	1	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0
22	BF3 b-1	*Micropholis longipedicellata*	502.73	12.00	5.26	0.42	4.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	1	1	2	0	0	0	0	0	0	0	0	0	0	0	1
23	BF3 b-2	*Micropholis garciniifolia*	502.73	12.00	5.26	0.42	4.2	2	1	0	1	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
24	BF3 b-3	*Trachelospermum jasminoides*	502.73	12.00	5.26	0.42	4.2	1	1	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
25	BF3 c-1	*Micropholis garciniifolia*	486.54	16.21	5.26	0.51	4.2	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0	1	0
26	BF3 c-2	*Micropholis garciniifolia*	486.54	16.21	5.26	0.51	4.2	4	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1
27	BF3 c-3	*Micropholis garciniifolia*	486.54	16.21	5.26	0.51	4.2	2	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
28	BF4 a-1	*Diospyros sp.*	478.23	16.52	5.42	0.53	4.1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	2	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0
29	BF4 a-2	*Canarium ovatum*	478.23	16.52	5.42	0.53	4.1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	3	0	0	2	0	0	0	0	0	0	0	0	0	0
30	BF4 a-3	*Selaginella roxburghii*	478.23	16.52	5.42	0.53	4.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1
31	BF4 b-1	*Terminalia guyanensis*	506.28	17.52	5.42	0.50	4.1	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
32	BF4 b-2	*Canarium ovatum*	506.28	17.52	5.42	0.50	4.1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	2	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	BF4 b-3	*Reinwardtiodendron kinabaluense*	506.28	17.52	5.42	0.50	4.1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	2	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0
34	BF4 c-1	*Strombosia pustulata*	493.72	18.99	5.42	0.59	4.1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1	0	0	0	0	0	0	0	1	0	0	0	0	1
35	BF4 c-2	*Dapania racemosa*	493.72	18.99	5.42	0.59	4.1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	3	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	BF4 c-3	*Bhesa paniculata*	493.72	18.99	5.42	0.59	4.1	1	0	0	1	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	BO1 a-1	*Elaeis guineensis*	383.65	6.48	18.87	0.37	4.3	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1
38	BO1 b-4	*Elaeis guineensis*	432.91	7.10	18.87	0.32	4.3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	0	1
39	BO1 c-5	*Elaeis guineensis*	436.40	7.15	18.87	0.29	4.3	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
40	BO1 c-3	*Elaeis guineensis*	436.40	7.15	18.87	0.29	4.3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	1	0	0	0	0	0	0	0	1
41	BO2 b-1	*Elaeis guineensis*	429.75	8.18	13.92	0.43	4.5	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
42	BO2 c-2	*Elaeis guineensis*	429.27	7.42	13.92	0.23	4.5	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	2	0	0	0	1	0	0	0	0	0	1	0	0
43	BO3 a-5	*Elaeis guineensis*	429.56	7.11	12.74	0.31	4.5	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
44	BO3 b-2	*Elaeis guineensis*	423.44	6.32	12.74	0.32	4.4	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0

Table S3.3. Continued.

No	Plot ID	Phost plants	Environmental variables					AMF Abundance																																						
			C roots (mg/g)	N roots (mg/g)	Al roots (mg/g)	P roots (mg/g)	Soil pH	*Acaulospora lacunosa* VTX00024	*Acaulospora lacunosa 2*	*Ambisporaceae leptoticha*	*Ambispora leptoticha VTX00242*	*Archaeospora* PODO18.1	*Archaeospora* sp. VTX00005	*Claroideoglomus* VTX00193	*Claroideoglomus Torrecillas* VTX00193	*Claroideoglomus Torrecillas* VTX00056	*Diversispora Torrecillas* VTX00380	*Diversispora Torrecillas* VTX00354	*Scutellospora heterogama* VTX00255	*Scutellospora pellucida*	*Glomus* Alguacil VTX00280	*Glomus* Alguacil VTX00149	*Glomus constrictum* VTX00064	*Glomus intraradices*	*Glomus* Kottke08-7 VTX00069	*Glomus* sp. VTX00084	*Glomus* sp. VTX00194	*Glomus* sp. VTX00090	*Glomus* sp. VTX00064	*Glomus* sp. VTX0036	*Glomus* sp. VTX00291	*Glomus* sp.Voyria VTX00126	*Paraglomus* Alguacil12b VTX00350	*Paraglomus* Para2 VTX00308	*Paraglomus* sp. VTX00349	*Unknown Glomeromycota* 1	*Unknown Glomeromycota* 2	*Unknown Glomeromycota* 3	*Unknown Glomeromycota* 4	*Unknown Glomeromycota* 5	*Unknown Glomeromycota* 6	*Unknown Glomeromycota* 7	*Unknown Glomeromycota* 8	*Unknown Glomeromycota* 9	*Unknown Glomeromycota* 10	*Unknown Glomeromycota* 11
45	BO3 c-1	*Elaeis guineensis*	429.45	6.20	12.74	0.51	4.4	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
46	BO4 b-5	*Elaeis guineensis*	406.47	5.78	19.92	0.23	4.5	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0
47	BR1 b-4	*Hevea brasiliensis*	446.83	8.64	9.94	0.25	4.6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	1	0	1	1	0	0	0	0
48	BR1 c-2	*Hevea brasiliensis*	460.63	5.21	9.94	0.16	4.6	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	2	0	0	0	0	1	0	0	0	0	0	0	0
49	BR2 b-3	*Hevea brasiliensis*	426.69	12.45	14.22	0.39	4.5	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1
50	BR2 c-1	*Hevea brasiliensis*	424.74	12.86	14.22	0.33	4.5	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0
51	BR3 a-5	*Hevea brasiliensis*	433.43	11.38	16.37	0.35	4.5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
52	BR3 b-3	*Hevea brasiliensis*	418.83	11.83	16.37	0.33	4.5	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0
53	BR3 c-1	*Hevea brasiliensis*	430.11	11.46	16.37	0.37	4.5	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0
54	BR4 a-2	*Hevea brasiliensis*	412.68	9.82	16.39	0.41	4.4	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0
55	BR4 b-4	*Hevea brasiliensis*	421.47	11.10	16.39	0.46	4.4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	4	0	0	0	0	1	0	0	0	0	0	0	0
56	BR4 c-2	*Hevea brasiliensis*	440.09	9.63	16.39	0.42	4.4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0	2	0	0	0	0	1
57	HF1 a-1	*Pimenta pseudocaryophyllus*	494.24	14.53	3.37	0.38	4.4	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	1	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
58	HF1 a-2	*Syzygium sp.*	494.24	14.53	3.37	0.38	4.4	1	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0
59	HF1 a-3	*Nephelium mutabile*	494.24	14.53	3.37	0.38	4.4	0	1	0	0	0	1	0	0	0	1	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
60	HF1 b-1	*Shorea talura*	511.14	9.82	3.37	0.26	4.4	0	1	0	1	0	0	1	0	0	1	0	0	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
61	HF1 b-3	*Platanus orientalis*	511.14	9.82	3.37	0.26	4.4	1	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	1	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
62	HF1 b-4	*Dacryodes sp.*	511.14	9.82	3.37	0.26	4.4	0	0	0	0	0	2	0	0	0	0	0	0	1	0	2	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
63	HF1 c-1	*Dapania racemosa*	498.93	12.93	3.37	0.30	4.4	1	0	0	0	0	1	0	0	0	1	0	0	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
64	HF1 c-2	*Dapania racemosa*	498.93	12.93	3.37	0.30	4.4	1	0	0	0	0	1	0	0	0	0	1	0	1	0	1	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
65	HF1 c-3	*Gnetum diminutum*	498.93	12.93	3.37	0.30	4.4	0	0	0	0	0	0	0	0	0	0	1	0	1	0	2	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1
66	HF2 a-1	*Nephelium mutabile*	500.64	11.61	4.53	0.27	4.3	0	0	0	0	0	0	0	0	0	0	1	0	2	0	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
67	HF2 a-2	*Santiria trimera*	500.64	11.61	4.53	0.27	4.3	0	0	0	0	0	1	0	0	0	0	1	0	2	0	1	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
68	HF2 a-3	*Canarium ovatum*	500.64	11.61	4.53	0.27	4.3	2	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
69	HF2 b-1	*Micropholis longipedicellata*	502.74	10.20	4.53	0.24	4.3	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	1	2	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
70	HF2 b-2	*Micropholis garciniifolia*	502.74	10.20	4.53	0.24	4.3	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1
71	HF2 b-3	*Dacryodes* sp.	502.74	10.20	4.53	0.24	4.3	0	0	0	0	0	0	0	0	0	1	1	0	1	0	2	0	0	1	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
72	HF2 c-1	*Mitrella kentii*	510.29	13.11	4.53	0.31	4.3	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	2	0	1	0	0	1	1	0	0	0	0	0	0
73	HF2 c-2	*Artocarpus heterophyllus*	510.29	13.11	4.53	0.31	4.3	0	0	0	0	0	0	0	0	0	0	1	0	1	0	3	0	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
74	HF2 c-3	*Manilkara zapota*	510.29	13.11	4.53	0.31	4.3	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	1	0	0	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
75	HF3 a-1	*Shorea acuminata*	522.19	12.21	4.07	0.25	4.3	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	1
76	HF3 a-2	*Agrostistachys borneensis*	522.19	12.21	4.07	0.25	4.3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1
77	HF3 a-3	*Prunus brittoniana*	522.19	12.21	4.07	0.25	4.3	0	0	0	0	0	1	0	0	0	0	2	0	4	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
78	HF3 b-1	*Castanopsis lucida*	474.42	13.39	4.07	0.28	4.3	0	0	0	0	1	0	0	0	0	0	1	1	2	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
79	HF3 b-2	*Manilkara zapota*	474.42	13.39	4.07	0.28	4.3	0	0	0	0	0	1	0	0	0	0	1	0	2	0	3	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
80	HF3 b-3	*Casearia nitida*	474.42	13.39	4.07	0.28	4.3	0	0	0	0	0	1	0	0	0	0	1	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
81	HF3 c-1	*Maesobotrya vermeulenii*	509.39	11.38	4.07	0.25	4.3	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	1	1	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
82	HF3 c-2	*Shorea tumbuggaia*	509.39	11.38	4.07	0.25	4.3	1	1	0	1	0	0	1	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
83	HF3 c-3	*Trattinnickia demerarae*	509.39	11.38	4.07	0.25	4.3	0	0	0	0	0	0	0	0	0	0	1	0	1	0	2	0	1	1	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
84	HF4 a-1	*Syzygium cumini*	492.41	10.06	4.39	0.22	4.2	0	0	0	0	0	0	0	0	0	0	1	0	1	0	3	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85	HF4 a-2	*Syzygium rowlandii*	492.41	10.06	4.39	0.22	4.2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	1	0	1	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
86	HF4 a-3	*Syzygium rowlandii*	492.41	10.06	4.39	0.22	4.2	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	3	0	0	1	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
87	HF4 b-1	*Spatholobus* sp.	503.53	13.49	4.39	0.25	4.2	0	0	0	0	0	2	0	0	0	0	1	0	3	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
88	HF4 b-2	*Canarium oleiferum*	503.53	13.49	4.39	0.25	4.2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	1

Table S3.3. Continued.

No	Plot ID	Phost plants	Environmental variables					AMF Abundance																																						
			C roots (mg/g)	N roots (mg/g)	Al roots (mg/g)	P roots (mg/g)	Soil pH	*Acaulospora lacunosa* VTX00024	*Acaulospora lacunosa* 2	*Ambisporaceae leptoticha*	*Ambispora leptoticha* VTX00242	*Archaeospora* PODO18.1	*Archaeospora* sp. VTX00005	*Claroideoglomus* VTX00193	*Claroideoglomus Torrecillas* VTX00193	*Claroideoglomus Torrecillas* VTX00056	*Diversispora Torrecillas* VTX00380	*Diversispora Torrecillas* VTX00354	*Scutellospora heterogama* VTX00255	*Scutellospora pellucida*	*Glomus* Alguacil VTX00280	*Glomus* Alguacil VTX00149	*Glomus constrictum* VTX00064	*Glomus intraradices*	*Glomus* Kottke08-7 VTX00069	*Glomus* sp. VTX00084	*Glomus* sp. VTX00194	*Glomus* sp. VTX00090	*Glomus* sp. VTX00064	*Glomus* sp. VTX0036	*Glomus* sp. VTX00291	*Glomus* sp.Voyria VTX00126	*Paraglomus* Alguacil12b VTX00350	*Paraglomus* Para2 VTX00308	*Paraglomus* sp. VTX00349	*Unknown Glomeromycota* 1	*Unknown Glomeromycota* 2	*Unknown Glomeromycota* 3	*Unknown Glomeromycota* 4	*Unknown Glomeromycota* 5	*Unknown Glomeromycota* 6	*Unknown Glomeromycota* 7	*Unknown Glomeromycota* 8	*Unknown Glomeromycota* 9	*Unknown Glomeromycota* 10	*Unknown Glomeromycota* 11
89	HF4 b-3	*Canarium zeylanicum*	503.53	13.49	4.39	0.25	4.2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	2	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1
90	HF4 c-1	*Protium gallicum*	507.39	13.09	4.39	0.28	4.2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	2	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
91	HF4 c-2	*Nephelium mutabile*	507.39	13.09	4.39	0.28	4.2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	4	0	0	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
92	HF4 c-3	*Shorea tumbuggaia*	507.39	13.09	4.39	0.28	4.2	0	0	1	0	0	0	0	0	1	0	0	0	0	0	3	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
93	HO1 a-3	*Elaeis guineensis*	404.83	7.14	6.74	0.34	4.7	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	1	0	0	0	0	0	1	0	0	0	0
94	HO1 b-2	*Elaeis guineensis*	411.46	7.63	6.74	0.32	4.7	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	0	0	0	0	2	1	0	0	0	0	0	0	0	0
95	HO1 c-2	*Elaeis guineensis*	416.77	7.37	6.74	0.35	4.7	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	1	1	0	0	0	0	0	0	0	1
96	HO2 a-4	*Elaeis guineensis*	450.80	6.58	9.58	0.17	4.7	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	1	1	0	1	0	0	0	0	0	0
97	HO2 b-3	*Elaeis guineensis*	411.11	6.93	9.58	0.22	4.5	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
98	HO2 c-2	*Elaeis guineensis*	434.53	5.76	9.58	0.20	4.5	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0
99	HO3 a-2	*Elaeis guineensis*	433.98	6.56	12.94	0.27	4.4	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	HO3 b-1	*Elaeis guineensis*	430.58	8.30	12.94	0.41	4.4	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	1	0	1	0	0	0
101	HO3 c-3	*Elaeis guineensis*	444.71	8.56	12.94	0.82	4.4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	0	0	0	0	0	1	0	0	0	1	1	0	0	0
102	HO4 a-1	*Elaeis guineensis*	457.89	6.90	13.31	0.29	4.5	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	1
103	HR1 b-3	*Hevea brasiliensis*	384.74	11.67	15.09	0.40	4.8	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	1	1
104	HR1 c-3	*Hevea brasiliensis*	393.87	13.09	15.09	0.51	4.8	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0
105	HR2 a-3	*Hevea brasiliensis*	445.52	11.19	6.74	0.43	4.8	1	1	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
106	HR2 c-4	*Hevea brasiliensis*	466.85	10.53	6.74	0.36	4.5	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1
107	HR3 a-1	*Hevea brasiliensis*	422.80	13.93	9.58	0.62	4.4	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
108	HR3 b-2	*Hevea brasiliensis*	457.98	15.92	9.58	0.79	4.4	0	1	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
109	HR3 c-1	*Hevea brasiliensis*	461.67	14.66	9.58	0.69	4.4	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0
110	HR4 a-2	*Hevea brasiliensis*	442.83	13.01	12.94	0.39	4.3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
111	HR4 b-2	*Hevea brasiliensis*	460.33	13.97	12.94	0.38	4.3	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
112	HR4 c-1	*Hevea brasiliensis*	467.03	13.59	12.94	0.42	4.3	1	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Data with grey background color were used for NMDS analysis (10 trees per system).

Table S3.4. Molecular identification of arbuscular mycorrhizal plant host species in Bukit Duabelas National Park transformation systems.

Plot ID	Family	Genus	Species	E-Value	Similarity
Forest					
BF1 a-1	Apocynaceae	*Trachelospermum*	*jasminoides*	0	98.93
BF1 a-2	Apocynaceae	*Trachelospermum*	*jasminoides*	0	98.93
BF1 a-3	Pandaceae	*Galearia*	*celebica*	0	99.77
BF1 b-1	Pandaceae	*Galearia*	*celebica*	0	99.77
BF1 b-2	Combretaceae	*Terminalia*	*guyanensis*	0	100
BF1 b-3	Sapotaceae	*Micropholis*	*longipedicellata*	0	100
BF1 c-1	Moraceae	*Ficus*	*fulva*	0	100
BF1 c-2	Moraceae	*Ficus*	*fulva*	0	99.83
BF1 c-3	Combretaceae	*Terminalia*	*guyanensis*	0	100
BF2 a-1	Moraceae	*Ficus*	*subpisocarpa*	0	100
BF2 a-2	Moraceae	*Ficus*	*subpisocarpa*	0	100
BF2 a-3	Selaginellaceae	*Selaginella*	*roxburghii*	0	99.47
BF2 b-1	Fabaceae	*Butea*	*monosperma*	0	98.71
BF2 b-2	Fabaceae	*Acacia*	*tenuifolia*	0	99.47
BF2 b-3	Fabaceae	*Butea*	*monosperma*	0	98.92
BF2 c-1	Arecaceae	*Plectocomiopsis*	*geminiflora*	0	100
BF2 c-2	Fabaceae	*Acacia*	*tenuifolia*	0	99.47
BF2 c-3	Moraceae	*Ficus*	*subpisocarpa*	0	100
BF3 a-1	Burseraceae	*Canarium*	*ovatum*	0	99.83
BF3 a-2	Malvaceae	*Cola*	*acuminata*	0	100
BF3 a-3	Malvaceae	*Cola*	*acuminata*	0	100
BF3 b-1	Sapotaceae	*Micropholis*	*longipedicellata*	0	99.8
BF3 b-2	Sapotaceae	*Micropholis*	*garciniifolia*	0	100
BF3 b-3	Apocynaceae	*Trachelospermum*	*jasminoides*	0	98.93
BF3 c-1	Sapotaceae	*Micropholis*	*garciniifolia*	0	100
BF3 c-2	Sapotaceae	*Micropholis*	*garciniifolia*	0	100
BF3 c-3	Sapotaceae	*Micropholis*	*garciniifolia*	0	100
BF4 a-1	Ebenaceae	*Diospyros*	*sp*	2.58E-133	100
BF4 a-2	Burseraceae	*Canarium*	*ovatum*	0	99.83
BF4 a-3	Selaginellaceae	*Selaginella*	*roxburghii*	0	99.47
BF4 b-1	Combretaceae	*Terminalia*	*guyanensis*	0	100
BF4 b-2	Burseraceae	*Canarium*	*ovatum*	0	99.83
BF4 b-3	Meliaceae	*Reinwardtiodendron*	*kinabaluense*	0	100
BF4 c-1	Erythropalaceae	*Strombosia*	*pustulata*	0	100
BF4 c-2	Oxalidaceae	*Dapania*	*racemosa*	0	99.27
BF4 c-3	Centroplacaceae	*Bhesa*	*paniculata*	0	100

Table S3.4. Continued

Plot ID	Family	Genus	Species	E-Value	Similarity
Rubber					
BR1 a-1	Euphorbiaceae	*Hevea*	*brasiliensis*	0	100
BR1 b-1	Euphorbiaceae	*Hevea*	*brasiliensis*	0	100
BR2 a-2	Euphorbiaceae	*Hevea*	*brasiliensis*	0	100
BR2 c-1	Euphorbiaceae	*Hevea*	*brasiliensis*	0	99
BR3 a-1	Euphorbiaceae	*Hevea*	*brasiliensis*	5.42E-176	100
BR3 b-1	Euphorbiaceae	*Hevea*	*brasiliensis*	2.64E-161	99.73
BR3 c-1	Euphorbiaceae	*Hevea*	*brasiliensis*	1.00E-145	99
BR4 a-2	Euphorbiaceae	*Hevea*	*brasiliensis*	0	100
BR4 b-1	Euphorbiaceae	*Hevea*	*brasiliensis*	0	99
BR4 c-2	Euphorbiaceae	*Hevea*	*brasiliensis*	0	100
Oil palm					
BO1 a-1	Arecaceae	*Elaeis*	*guineensis*	0	99.63
BO1 b-3	Arecaceae	*Elaeis*	*guineensis*	0	99.63
BO1 c-1	Arecaceae	*Elaeis*	*guineensis*	2.00E-92	98
BO2 a-1	Arecaceae	*Elaeis*	*guineensis*	0	99
BO2 b-1	Arecaceae	*Elaeis*	*guineensis*	0	99
BO2 c-2	Arecaceae	*Elaeis*	*guineensis*	0	99
BO3 a-1	Arecaceae	*Elaeis*	*guineensis*	3.00E-127	99
BO3 b-2	Arecaceae	*Elaeis*	*guineensis*	4.00E-105	100
BO3 c-1	Arecaceae	*Elaeis*	*guineensis*	0	99
BO4 b-1	Arecaceae	*Elaeis*	*guineensis*	0	99

Table S3.5. Molecular identification of arbuscular mycorrhizal plant host species in Harapan transformation systems.

Plot ID	Family	Genus	Species	E-Value	Similarity
HF1 a-1	Myrtaceae	*Pimenta*	*pseudocaryophyllus*	0	99.83
HF1 a-2	Myrtaceae	*Syzygium*	sp.	0	99.82
HF1 a-3	Sapindaceae	*Nephelium*	*mutabile*	0	99.66
HF1 b-1	Dipterocarpaceae	*Shorea*	*talura*	0	99.48
HF1 b-3	Platanaceae	*Platanus*	*orientalis*	1.00E-120	98
HF1 b-4	Burseraceae	*Dacryodes*	sp.	0	99.5
HF1 c-1	Oxalidaceae	*Dapania*	*racemosa*	0	99.32
HF1 c-2	Oxalidaceae	*Dapania*	*racemosa*	0	99.26
HF1 c-3	Gnetaceae	*Gnetum*	*diminutum*	0	99.33
HF2 a-1	Sapindaceae	*Nephelium*	*mutabile*	0	99.66
HF2 a-2	Burseraceae	*Santiria*	*trimera*	0	95.11
HF2 a-3	Burseraceae	*Canarium*	*ovatum*	0	99.83
HF2 b-1	Sapotaceae	*Micropholis*	*longipedicellata*	0	100
HF2 b-2	Sapotaceae	*Micropholis*	*garciniifolia*	1.21E-129	100
HF2 b-3	Burseraceae	*Dacryodes*	sp.	0	99.49
HF2 c-1	Annonaceae	*Mitrella*	*kentii*	0	99.65
HF2 c-2	Moraceae	*Artocarpus*	*heterophyllus*	0	100
HF2 c-3	Sapotaceae	*Manilkara*	*zapota*	0	99.47
HF3 a-1	Dipterocarpaceae	*Shorea*	*acuminata*	0	99.81
HF3 a-2	Euphorbiaceae	*Agrostistachys*	*borneensis*	0	99.3
HF3 a-3	Rosaceae	*Prunus*	*brittoniana*	0	99.83
HF3 b-1	Fagaceae	*Castanopsis*	*lucida*	0	99.83
HF3 b-2	Sapotaceae	*Manilkara*	*zapota*	0	100
HF3 b-3	Flacourtiaceae	*Casearia*	*nitida*	0	99
HF3 c-1	Phyllanthaceae	*Maesobotrya*	*vermeulenii*	0	99.48
HF3 c-2	Dipterocarpaceae	*Shorea*	*tumbuggaia*	0	89.26
HF3 c-3	Burseraceae	*Trattinnickia*	*demerarae*	0	99.12
HF4 a-1	Myrtaceae	*Syzygium*	*cumini*	0	99.83
HF4 a-2	Myrtaceae	*Syzygium*	*rowlandii*	0	99.82
HF4 a-3	Myrtaceae	*Syzygium*	*rowlandii*	0	100
HF4 b-1	Fabaceae	*Spatholobus*	sp.	0	99.63
HF4 b-2	Burseraceae	*Canarium*	*oleiferum*	0	100
HF4 b-3	Burseraceae	*Canarium*	*zeylanicum*	0	99.64
HF4 c-1	Burseraceae	*Protium*	*gallicum*	0	99.29
HF4 c-2	Sapindaceae	*Nephelium*	*mutabile*	0	99.66
HF4 c-3	Dipterocarpaceae	*Shorea*	*tumbuggaia*	0	89.26

Table S3.5. Continued

Plot ID	Family	Genus	Species	E-Value	Similarity
Rubber					
HR1 b-3	Euphorbiaceae	*Hevea*	*brasiliensis*	0	99
HR1 c-3	Euphorbiaceae	*Hevea*	*brasiliensis*	0	99
HR2 a-3	Euphorbiaceae	*Hevea*	*brasiliensis*	0	99
HR2 c-4	Euphorbiaceae	*Hevea*	*brasiliensis*	0	99
HR3 a-1	Euphorbiaceae	*Hevea*	*brasiliensis*	0	100
HR3 b-2	Euphorbiaceae	*Hevea*	*brasiliensis*	0	100
HR3 c-1	Euphorbiaceae	*Hevea*	*brasiliensis*	0	99
HR4 a-2	Euphorbiaceae	*Hevea*	*brasiliensis*	0	100
HR4 b-2	Euphorbiaceae	*Hevea*	*brasiliensis*	0	100
HR4 c-1	Euphorbiaceae	*Hevea*	*brasiliensis*	0	100
Oil palm					
HO1 a-3	Arecaceae	*Elaeis*	*guineensis*	2.00E-180	99
HO1 b-2	Arecaceae	*Elaeis*	*guineensis*	0	99
HO1 c-2	Arecaceae	*Elaeis*	*guineensis*	5.00E-78	100
HO2 a-4	Arecaceae	*Elaeis*	*guineensis*	1.00E-116	98
HO2 b-3	Arecaceae	*Elaeis*	*guineensis*	0	99
HO2 c-2	Arecaceae	*Elaeis*	*guineensis*	0	99
HO3 a-2	Arecaceae	*Elaeis*	*guineensis*	6.00E-170	99
HO3 b-1	Arecaceae	*Elaeis*	*guineensis*	0	99
HO3 c-3	Arecaceae	*Elaeis*	*guineensis*	0	99
HO4 a-1	Arecaceae	*Elaeis*	*guineensis*	0	99

Table S3.6. Arbuscular mycorrhizal species rich and frequency detected in Bukit and Harapan land use systems.

OTU ID	AMF OTUs	Species richness	Frequency (%)	Association specificity (*d'*)
OTU_1	*Acaulospora lacunosa* VTX00024	78	8.71	0.21
OTU_2	*Acaulospora* sp. VTX00227	41	4.58	0.15
OTU_3	*Ambispora leptoticha*	3	0.33	0.34
OTU_4	*Ambispora leptoticha* VTX00242	18	2.01	0.23
OTU_5	*Archaeospora* PODO18.1	7	0.78	0.27
OTU_6	*Archaeospora* sp. VTX00005	20	2.23	0.27
OTU_7	*Claroideoglomus* NF25 VTX00193	8	0.89	0.28
OTU_8	*Claroideoglomus* Torrecillas12b Glo G1 VTX00193	5	0.56	0.37
OTU_9	*Claroideoglomus* Torrecillas12b Glo G3 VTX00056	8	0.89	0.30
OTU_10	*Diversispora* Torrecillas12b Div2 VTX00380	13	1.45	0.21
OTU_11	*Diversispora* Torrecillas12b Div3 VTX00354	25	2.79	0.28
OTU_12	*Scutellospora heterogama* VTX00255	11	1.23	0.25
OTU_13	*Scutellospora pellucida*	57	6.36	0.19
OTU_14	*Glomus* Alguacil12a Glo G9 VTX00280	8	0.89	0.31
OTU_15	*Glomus* Alguacil12b GLO G11 VTX00149	92	10.27	0.30
OTU_16	*Glomus constrictum* VTX00064	7	0.78	0.44
OTU_17	*Glomus intraradices* VTX00100	61	6.81	0.24
OTU_18	*Glomus* Kottke08-7 VTX00069	28	3.13	0.28
OTU_19	*Glomus* sp. VTX00084	6	0.67	0.35
OTU_20	*Glomus* sp. VTX00194	50	5.58	0.15
OTU_21	*Glomus* sp. RF1 VTX00090	9	1.00	0.33
OTU_22	*Glomus* sp. VTX00064	7	0.78	0.26
OTU_23	*Glomus* Alguacil12a Glo G8 VTX00363	95	10.60	0.17
OTU_24	*Glomus* sp. VTX00291	5	0.56	0.51
OTU_25	*Glomus* Voyria symbiont type 2 VTX00126	67	7.48	0.39
OTU_26	*Paraglomus* Alguacil12b PARA2 VTX00350	5	0.56	0.41
OTU_27	*Paraglomus* Para2 VTX00308	43	4.80	0.37
OTU_28	*Paraglomus* sp. VTX00349	4	0.45	0.32
OTU_29	Unknown OTU1	25	2.79	0.10
OTU_30	Unknown OTU2	8	0.89	0.04
OTU_31	Unknown OTU3	9	1.00	0.02
OTU_32	Unknown OTU4	2	0.22	0.30
OTU_33	Unknown OTU5	8	0.89	0.07
OTU_34	Unknown OTU6	15	1.67	0.09
OTU_35	Unknown OTU7	4	0.45	0.16
OTU_36	Unknown OTU8	4	0.45	0.09
OTU_37	Unknown OTU9	9	1.00	0.20
OTU_38	Unknown OTU10	3	0.33	0.14
OTU_39	Unknown OTU11	28	3.13	0.08

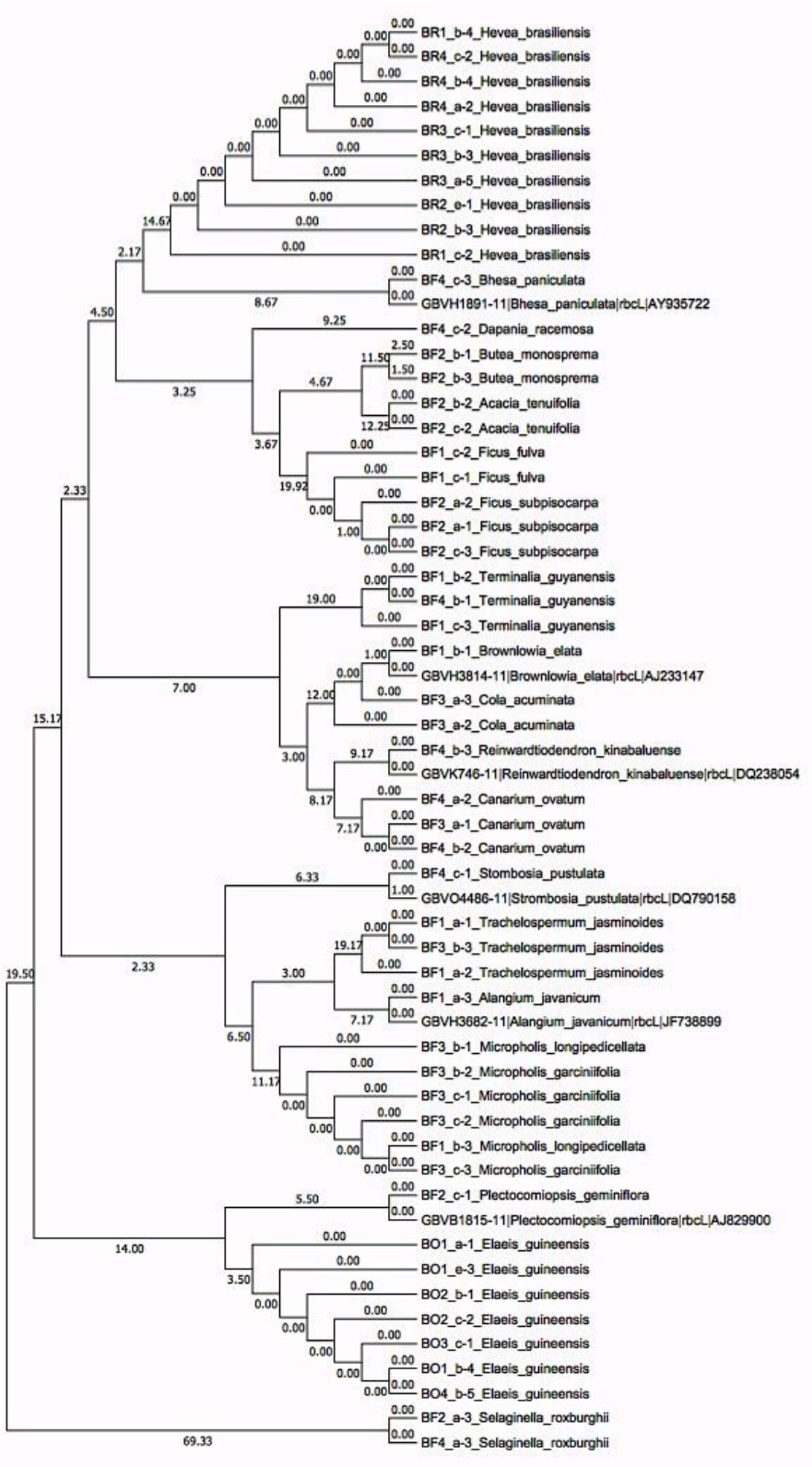

Figure S3.1. Phylogenetic tree of host plants of arbuscular mycorrhizal fungi in Bukit Duabelas National Park transformation systems based maximum parsimony.

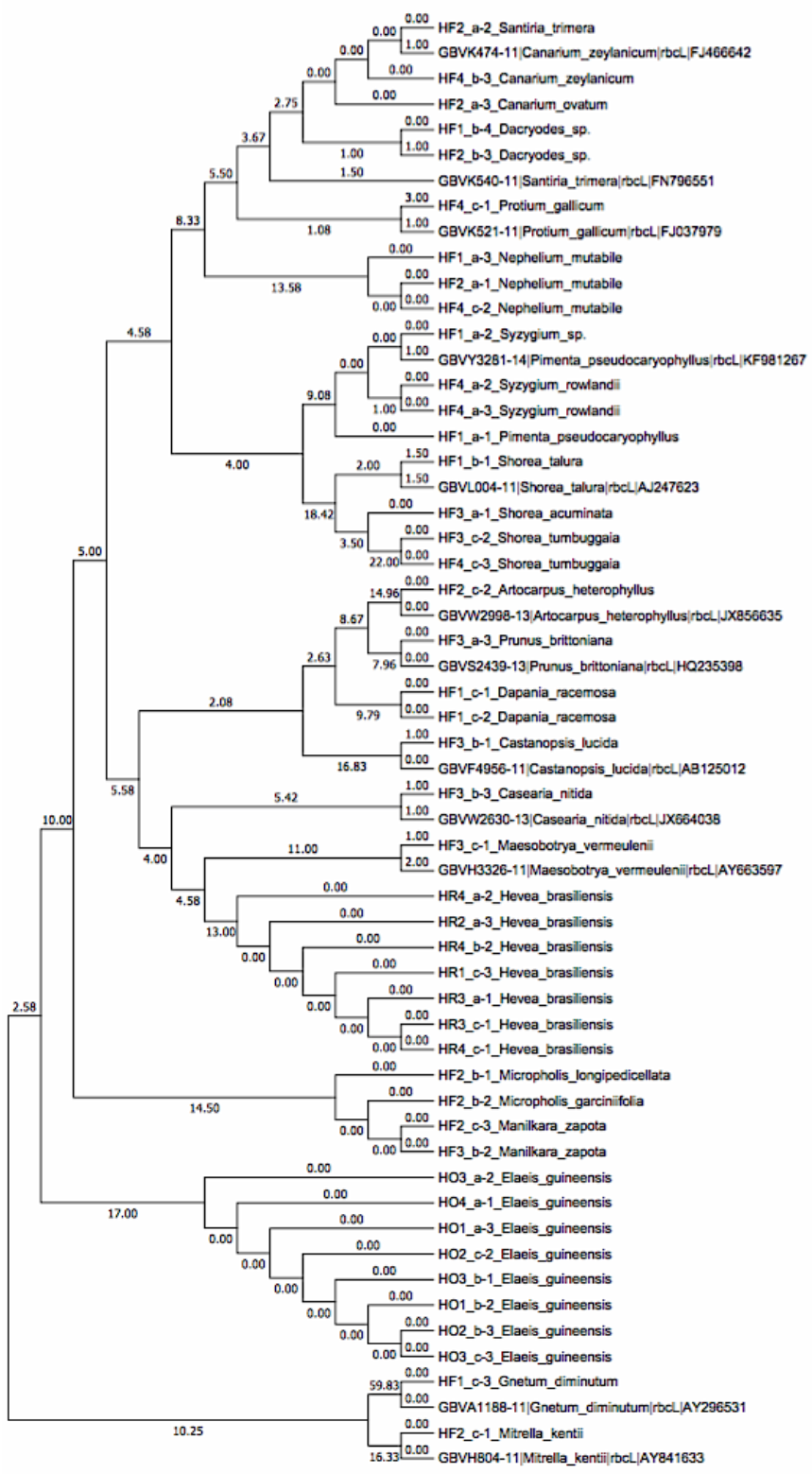

Figure S3.2. Phylogenetic tree of host plants of arbuscular mycorrhiza in Harapan transformation systems based on maximum parsimony.

Chapter 4
Conclusion and Outlook

4.1. Conclusion

This study was set out to explore the community structure of AMF influenced by management practice in temperate grassland and tropical land use systems. The experiment in temperate grassland was sought to know whether fertilization, cutting frequency, and herbivore in different swards (species rich, dicots, and monocots) result in change of AMF community structure and species richness. In tropical land use systems, the impact of conversion of rainforest to oil palm and rubber plantations on AMF community structure and species richness was observed.

The main empirical findings are presented as specific chapters and were summarized within the following respective chapters. In the chapter two, the impact of land management and herbivory on AMF in uplands permanent grassland has been described. This section answered the question that whether fertilization and combination management practices altered pattern of AMF community structure in grassland ecosystem. Under fertilization, arbuscules and vesicles as key structures for nutrient exchange between plant and fungus were decreased. Land management of different sward, fertilization, and utilization also significantly decreased the relative abundance of arbuscule. This funding suggests that mycorrhizal growth and storage reserves are unsettle caused by fertilization and combination treatments of land management practice. The

study has offered a perspective that management practices could negate the disadvantages on AMF.

In the chapter three, we studied the impact of forest transformation to managed oil palm and rubber plantations. Land use change by forest conversion also altered the AMF community structures. In Indonesia, oil palm and rubber plantations puts pressure on natural resources. These mono plantations are often planted into area which were forest previously. Change of land use, consequently alters the plant ecosystem and soil properties. In this study, forest conversion into oil palm and rubber plantations resulted in change of AMF community structure and decreased the species richness. The different environmental variables in plant roots and soil appear as the most likely factor shaping the structure of AMF community in transformation of lowland rainforest Sumatra.

4.2. Outlook

The AMF community structure is now being recognized, particularly with respect to management practices in grassland and land use change in tropical rainforest. Relation between management practice and land use change on AMF community structure suggest a feedback that plant and AMF association in different ecosystems may play a fundamental role in determining the AMF species composition and diversity. However, the interpretation of functional AMF community in ecosystem is still limited by lack of knowledge. The conclusion of relationship between management

practices and land use change on AMF will be more possible if future work include the study of functional diversity of AMF in relation to their symbioses. The use of high throughput sequencing will be more reliable in covering the abundance of AMF.

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Indonesia for my second experiment that has been presented as the chapter 3 in this dissertation. The project is a collaboration between Georg-August-University Göttingen, University of Jambi, Tadulako University, Bogor Agricultural University, and Indonesian Institute for Science (LIPI) in the framework of Collaborative Research Center (CRC) 990. Therefore, I would also like to thank the support from Prof. Dr. Sri Wilarso Budi, Dr. Henry Barus, Dr. Bambang Irawan, and Dr. Upik Yelianti as our research counterparts in Indonesia and the help they rendered to carry out the research activities during the experiment in Indonesia. I also thank Wolfram Lorenz and CRC 990 management in Bogor as well as Dr. Barbara Wick and Ivonne Hein for providing assistance with the administrative tasks in the framework of CRC project. I thank Khairul Anwar, Happy Chandra, Nover and Arif who helped us with the fieldwork in Jambi Indonesia.

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Appendix 1

Root community traits as indicator for transformation of tropical lowland rain forests into oil palm and rubber plantations

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Abstract

Conversion of tropical forests into intensely managed plantations is a threat to ecosystem functions. On Sumatra, Indonesia, oil palm (*Elaeis guineensis*) plantations are rapidly expanding, displacing rain forest and extensively used rubber (*Hevea brasiliensis*) agro-forests. The effect of forest transformation on root traits is unknown. Here, we hypothesized that chemical and performance traits of root communities vary with forest transformation and that degradation of traits is linked with loss of ecosystem functions. To test these hypotheses root functional traits were determined as root community functional parameters (RCFP). Carbon and nitrogen in soil and litter, phosphorus availability, base cations and pH were determined as proxy for ecosystem functions. The study was conducted in secondary lowland rain forests and in secondary forests enriched with rubber (jungle rubber) as well as in rubber and oil palm plantations in two landscapes (Bukit Duabelas and Harapan, Sumatra). Ectomycorrhizas were rare and only detected in jungle rubber and lowland rain forest. Arbuscular mycorrhizal root colonization was abundant and generally unaffected by the land use system. Root vitality was lower and spore abundance higher in oil palm plantations than in other land use systems. Multivariate analysis with RCFPs uncovered ordering of the sites according to land use (oil palm plantation < rubber plantation < jungle rubber < rain forest) with aluminium, iron, mortality and soil spores as negative and root mass, root carbon, nitrogen and base cations as major positive loadings. The ordination scores were used as transformation

indices and showed significant correlation with ecosystem properties (positive: soil nitrogen and litter carbon, negative: soil pH). As the transformation indices were determined by contrasting behavior of RCFPs and not by the loss of traits abundance *per se* our results suggest that any measure that improves root vitality may enhance the ecological functions of intense tropical production systems.

Introduction

Globally, tropical rain forests are rapidly converted to plantation agriculture (Hansen et al. 2008). In Indonesia, which is together with Malaysia the world´s largest producer of palm oil (Carrasco et al. 2014), 40% of the forest (64 Mio ha) was lost since the countries´ independence in 1945 (FAO 2010). In the 1950s rubber (*Hevea brasiliensis*) was introduced as a crop tree and is currently cultivated in two systems, in intense monocultures often with high yielding clones (rubber plantation) or as jungle rubber. Jungle rubber is a complex, extensive form of agro-forestry, usually established after swidden agriculture, where rubber trees are grown together with naturally established secondary forest (Guoyon et al. 1993, Tata et al. 2008). Tree species richness is slightly lower, but the forest structure of jungle rubber is similar to that of unmanaged lowland rain forests (Guoyon et al. 1993, Murdiyarso et al. 2002, Tata et al. 2008). Pristine lowland rain forests exists only in fragments and most of unmanaged forests, even in protected areas, are secondary forests. Since

the 1990s with the introduction of oil palms (*Elaeis guineensis*), expansion of plantation area at the expense of primary and secondary forests has drastically increased (Ministry of Agriculture, 2010), with particularly high rates (> 2% per year) on Sumatra (Erasmi et al. 2010). Because of the world´s increasing demand for biofuel, chemical raw materials and edible oil, palm oil production is now a major driver for tropical forest conversion (Carrasco et al. 2014). The ecological consequences of this rapid transformation process are severe, including for example massive loss in biodiversity, soil degradation, reduction in carbon storage, decreased energy flux, increases in greenhouse gas emissions, etc. (Dechert et al. 2004, Wilcove et al. 2010, Carlson et al. 2012, Barnes et al. 2014). While the alterations of above-ground ecosystem properties and processes have been intensively studied, much less is known about the below-ground plant responses to these massive changes.

Roots and associated mycorrhizal fungi play a central role for nutrient uptake and allocation to the above-ground parts; they further mediate carbon transfer to the soil, thereby, eventually affecting biogeochemical cycles (Godbold et al. 2006, Forana et al. 2009, Orwin et al. 2010, Clemmensen et al. 2013). In tropical forests, most tree species including the introduced rubber and oil palms form symbioses with arbuscular mycorrhizal (AM) fungi, but in lowland tropical forests also a number of native species occur, e.g. dipterocarps and Fagaceae that associate with ectomycorrhizal (EM) fungi (Habib et al. 2013).

The ability of tree roots to form mutualistic AM or EM associations is a typical species-related trait that can mediate differences in plant nutrition, especially of phosphorus and nitrogen (Smith and Read 2008). Root functional traits have often been studied in agroecological systems (Garnier and Navas 2012), but only little information is available for forest trees, especially regarding the chemical root traits. In tropical ecosystems with potentially 100s of species per hectare (Murdiyarso et al. 2002, Tata et al. 2008) *in situ* root traits are difficult to measure, because a trait is defined as a feature of a species (Violle et al. 2007). Instead, information on root traits can be gathered at the community level of the co-occurring species and is then defined as a "root community functional parameter" (RCFP) according to Violle et al. (2007). Only few studies addressed the variation of RCFPs . Prieto et al (2014) found that RCFPs related to resource acquisition (root morphology) and conservation (degradability) co-varied with land use across tropical, mediterranean and montane climate. In grassland ecosystems RCFPs were correlated with plant productivity and ecosystem functions (Fornara et al. 2009, Orwin et al. 2010). We, therefore, anticipated that RCFPs were useful indicators of land transformation and of functional ecosystem properties in response to tropical forest conversion. However, these relationships have not yet been investigated.

Here, we expected profound effects of forest transformation on functional traits of the root communities and that RFPCs were linked with ecosystem

properties such as soil fertility. Specifically, we hypothesized that (i) chemical and performance parameters of root communities vary with forest transformation, that (ii) the RCFPs can be used to derive transformation indices and that (iii) the transformation indices are correlated with ecosystem functional properties. To test our hypotheses we selected four forest types (oil palm plantations, rubber monoculture, rubber jungle and rain forest) in two landscapes on Sumatra and investigated root functional parameters at the community level (root element composition, root vitality, EM and AM colonization and function [AM vesicles, AM arbuscules, AM spores]) in addition to ecosystem functions related to soil fertility (soil and litter carbon and nitrogen concentrations, available phosphorus, base cations, and soil pH). Multivariate analyses extracted informative RCFPs as indicators for forest transformation (higher root nutrient concentrations and higher fine root mass in forest plots opposed to higher root concentrations of Al and Fe, higher root mortality and high spore number of AM fungi in soil in oil palm plots) and ordered them according to land use (oil palm < rubber plantation < rubber jungle < rain forest). A general linear model with the ordination scores of the RCFPs as dependent variables identified significantly correlated ecosystem properties (positive: soil nitrogen and litter carbon, negative: soil pH).

Materials and Methods

Site description

The study sites were located on Sumatra, Province of Jambi (Indonesia) in two landscapes, i.e., the area of Harapan Rainforest and the area of the National Park Bukit Dua Belas (Fig. 1A,B). In each landscape four forest types were selected: secondary rain forest, jungle rubber, rubber plantations and oil palm plantations. The study areas were in the lowlands (below 100m a.s.l.) on deep, well drained, acid soil with low fertility (Murdiyarso et al. 2002). The climate is tropical with annual precipitation > 2000mm and only two months with less than 100 mm rain fall. In the Harapan area the annual mean temperature is 26.9 °C and the annual precipitation 2332mm (location: Dusun Baru, http://en.climate-data.org/location/595657/); in the Bukit Duabelas area the mean annual temperature is 26.8°C and the precipitation sum is 2860mmm (location: Lubuk Kepayang, http://en.climate-data.org/location/587840/).

Sampling and export permission

Research permit (Kartu Izin Peneliti Asing, permission number: 333/SIP/FRP/SM/IX/2012) was issued by the Ministry of Research and Technology RISTEK (Kementrian Ristek dan Teknologi, Jakarta, Indonesia). The Research Center for Biology of the Indonesian Institute of Science LIPI (Lembaga Ilmu Pengetahuan Indonesia, Jakarta, Indonesia) recommended issuing a sample collection permit (Rekomendasi Ijin Pengamblian dan Ankut (SAT-DN) Sampel Tanah dan Akar, number:

2696/IPH.1/KS:02/XI/2012). Collection permit (number: S.16/KKH-2/2013) and export permit (reference number: 48/KKH-5/TRP/2014) were issued by the Directorate General of Forest Protection and Nature Conservation PHKA (Perlindungan Hutan dan Konservasi Alam, Jakarta, Indonesia) under the Ministry of Forestry of the Republic of Indonesia. The Chamber of Agriculture of Lower Saxony (Plant Protection Office, Hannover, Germany) issued the import permits (Letter of Authority, numbers: DE-NI-12- 69 -2008-61-EC, DE-NI-14- 08 -2008-61-EC).

Sampling design

In each of the two landscapes and in each forest type four plots (50m x 50m) were installed resulting in 32 sampling sites (Table 1). Oil palm, rubber plantations and rubber jungle were sampled in October and November 2012 and rain forest in November and December 2013. In each plot, subplots of 5m x 5m were defined and soil samples were collected in three of these subplots (designated as a,b,c). In each subplot five soil cores (0.04 m diameter and 0.20 m depth) were extracted (four towards the corners and one in the centre of the subplot) at a distance of more than 1m. Leaf litter was removed before soil sampling and kept separately. In total 480 soil cores were taken in both landscapes (2 landscapes x 16 plots x 3 subplots x 5 soil cores). Soil cores and litter samples were stored individually in plastic bags in cool bags and transported to the University of Jambi, where they were stored at 4°C until processing.

Sample preparation

Each soil core was weighed, sieved subsequently through two sieves with 10 and 5 mm mesh size and separated into roots and bulk soil. The five samples from the same subplot were pooled and well mixed yielding one root and one bulk soil sample per subplot. Litter samples of a subplot were also pooled yielding a total number of 96 pooled samples per fraction.

Fresh bulk soil samples (about 20 g) were initially air dried and then oven dried (105°C for 48h) to determine the soil water content according to the following equation:

$$\text{Relative soil water content (g/g soil)} = \left(\frac{\text{weight fresh soil (g)} - \text{weight air dried soil (g)}}{\text{weight fresh soil(g)}}\right) + \left(\frac{\text{weight air dried soil (g)} - \text{weight oven dried soil (g)}}{\text{weight air dired soil (g)}}\right)$$

Pooled root samples were washed and patted dry with tissue paper. The fresh root mass of the sample was weighed. The roots were separated into coarse and fine roots according to the root diameter. Fine roots (diameter ≤ 2 mm) were weighed, stored in wet tissue paper at 4°C, used for root vitality and mycorrhizal analysis, and were subsequently oven-dried at 60°C for 48h. Fine root dry mass was calculated as:

$$\text{Fine root mass (g/kg soil)} = \left(\frac{\text{dry weight pooled fine roots (g/subplot)}}{\text{dry weight pooled soil (g/subplot)}}\right) * 1000$$

Litter samples were dried in an oven at 80°C for 48h. Dry aliquots of soil, roots and litter were stored in 50ml reaction tubes (Falcon tube 50ml, 115 x 28mm, Sarstedt, Nümbrecht, Germany). Before closing the screw cap, a small reaction tube (Eppendorf micro tube, 1.5ml, Sarstedt, Nümbrecht, Germany) with perforated walls containing silica gel (10 g (40 x 90 mm) desiccant bag silica gel orange, Carl Roth, Karlsruhe, Germany) was added. The samples were shipped to the University of Göttingen (Göttingen, Germany), IBP Bogor Agricultural University (Bogor, Indonesia) and Tadulako University (Palu, Indonesia) for further analysis.

Analysis of root vitality and ectomycorrhizal (EM) colonization

The root tips of fresh fine roots were inspected using a dissecting microscope with an integrated camera (Leica EZ4HD, Wetzlar, Germany) at 35-fold magnification. Aliquots of fine roots were placed in a water-filled Petri dish (Petri dish 92 x 16 mm, Sarstedt, Nümbrecht, Germany). In general, 250 roots tips were counted and scored as vital and dead root tips after colour of vascular tissue, strength and flexibility as described by Allen et al. (2000). On the vital root tips the number of EM root tips was counted. EM root tips were recognized by presence of “a sheath or mantle of fungal tissue which encloses the root” and hyphal elements (Smith and Read, 2008). Dead, non-EM, and vital EM root tips were documented by photos taken with the microscope camera.

Arbuscular mycorrhizal (AM) colonization

Up to 25 fine root fragments per subplot with a length of 20 to 30 mm measured from the root tip were stored in reaction tubes (Eppendorf micro tube 2ml, Sarstedt, Nümbrecht, Germany) containing 70% ethanol (Rotisolv HPLC Gradient, Carl Roth, Karlsruhe, Germany). Roots were stained following the method of Vierheilig *et al.* (1998). The root segments were washed several times with ultra-purified water (ultra-pure water system, Arium 611, Sartorius, Göttingen, Germany), briefly surfaced-dried on tissue paper and then bleached in 2 ml of 10% potassium hydroxide (KOH, Merck, Darmstadt, Germany) for 90 min at 90°C. Because not all roots were bleached after one KOH treatment, this step was repeated with variation of the incubation time and temperature until the objective was achieved. The bleached roots were carefully washed up to three times with ultra-purified water to remove the KOH and then stained in 2 ml of a vinegar-ink-solution (10% acetic acid (Merck, Darmstadt, Germany), black ink (Sheaffer Skrip, Shelton, USA) and ultra-purified water with a ratio of 1:1:8 for 45 min at room temperature. The stained roots were washed with ultra-purified water to remove superfluous dye. Roots were preserved up to eight weeks in lactoglycerol consisting of 86% Glycerol (Carl Roth, Karlsruhe, Germany), 80% lactic acid (Carl Roth, Karlsruhe, Germany) and ultra-purified water with a ratio of 1:1:1 before preparing microscope object slides.

For microscopic analysis, roots were cut into small segments (10 mm) and arranged with forceps in a drop of lactoglycerol as the mountant on a

microscope object slide. Cover slides were gently pressed on root segments and flattened overnight using a lead weight (weight between 40 and 50 g). Subsequently, the cover slides were sealed with colorless nail polish to protect the specimen from drying. Three slides per sample were prepared and analyzed.

The gridline intersection method after McGonigle et al. (1990) was used to determine AM colonization. The slides were placed under a compound microscope (Axio Observer Z.1, Zeiss, Jena, Germany). With the computer program AxioVision LE (Zeiss, Jena, Germany) a gridline was generated on the considered section (magnification 400x, distance between the intersects 100µm) and the presence or absence of the following structures was recorded in 120 intersects per sample: AM hyphae, arbuscules, and vesicles. For each recorded arbuscule and vesicle, a hypha was also counted because these structures are always co-occurring. For each sample 120 intersects were counted. AM colonization was calculated as:

$$\mathrm{AM-colonization}\ (\%) = \frac{\mathrm{number\ of\ hyphae}}{\mathrm{total\ number\ of\ intersects}} * 100\%$$

The relative abundance of arbuscules and vesicles was calculated correspondingly.

Determination of arbuscular mycorrhizal spore abundance

Air dried samples of bulk soil were stored in sealed plastic bags at 4ºC. Spores from each soil samples (n = 480) were isolated as described by Gerdemann and Nicolson (1963). Twenty gram of soil of each sample was suspended in 500 ml of water, stirred manually with hand for 10 minutes. The suspension was passed through sieves, which were arranged in a descending order from 250 µm, 125 µm and 63 µm and washed with tap water. The material retained on the sieves were layered onto a a water-sucrose solution (50%) gradient and centrifuged at 900 x g for 2 min (Ohms, 1957). The supernatant was washed with tap water for 3 minutes in a 63 µm sieve, filtrated onto a gridded filter paper, than placed in a 90 mm diameter Petri dish. The spores obtained from all sieves were counted under a binocular stereomicroscope with 100- 400 magnification (Olympus SZ61, Osaka, Japan). The number of spores were expressed as spores per 20 g soil sample.

Element analyses in plant and soil fractions

Dry samples of soil, roots and litter were ground to a fine powder in a ball mill (MM 2000, Retsch, Haan, Germany). Aliquots of 0.7 to 0.9 mg per sample were weighed into tin capsules (5 x 9mm, HEKAtech, Wegberg, Germany) and used for carbon and nitrogen analyses in an Elemental Analyzer (EA 1108, Carlo Erba Instruments, Milan, Italy). Acetanilide (C: 71.09 %, N: 10.36 %, HEKAtech, Wegberg, Germany) was used as the standard.

For analyses of further elements Al, Ca, Fe, K, Mg, Mn, Na, P and S (aluminum, calcium, iron, potassium, magnesium, manganese, sodium and sulfur) a milled aliquot of 50 mg of dry soil or fine roots of each sample was digested in 2 ml of 65% nitric acid (HNO_3, Merck, Darmstadt, Germany) for 14h at 200°C (Heinrichs *et al.* 1986). Afterwards each extract was completely transferred into an Erlenmeyer flask. The polytetrafluoroethylene tubes (Loftfields Analytische Lösung, Neu Eichenberg, Germany) used for the extraction were washed with HPLC grade water (Chromanorm, VWR, Darmstadt, Germany), the washing solution was filtered through black ribbon filter paper (filter papers MN 640w, ⌀ 90mm, ashless, Macherey-Nagel, Düren, Germany) into the Erlenmeyer flask and the volume was adjusted to 25 ml with HPLC grade water. Then elements in the extract were analysed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES, iCAP 6300 Series, Thermo Fischer Scientific, Dreieich, Germany).

$$\text{Standard nutrient level (mg/g)} = \frac{\text{nutrient content (mg/l)} * \text{volume (ml)}}{\text{net weight nutrient (g)}}$$

To calculate the sum of base cations, the concentrations of potassium, magnesium and calcium were converted from mg g^{-1} into µmol g^{-1} and then added.

For the extraction of available phosphorus in soil the method of Bray and Kurtz (1945) was used. Air dried soil samples were sieved through a 2mm mesh. Two grams of soil from each sample were mixed with 15ml of Bray

solution containing 0.03N NH_4F and 0.025N HCl and were shaken (Finofors AG, Basel, Switzerland) for 5 min at 180 rmp at room temperature. After shaking, the suspensions were filtered through a phosphorus-free folded filter (filter papers MN 280 ¼ 125mm, Macherey-Nagel, Düren, Germany). Phosphorus concentrations of the filtrates were analysed by ICP OES (iCAP 6300 Series, Thermo Fischer Scientific, Dreieich, Germany).

Determination of soil pH

Soil pH was determined at a depth of 0.01m. Soil was mixed with deionized water (1:4) and used for pH measurements.

Data analysis

The samples of each subplot (3 per plot) were analyzed individually. In rare cases (4 of 96 only 1 or 2 samples per subplot) were available. All data were included. Means per subplot were calculated (supplement Table S1) and used as input parameters to construct the data matrices for principle component analysis (PCA) and non-metric multidimensional scaling (NMDS, similarity measure: Gover). Multivariate analyses were conducted with the PAST free software package 2.17c (http://folk.uio.no/ohammer/past/, Hammer et al., 2001). The data were subjected to test the requirement of normal distribution by the Shapiro Wilks test ($P \geq 0.05$). When the P value of the Shapiro Wilks test was < 0.05, data were ln- or (-1/square-root)-transformed to achieve normal

distribution. In one case (ectomycorrhizal colonization), it was not possible to satisfy this criterion. The data were nevertheless included, but their in- or exclusion did not affect the final result. Because the data had different units and were subjected to different transformation procedures, the resulting matrix was z-score normalized and then used for the analyses. Because of the use of normalized data, the relative importance of individual factors was not considered, but their correlation coefficient R^2 with the PCs. To link root community functional parameters with environmental properties, general linear models (GLM) were tested with the first NMDS coordinate as dependent variable and soil and litter properties as independent variables. ANOVA were conducted using the plots (one-way ANOVA) or landscape and forest type (two-way ANOVA) as factors. When the ANOVA indicated significant differences among the means with $P < 0.05$, a post-hoc test (Tukey HSD) was conducted.

Data deposition and availability

The raw data of this study are deposited and available in the Dryad repository under

doi:10.5061/dryad.qf362

Results

Carbon and nitrogen in fine roots, litter and soil differ among forest types

To test our underlying assumption that forest transformation leads to changes in ecologically relevant biotic and abiotic properties, we measured the concentrations of carbon and nitrogen in fine roots, litter and soil in four forest types in two landscapes on Sumatra (Figure 2). The carbon concentrations of fine roots were lower in oil palm and rubber plantations than in rain forest or jungle rubber (Fig. 2A). Less carbon was also present in litter in oil palm plantations than in that of other (agro)-forest types (Fig. 2B). Total soil carbon was lower in oil palm and in rubber plantations than in jungle rubber (Fig. 2C). In Harpan, the carbon content in the rain forest soil was higher than in the agro-systems, while in Bukit Duabelas no difference to those systems was found (Fig. 2C). The carbon content in the standing fine root biomass was low compared with total soil carbon (Fig. 2C, grey stacked bars).

The fine root nitrogen concentration in oil palm plantations was almost twice lower than in fine roots of other forest types (Fig. 2D). Nitrogen in litter showed no clear change with forest type (Fig. 2E). Nitrogen in soil was unaffected by forest type within each landscape with the exception of jungle rubber in Bukit Duabelas, where nitrogen was enriched compared with the other forests (Fig. 2 C,F). The nitrogen content in the standing fine

root biomass was low compared with total soil nitrogen (Fig. 2C, grey stacked bars).

Both landscapes differed in total carbon and nitrogen contents of the soil, with higher values in Bukit Duabelas than in Harapan (28 and 19 g carbon kg^{-1} $soil_{dw}$, $F_{1,16} = 23.1$, $P < 0.001$, 2.4 and 1.5 g nitrogen kg^{-1} $soil_{dw}$, $F_{1,16} = 37.6$, $P < 0.001$).

Root community functional parameters reflect forest type

We determined chemical traits (C, N, base cations (= sum of Mg, Ca and K), Mn, Na, Fe, Al, P, S) and performance traits (fine root mass, colonization by ectomycorrhizal and AM fungi, AM vesicles, AM arbuscles, AM spores in soil, dead root tips) of the roots at the plot level (supplement Table S1). On all plots the roots were a mixture of the vegetation present. Thus, our measurements represent RCFPs, but in oil palm and rubber plantations the roots were mainly from the crop trees because weeds or other vegetation were sparse and therefore also represent traits according their original definition (Violle et al. 2007). To find out whether RCFPs varied with forest types or landscapes a PCA was conducted (Fig. 3). Broken stick analysis indicated that only the first two PCs, which explained together 56.9% of the variance, were significant (not shown). PC1 (35.1%) separated the forest types along a gradient with the rain forests exhibiting the most positive and oil palm plantations the most negative scores (one-

way ANOVA on PC1 scores of plots, $F_{7,24} = 46.7$, $P < 0.001$). Positive PC1 loadings with correlations of $R \geq 0.5$ were C, N, S, base cations, Mn, and fine root mass (Table 2). Negative PC1 loadings with $R \leq -0.5$ were AM spores, dead root tips, Al and Fe (Table 2). The plot means of different forest types showed highest values for AM spores, dead root tips, Al and Fe in roots of oil palm plantations, and highest values for root mass and root nutrient concentrations for rain forest (Table 3, Fig. 2). RCFPs related to mycorrhizal colonization or function (arbuscules and vesicles) and to phosphorus were not strongly correlated with PC1 (Fig. 3, Table 2).

PC2 (21.8%) separated the two landscapes for oil palms and rain forests ($F_{7,24} = 3.6$, $P < 0.01$, Fig. 3), but any kind of rubber cultivation showed strong overlap with both oil palm and rain forest. Therefore, the loadings on PC2 (P concentrations, AM colonization, AM vesicles) were not useful to distinguish forest types (Fig. 3, Table 2).

The low significance of the mycorrhizal colonization-related traits was unexpected. However, mean AM colonization across all forest types was relatively stable 74.4 ± 1.7% with the exception of the oil plantations in Harapan (51.8 ± 7.5%) (Supplement Table 1). EM colonization was detected in some plots in Harapan rain forest (1.2% of the root tips with the maximum of 6% in one plot) and in rubber jungle systems in both landscapes (0.9 and 1.7% of the root tips in Bukit Dualbelas and Harapan, respectively), but the overall abundance was rare (Supplement Table 1).

Root community functional parameters are linked with ecosystem properties

We used eight environmental properties (the sum of base cations in soil, available phosphorus, soil pH and soil water content, soil carbon, soil nitrogen, litter carbon, litter nitrogen) as a proxy for ecosystem function and explored their response to forest transformation. We used the RCFPs that were informative for forest transformation (RCFPs with -0.5 > R > 0.5 on PC1, Table 2, Table 3) to conduct an NMDS and plotted the environmental variables as explanatory vectors (Fig. 4). As expected, the NMDS scores showed a separation of the forest types similar to that of the PCA (Fig. 3), but more succinct because of the exclusion of uninformative traits (Fig. 4). The separation of the forest types and, thus, land transformation systems was significant for the NMDS scores of coordinate 1 (Table 4). The environmental variables (which are not part of the calculation of the NMDS scores) indicated that soil pH was related to the negative scores of oil palm and rubber plantations, while litter and soil carbon and nitrogen were related to the positive scores of rain forest and jungle rubber (Fig. 4). It should be noted that the pH of forest plots (4.25 ± 0.03) was lower (P = 0.002) than that of the other plots (oil palm/rubber/jungle rubber 4.50 ± 0.05 / 4.47± 0.04 / 4.40 ± 0.04). Overall, the pH differences between the plots were small. Low scores on coordinate 1 were obtained for available phosphorus (scores on coordinate 1/coordinate 2: 0.06/-0.36), base cations in soil (0.12/-0.42)

and soil water content (0.21/-0.35) suggesting that these variables were not linked with the scores for forest types.

To test whether coordinate 1 scores represent transformation indices that can be quantitatively related to ecosystem functions, we conducted GLM analyses. Coordinate 1 scores were used as dependent variable and the eight environmental properties independent variables, allowing models with up to five environmental variables. This resulted in 219 models among which a model with three environmental properties exhibited the lowest AIC value (-4.63). The significant components in this model were soil nitrogen concentration, soil pH and litter carbon concentration. The model explained 67% (R^2 adjusted for d.f.) of the variation (Table 5). The *P*-value of the Durbin-Watson statistic was > 0.05 and therefore the model was not significantly affected by serial autocorrelation in the residuals.

Discussion

Root community functional parameters and soil properties vary with forest transformation

Recent studies highlight the importance of functional structures of communities rather than their biodiversity for ecosystem functioning (Moulliot et al. 2011, Katabuchi et al. 2012, Finegan et al. 2014). Our study clearly demonstrates a decline of positive RCFPs such as high root mass and high nutrient concentrations in mono-culture oil palm plantations compared with rain forest. Based on our design we cannot distinguish

whether the enhanced properties of the root communities in the rain forest were the result of tree phylogenic diversity or of trait-enrichment due to the presence of distinct forest tree species. We expected that the impact of dominant trees might have been traced by an effect of the associated EM on RCFPs, because the root nutrient status of forest trees is affected by symbioses with AM or EM fungi and fungal species identities (Lang and Polle 2011, Seven and Polle 2014, Pena and Polle 2014). However, our data did not reveal on influence of the mycorrhizal life traits on RCFPs. In contrast to the relatively stable AM colonization, AM spore abundance varied strongly with transformation system. Fungi are propagated by spores, but spores are also resting structures, by which the fungi survive unfavorable conditions (Wyatt et al. 2013). In tropical systems increased spore abundance correlated with decreased soil fertility (Lovelock et al. 2003). The increased AM spore abundance in oil palm and rubber monocultures, thus, points to a negative influence of these agricultural systems on ecologically important life traits.

A negative impact of monoculture oil palms was also evident on ecosystem properties such as soil carbon and nitrogen contents. Conversion of tropical forests into agricultural production systems has often been shown to result in decreased soil carbon and nitrogen pools (van Noordwick et al. 1997, Murty et al. 2002, Schroth et al. 2002, Smiley and Kroschel 2008, Leuschner et al. 2013). The magnitude of this effect in our study was similar to that in other tropical transformation system, e.g. in

cash crops such as maize on Central Sulawesi (Indonesia) (Dechert et al. 2004). In comparison with agricultural land use, agro-forestry systems recovered soil fertility (Dechert et al. 2004). Such a beneficial effect was also confirmed in our study for jungle rubber because the carbon and nitrogen concentrations in soil of this agro-forest system were even higher or, at least, as high as in rain forest soil. This finding is important because soil fertility has direct consequences for ecosystem services such biomass production, carbon cycling and carbon sequestration and has been identified as the major regulator of forest carbon balance (Fernandéz-Martínez et al. 2014).

The transformation index of root community traits is linked with ecosystem properties

Soil properties and vegetation mutually influence each other because both compartments are connected by matter flux. Alterations in plants traits are transmitted to the soil by the input of degrading leaf and root litter as well as by root physiological activities (exudation of carbohydrates, organic acids, nutrient uptake) (Mellilo et al. 1989, Prescott 2010). Therefore, RCFPs and soil properties are to some extent inter-dependent. Our study provides some insights into the nature of the links between soil properties and RCFPs for tropical forest transformation: (i) The ordination scores of RCFPs varied with the extent of forest transformation in the order rain forest > jungle rubber > rubber plantation > oil palm plantation. This finding

suggests that multiple RCFPs rather than single parameters were useful indicators of forest transformation and aggregate the functional and metabolic trait diversity of the community of different species. (ii) The scores can be regarded as transformation indices because they were quantitatively correlated with functional ecosystem properties, in particular with soil pH, litter carbon and soil nitrogen concentrations. The latter two parameters are especially interesting because litter carbon is the result of litter degradability, which in turn is driven by plant functional traits (Cornwell et al. 2008); soil nitrogen is important for soil fertility and forest productivity and therefore, eventually has strong impact on forest carbon cycling (Fernandéz-Martínez et al. 2014). Our findings, thus, link functional structures of root communities with ecosystem functions, notably those functions that are more important for carbon sequestration than climate or the rising atmospheric CO_2 concentration (Cornwell et al. 2008, Fernandéz-Martínez et al. 2014). This finding implies that RFCPs could be an important indicator for the functionality of above- and below-ground ecosystem interactions. Based on the present data, the cause-effect relationships remains unknown because mono-culture species with unfavorable root traits could affect soil properties or management could alter soil properties with negative consequences for root traits. Regardless the ultimate reason, our results suggest that the loss in ecosystem functionality in mono-cultures was related to complex and not to single-factor alterations of the root functional structures. One may have expected that negative transformation indices were driven by diminished nutrient

concentrations (carbon, nitrogen, cations) and low root mass in monocultures, but here we demonstrated concomitant increases in potentially phytotoxic metals (Al, Fe) and root tip mortality. The transformation indices were, thus, determined by contrasting behavior of RCFPs and not by the loss of traits abundance *per se.* Consequently, we may expect that any measure that improves root vitality may, eventually, enhance the ecological functions of tropical production systems. It will be important to investigate this suggestion in future studies

Degradation of root health is related to accumulation of plant toxic elements

Chemical root traits that distinguished the monocultures, especially the oil palms, from ecosystems with higher tree diversity were the enrichments in Fe and Al. Excess Al accumulation is known to limit plant performance and affects root growth (Delhaize and Ryan 1995, Kochian et al. 2005, Horst et al. 2010). In fact, the morphological appearance of the oil palm roots on our plots resembled the symptoms of Al toxicity such as stubby root systems lacking fine root branches with many brownish, distorted root tips (Rout et al. 2001). Plant availability of Al is modulated by soil acidity (Brunner and Sperisen 2013). The soils in the Jambi lowland region are oxisols and ultisols (Tata et al. 2008), i.e., acid soil classes that are commonly used for oil palm cultivation (Corley and Tinker 2003). In oil palm plantations a negative correlation between exchangeable Al in soil and root density was found (Cristancho et al. 2007). Controlled studies

confirmed the negative impact of Al on oil palm roots, especially on the length of the lateral roots and number of root tips (Cristancho et al. 2011). Cristancho et al. (2011) further showed that Al-stressed oil palms excreted significant concentrations of oxalic acids. Plant exudation of organic acids influences the availability of other soil elements and mobilizes for example phosphorus and Fe (Ma et al. 2001, Jones 1998). Here, we found high Fe concentrations in roots, whereas soil phosphorus availability was low and root phosphorus concentrations unaffected by the forest type. Excess Fe causes oxidative stress leading to cell destruction (Jones 1998) and may have caused here together with Al enhanced root mortality. It is important to note that the pH across all forest types was low, but not lower in plantations than in forest soil. Therefore, low pH may be pre-requisite, but was not the immediate reason for the observed decline in root health.

Currently, we can only speculate about the reason for root distortion in oil palm plantations. One possibility is that mono-cultures alter the soil microbial flora with negative effects on Al or Fe solubilization and plant availability as found in other countries (Fankem et al. 2006). AM colonization protects plant roots from Al stress (Seguel et al. 2013), but here variation in AM abundance was unrelated to Al concentrations. Phylogenetic analyses have shown high Al tolerance in tropical forest trees (Masunaga et al. 1998, Nguyen et al. 2003, Jansen et al. 2004, Ryan and Delhaize 2010). Therefore, it is also possible that introduced crop trees are not well-adapted to the prevalent soil conditions and accumulate

phytotoxic concentrations of Al and Fe over the years. As a consequence, roots will be damaged, soil exploration will decline and root litter input decrease, thereby, eventually leading to alterations in soil properties. To disentangle the underlying mechanisms, experimental studies with mixtures of oil palm, rubber and native forest species are necessary. Thereby, feed-back effects between ecosystem functions and functional traits of distinct tree species and their communities can be uncovered and used to develop improved management strategies.

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References

Allen AS, Andrews JA, Finzi AC, Matamala R, Richter DD, Schlesinger WH (2000): Effects of free-air CO_2 enrichment (FACE) on belowground processes in a *Pinus taeda* forest. Mycorrhiza 11: 107 – 114

Barnes AD, Jochum M, Mumme S, Haneda NF, Farajallah A Farajallah A, Widarto TH, Brose U (2014) Consequences of tropical land use for multitrophic biodiversity and ecosystem functioning. Nat Comms 5:5351

Bray RH, Kurtz LT (1945): Determination of total, organic and available forms of phosphorus in soils. Soil Science 59: 39 – 45

Brunner I and Sperisen C (2013) Aluminum exclusion and aluminum tolerance in woody plants. *Front. Plant Sci.* 4:172. doi: 10.3389/fpls.2013.00172

Carlson KM, Curran LM, Ratnasari D, Pittman AM, Soares-Filho BS, Asner GP, Trigg SN, Gaveau DA, Lawrence D, Rodrigues HO (2012) Committed carbon emissions, deforestation, and community land conversion from oil palm plantation expansion in West Kalimantan, Indonesia. Proc Natl Acad Sci USA 109: 7559–7564.

Carrasco LR, Larrosa C, Milner-Gulland EJ , Edwards DP (2014) A double-edged sword for tropical forests. Science 346: 38-40

Clemmensen, K.E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., Stenlid, J., Finlay, R.D., Wardle, D. a. & Lindahl, B.D. (2013) Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science*, 339, 1615–1618.

Corley R.H., Tinker P.B. (2003) The oil palm. Blackwell Science, Oxford, UK

Cornwell WK, Cornelissen JHC, Amatangelo K, Dorrepaal E, Eviner VT ., Godoy, O., Hobbie, S. E., Hoorens, B., Kurokawa, H., Pérez-Harguindeguy, N., Quested, H. M., Santiago, L. S., Wardle, D. A., Wright, I. J., Aerts, R., Allison, S. D., Van Bodegom, P., Brovkin, V., Chatain, A., Callaghan, T. V., Díaz, S., Garnier, E., Gurvich, D. E., Kazakou, E., Klein, J. A., Read, J., Reich, P. B., Soudzilovskaia, N. A., Vaieretti, M. V. and Westoby, M (2008) Plant species traits are the predominant control on litter

decomposition rates within biomes worldwide. Ecology Letters 11: 1065–1071.

Cristancho R.J.A., Munevar M.F., Acosta G.A., Santacruz A.L., Torres V.M. (2007) Relationship between soil characteristics and the distribution of mature oil palm (Elaeis guineensis Jacq) root system. Palmas, 28, 24–30

Cristancho RJA, Hanafi MM, Syed Omar SR, Rafii MY (2011) Variations in oil palm (Elaeis guineensis Jacq.) progeny response to high aluminium concentrations in solution culture. Plant Biology 13 (2011) 333–342

Dechert G, Veldkamp E, Anas I (2004) Is soil degradation unrelated to deforestation? Examining soil parameters of land use systems in upland Central Sulawesi, Indonesia. *Plant and Soil* 265: 197–209.

Delhaize E., Ryan P.R. (1995) Aluminum toxicity and tolerance in plants. Plant Physiology, 107, 315–321

Erasmi S, Ardiansyah M, Propastin P, Huete A (2010) Spatiotemporal trends of forest cover change in Southeast Asia. In: Tscharntke T, Leuschner C, Veldkamp E, Faust H, Guhardja E, Bidin A (eds) Tropical rainforests and agroforests under global change. Ecological and socio-economic valuations. Springer, Berlin, pp 269–291.

Fankem H, Nwaga D, Deubel A, Dieng L, Merbach W and Etoa FX 2006 Occurrence and functioning of phosphate solubilizing microorganisms from oil palm tree (*Elaeis guineensis*) rhizosphere in Cameroon. African Journal of Biotechnology Vol. 5 (24), pp. 2450-2460

FAO (2010) Global Forest Resources Assessment 2010. FAO Forestry Paper No. 163. Food and Agriculture Organization of the United Nations, Rome

Fernandez-Martinez, M; Vicca, S; Janssens, IA; Sardans, J ; Luyssaert, S ; Campioli, M; Chapin, FS; Ciais, P; Malhi, Y; Obersteiner, M et al. 2014 Nutrient availability as the key regulator of global forest carbon balance NATURE CLIMATE CHANGE, 4, 6: 471-476

Finegan, B., Peña-Claros, M., de Oliveira, A., Ascarrunz, N., Bret-Harte, M. S., Carreño-Rocabado, G., Casanoves, F., Díaz, S., Eguiguren Velepucha, P., Fernandez, F., Licona, J. C., Lorenzo, L., Salgado Negret, B., Vaz, M., Poorter, L. (2015), Does functional trait diversity predict above-ground biomass and productivity of tropical forests? Testing three alternative

hypotheses. Journal of Ecology, 103: 191–201. doi: 10.1111/1365-2745.12346

Fornara, D.A, Tilman, D. & Hobbie, S.E. (2009) Linkages between plant functional composition, fine root processes and potential soil N mineralization rates. *Journal of Ecology*, 97, 48–56.

Garnier E, Navas ML. 2012 A trait-based approach to comparative functional plant ecology: concepts, methods and applications for agroecology. A review. Agronomy for Sustainable Development 32 (2): 365-399.

Gerdemann JW, Nicolson TH (1963):Spores of mycorrhizal Endogonespecies extracted from soil by wet sieving and decanting. Transaction of the British Mycological Society 46 :235 – 244

Godbold DL, Hoosbeek MR, Lukac M, Cotrufo MF, Janssens IA, Ceulemans R, Polle A, Velthorst EJ, Scarascia-Mugnozza G, DeAngelis P, Miglietta F, Peressotti F (2006) Mycorrhizal hyphal turnover as a dominant process for carbon input into soil organic matter. Plant and Soil 281: 15-24

Gouyon A, H de Foresta, P Levang 1993 Does 'jungle rubber'deserve its name? An analysis of rubber agroforestry systems in southeast Sumatra. Agroforestry systems, 1993 22: 181-205.

Habib MT, Heller T, Polle A (2013) Molecular physiology of tree ectomycorrhizal interactions. In: Plant roots: the hidden half. (eds. A. Eshel, T. Beeckman), CRC Press, Boca Raton, 39-1 – 39-21

Hammer, O., Harper, D. A. T., and Ryan, P. D. (2001). PAST: palaeontological statistics software package for education and data analysis. *Palaeontol Electron* 4, 9–17.

Hansen MC, Stehman SV, Potapov PV, Loveland TR, Townshend JRG, DeFries RS, Pittman KW, Arunarwati B, Stolle F, Steininger MK, Carroll M, DiMiceli C (2008) Humid tropical forest clearing from 2000 to 2005 quantified by using multitemporal and multiresolution remotely sensed data. Proc Nat Acad Sci USA 105:9439–9444

Heinrichs H, Brumsack HJ, Loftfield N, König N (1986): Verbessertes Druckaufschlussystem für biologische und anorganische Materialien. Zeitschrift für Pflanzenernährung und Bodenkunde 149: 350 – 353

Horst, W. J., Wang, Y., and Eticha, D. (2010). The role of the root apoplast in aluminium-induced inhibition of root elongation and in aluminium resistance of plants: a review. *Ann. Bot.* 106, 185–197

Jansen S, Watanabe T, Dessein S, Robbrecht E, Smets E (2004) The evolution of aluminum accumulation in angiosperms. In: Hemsley AR, Poole I, editors. The Evolution of plant physiology. Amsterdam: Elsevier Academic Press. pp. 467–479.

Jones, D L. Organic acids in the rhizosphere–a critical review. *Plant and soil*, 1998, 205. Jg., Nr. 1, S. 25-44

Katabuchi, M., Kurokawa, H., Davies, S. J., Tan, S. and Nakashizuka, T. (2012), Soil resource availability shapes community trait structure in a species-rich dipterocarp forest. Journal of Ecology, 100: 643–651. doi: 10.1111/j.1365-2745.2011.01937.x

Kochian, L. V., Pineros, M. A., and Hoekenga, O. A. (2005). The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. *Plant Soil* 274, 175–195. doi: 10.1007/s11104-004-1158-7

Lang C, Polle A (2011) Ectomycorrhizal fungal diversity, tree diversity and root nutrient relations in a mixed Central European forest. Tree Physiology 31: 531-538

Leuschner C, Moser G, Hertel D, Erasmi S, Leitner D, Culmsee H, Schuldt B, Schwendenmann L (2013) Conversion of tropical moist forest into cacao agroforest: consequences for carbon pools and annual C sequestration. Agroforest Syst (2013) 87:1173–1187

Lovelock, C.E., Andersen, K. & Morton, J.B. (2003) Influence of host tree species and environmental variables on arbuscular mycorrhizal communities in tropical forests. *Oecologia*, 135, 268–279

Ma, J. F., Ryan, P. R., and Delhaize, E. (2001). Aluminum tolerance in plants and the complexing role of organic acids. *Trends Plant Sci.* 6, 273–278. doi: 10.1016/S1360-1385(01)01961-6

Masunaga, T., Kubota, D., Hotta, M., and Wakatsuki, T. (1998). Mineral composition of leaves and bark in aluminium accumulators in a tropical rain forest in Indonesia. *Soil Sci. Plant Nutr.* 44, 347–358. doi:

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

Melillo JM, Aber JD, Linkins AE, Ricca A, Fry B, Nadelhoffer KJ (1989) Carbon and nitrogen dynamics along the decay continuum: Plant litter to soil organic matter. In: Ecology of Arable Land — Perspectives and Challenges. Developments in Plant and Soil Sciences 39, pp 53-62

Ministry of Agriculture (2010) Area and Production by Category of Producers: Oil Palm, 1967-2010 (Indonesian Ministry of Agriculture, Jakarta, Indonesia).

Mouillot D, Villéger S, Scherer-Lorenzen M, Mason NWH (2011) Functional Structure of Biological Communities Predicts Ecosystem Multifunctionality. PLoS ONE 6(3): e17476. doi:10.1371/journal.pone.001747

Murdiyarso D, M. Van Noordwijk, U. R. Wasrin, T. P. Tomich, and A. N. Gillison (2002) Environmental benefits and sustainable land-use options in the Jambi transect, Sumatra. Journal of Vegetation Science, 13(3):429-438.

Murty D, Kirschbaum MUF, McMurtie RE, McGilvray H (2002) Does conversion of forest to agricultural land change soil carbon and nitrogen? A review of the literature. Glob Change Biol 8:105–123

Nguyen, N. T., Nakabayashi, K., Thompson, J., and Fujita, K. (2003). Role of exudation of organic acids and phosphate in aluminum tolerance of four tropical woody species. *Tree Physiol.* 23, 1041–1050. doi: 10.1093/treephys/23.15.1041

Ohms, RE. (1957): A flotation method for collecting spores of a phycomycetous mycorrhizal parasite from soil. Phytophatology 47: 751 – 752

Orwin, K.H., Buckland, S.M., Johnson, D., Turner, B.L., Smart, S., Oakley, S. & Bardgett, R.D. (2010) Linkages of plant traits to soil properties and the functioning of temperate grassland. *Journal of Ecology*, 98, 1074–1083.

Pena R, Polle A (2014) Attributing functions to ectomycorrhizal fungal identities in assemblages for nitrogen acquisition under stress. The ISME Journal 8: 321-330

Prescott CE 2010 Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? Biogeochemistry Volume 101, Issue 1-3, pp 133-149

Prieto I, Roumet C, Cardinael R, Dupraz C, Jourdan C, Kim JH, Maeght JL, Mao Z, Pierret A, Portillo N, Roupsard O, Thammahacksa C and Stokes A (2015) Root community traits along a land use gradient: evidence of a community-level economics spectrum. Journal of Ecology, in the press, doi: 10.1111/1365-2745.12351

Rout G.R., Samantaray S., Das P. (2001) Aluminum toxicity in plants: a review. Agronomie, 21, 3–21.

Ryan, P. R., and Delhaize, E. (2010). The convergent evolution of aluminium resistance in plants exploits a convenient currency. *Funct. Plant Biol.* 37, 275–284. doi: 10.1071/FP09261

Schroth G, D'Angelo SA, Teixeira WG, Haag D, Lieberei R (2002) Conversion of secondary forest into agroforestry and monoculture plantations in Amazonia: consequences of biomass, litter and soil carbon stocks after 7 years. For Ecol Manage 163:131–150

Seguel, A; Cumming, JR.; Klugh-Stewart, K; Cornejo, P; Borie, F (2013) The role of arbuscular mycorrhizas in decreasing aluminium phytotoxicity in acidic soils: a review MYCORRHIZA 23: 167-183

Seven J, Polle A (2014) Subcellular nutrient element localization and enrichment in ecto- and arbuscular mycorrhizas of field-grown beech and ash trees indicate functional differences. PLoS ONE 9(12): e114672

Smiley GL, Kroschel J (2008) Temporal change in carbon stocks of cocoa-Gliricidia agroforests in Central Sulawesi, Indonesia. Agrofor Syst 73:219–231

Smith SE, Read D (2008) Mycorrhizal symbiosis, 3rd edn. Academic, London

Tata HL, Rasnovi S, van Noordwijk M and Werger MJA. 2008. Can rubber agroforests conserve biodiversity in Jambi (Sumatra)? *Proceedings of Indonesian Students' Scientific Meeting 2008, Delft, The Netherlands. h*ttps://portals.iucn.org/2012forum/sites/2012forum/files/can20rafconserve biodive-issmproceedings.pdf

van Noordwijk M, Cerri C, Woomer PL, Nugoho K, BernouxM (1997) Soil carbon dynamics in the humid tropical forest zone. Geoderma 79:187–225

Vierheilig H, Coughlan A, Wyss U, Piche Y (1998): Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Applied and Environmental Microbiology 64: 5004 – 5007

Violle, C., Navas, M.L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I. Garnier E (2007). Let the concept of trait be functional! Oikos, 116, 882–892

Wilcove DS, Giam X, Edwards DP, Fisher B, Koh I (2013) Navajot´s nightmare revisited: logging, agriculture and biodivity in Southeast Asia. Trends Ecol Evol 28: 531-540

Wyatt, Timon T.; Wosten, Han A. B.; Dijksterhuis, Jan 2013 Fungal Spores for Dispersion in Space and Time . Edited by: Sariaslani, S; Gadd, GM Advances In Applied Microbiology, Vol 85 Book Series: Advances in Applied Microbiology Volume: 85 Pages: 43-91 Published: 2013

Table 1: Geographic location of the research plots in two landscapes and four forest types on Sumatra (Indonesia). O = oil palm plantation, R = rubber plantation, J = jungle rubber, F = secondary rain forest.

	Bukit Dua Belas				Harapan		
plot	latitude	longitude	altitude (m asl)	plot	latitude	longitude	altitude (m asl)
BF1	S 01°59'42.5''	E 102°45'08.1''	83	HF1	S 02°09'09.9''	E 103°21'43.2''	76
BF2	S 01° 58'55.1''	E 102°45'02.7''	77	HF2	S 02°09'29.4''	E 103°20'01.5''	75
BF3	S 01°56’33.9''	E 102°34’52.7''	87	HF3	S 02°10'30.1''	E 103°19'57.8''	58
BF4	S 01°56’31.0''	E 102°34’50.3''	87	HF4	S 02°11'15.2''	E 103°20'33.4''	77
BJ1	S 02°08'25.6''	E 102°51'04.3''	74	HJ1	S 01°55'40.0''	E 103°15'33.8''	51
BJ2	S 02°01'49.7''	E 102°46'16.7''	76	HJ2	S 01°49'31.9''	E 103°17'39.2''	84
BJ3	S 02°03'46.7''	E 102°48'03.5''	89	HJ3	S 01°50'56.9''	E 103°17'59.9''	95
BJ4	S 02°00'57.3''	E 102°45'12.3''	60	HJ4	S 01°47'07.3''	E 103°16'36.9''	57
BR1	S 02°05'30.7''	E 102°48'30.7''	71	HR1	S 01°54'39.5''	E 103°16'00.1''	77
BR2	S 02°05'06.8''	E 102°47'20.7''	95	HR2	S 01°52'44.5''	E 103°16'28.4''	59
BR3	S 02°05'43.0''	E 102°46'59.6''	90	HR3	S 01°51'34.8''	E 103°18'02.1''	90
BR4	S 02°04'36.1''	E 102°46'22.3''	51	HR4	S 01°48'18.2''	E103°15'52.0''	71
BO1	S 02°04'26.1''	E 102°48'55.1''	75	HO1	S 01°54'35.6''	E 103°15'58.3''	81
BO2	S 02°04'32.0''	E 102°47'30.7''	84	HO2	S 01°53'00.7''	E 103°16'03.6''	55
BO3	S 02°04'15.2''	E 102°47'30.6''	71	HO3	S 01°51'28.4''	E 103°18'27.4''	64
BO4	S 02°03'01.5''	E 102°45'12.1''	34	HO4	S 01°47'12.7''	E 103°16'14.0''	48

Table 2. PCA loadings for correlations of root traits with PC1 and PC2

Trait name	Abbreviation	PC1	PC2
Sulfur	Sroot	0.8349	0.03783
Nitrogen	Nroot	0.8156	0.1825
Carbon	Croot	0.7011	-0.6564
Manganese	Mnroot	0.6661	0.5824
Fine root mass	FiRdw	0.6551	0.1073
Base cations	CatBroot	0.6232	0.5343
AM arbuscules	AMarb	0.4189	-0.1070
AM root colonization	AMR	0.3733	0.6448
Phosphorus	Proot	0.3470	0.5011
Ectomycorrhizal root tips	EMRT	0.3024	-0.3330
AM vesicles	AMves	0.0615	0.7670
Sodium	Naroot	-0.0807	-0.0838
Iron	Feroot	-0.5489	0.7291
Dead root tips	DeadR	-0.6545	-0.3144
Aluminium	Alroot	-0.7411	0.5369
AM spores in soil	AMspore	-0.8318	0.2911

Table 3: Means of root community functional parameters (RCFP ± SE) in four forest types (F = rain forest, J = jungle rubber, R = rubber plantation, O = oil palm plantation). Abbreviations for FCRF as in Table 2, *P* of one-way ANOVA with forest types as factor (n = 8). If not indicated otherwise data were expressed per gram of root dry mass.

RCFP	F	J	R	O	*P*
Sroot (mg g^{-1})	1.67 ±0.14	1.17± 0.07	1.12± 0.06	0.89± 0.06	< 0.001
Mnroot(mg g^{-1})	0.23 ±0.03	0.22± 0.05	0.18± 0.03	0.08± 0.01	0.019
FiRdw (g kg^{-1})[a]	3.31 ±0.32	3.16± 0.61	1.89± 0.15	1.72± 0.30	0.009
CatBroot (µmol g^{-1})	232 ± 31	272 ± 36	249 ± 25	136 ± 6	0.008
Feroot (mg g^{-1})	2.99 ±0.40	3.67± 0.48	7.54± 1.10	7.05± 0.87	< 0.001
DeadR (%)[b]	55.3 ±2.1 4	5.5± 3.8	48.7± 4.2 8	6.3± 0.7	< 0.001
Alroot (mg g^{-1})	5.12 ±0.48	7.11± 0.93	12.54± 1.23	14.90± 1.18	< 0.001
AMspore[c]	18.9 ±4.8 3	8.5± 2.5	77.0± 8.1	82.8± 5.8	< 0.001

[a] kg of dry soil,[b] % of all root tips,[c] number per 20 gram of air dried soil

Table 4: Mean NMDS scores of the forest types. Different letters in columns indicate significant differences with $P < 0.05$ (one way ANOVA, n = 4 per forest type). B = Bukit Duabelas, H = Harapan, O = oil palm, R = rubber plantation, J = jungle rubber, F = forest.

Forest type	Coordinate 1	Coordinate 2
BO	-0.226 a	0.000 abc
HO	-0.198 a	0.078 c
BR	-0.086 b	-0.097 a
HR	-0.002 c	-0.060 ab
HJ	0.060 d	0.047 bc
BJ	0.096 d	-0.029 abc
HF	0.168 e	0.082 c
BF	0.188 e	-0.022 abc

Table 5: Best general linear model (GLM) for the relationship of RCFP with ecosystem properties.

Analysis of Variance for RCFP

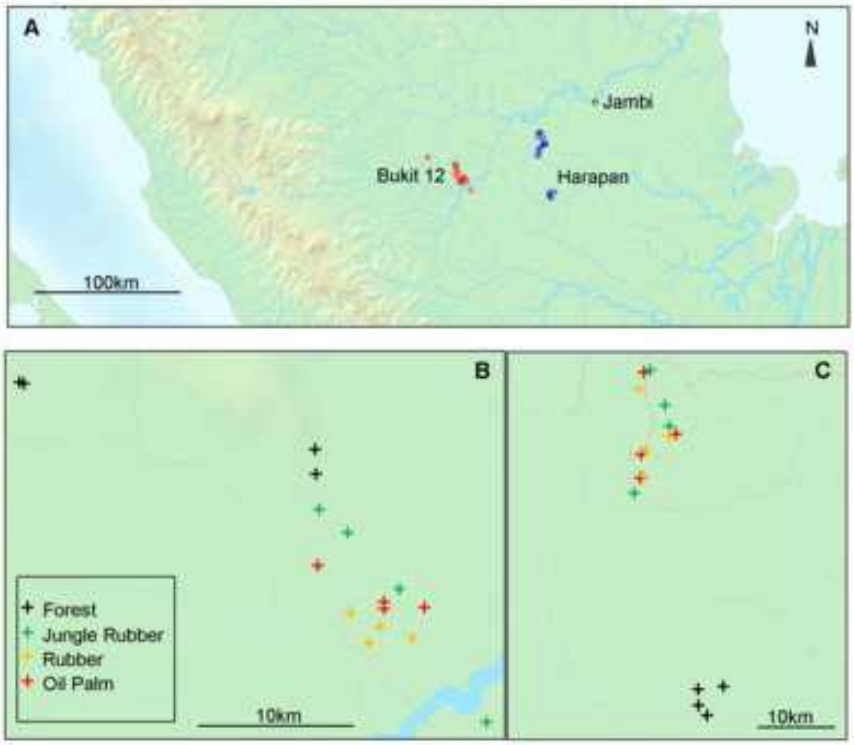
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	0.511	3	0.170	22.47	<0.0001
Residual	0.212	28	0.007		
Total (Corr.)	0.723		31		
Nsoil	0.062	1	0.063	8.31	0.0075
pH	0.102	1	0.103	13.56	0.0010
Clitter	0.249	1	0.249	32.88	<0.0001
Residual	0.212	28	0.007		
Total (corr.)	0.723	31			

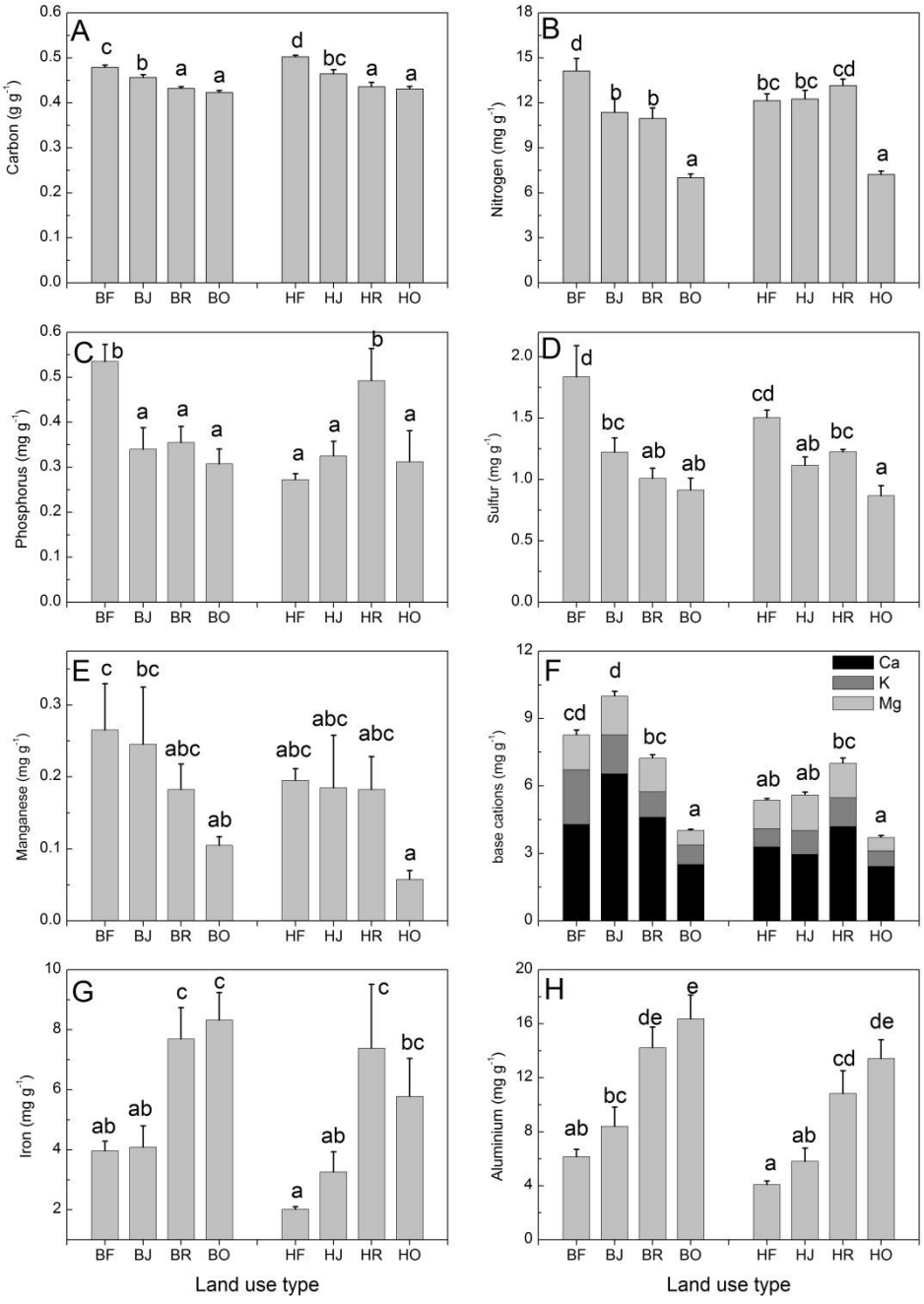
Figure 1. Maps of the landscapes Bukit Duablas (A) and Harapan (B) in the province Jambi on Sumatra (Indonesia). The locations of the research plots are indicated.

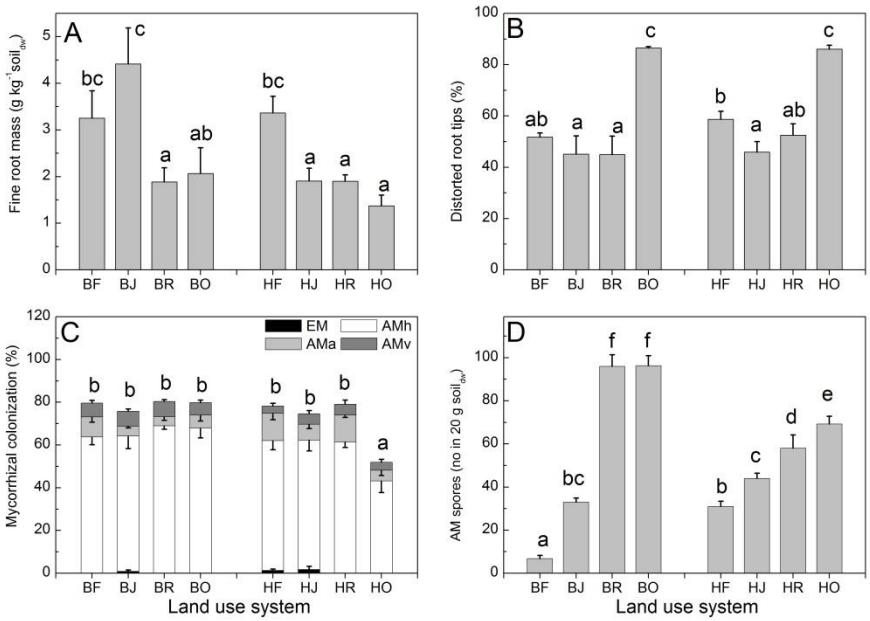
Figure 2. Carbon (A,B,C) and nitrogen concentrations (D,E,F) in roots, litter and soil in two landscapes and four forest types on Sumatra (Indonesia). To obtain the carbon or nitrogen content of fine roots, the root mass (g kg^{-1} soil) was multiplied with the fine root carbon or nitrogen concentration (g g^{-1} root mass). The carbon or nitrogen contents are shown by the grey stacked bars in panel C and F, respectively. Data indicate means (± SE). Different letters indicate significant differences at $P < 0.05$ (Tukey HSD test).). B = Bukit Duabelas, H = Harapan, O = oil palm, R = rubber plantation, J = jungle rubber, F = forest

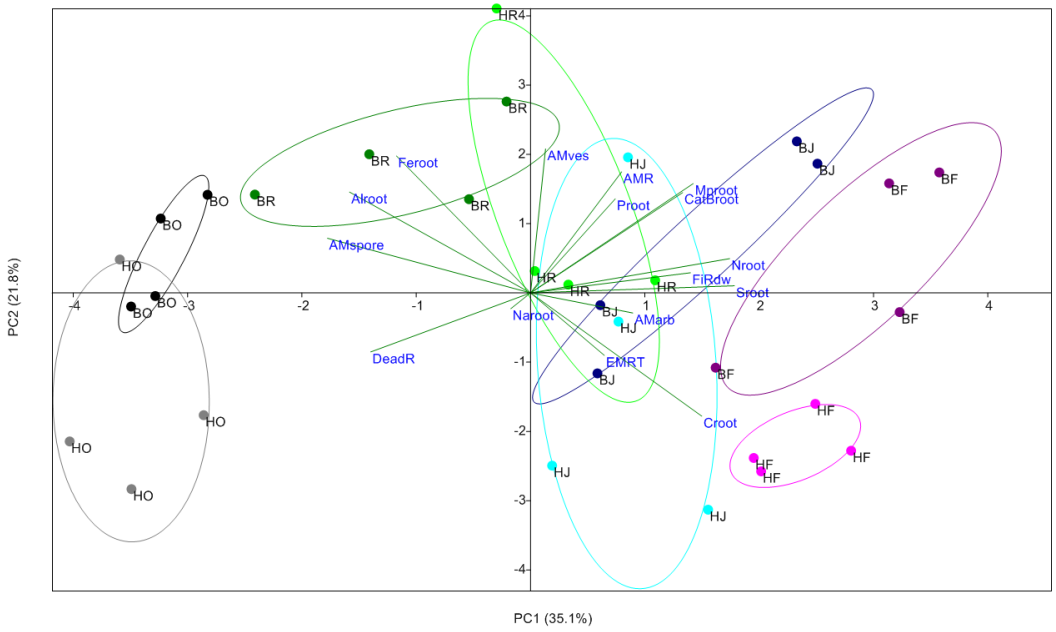
Figure 3. Principle component analysis of root community functional parameters. The parameters and their abbreviations are listed in Table 2. B = Bukit Duabelas, H = Harapan, O = oil palm, R = rubber plantation, J = jungle rubber, F = forest

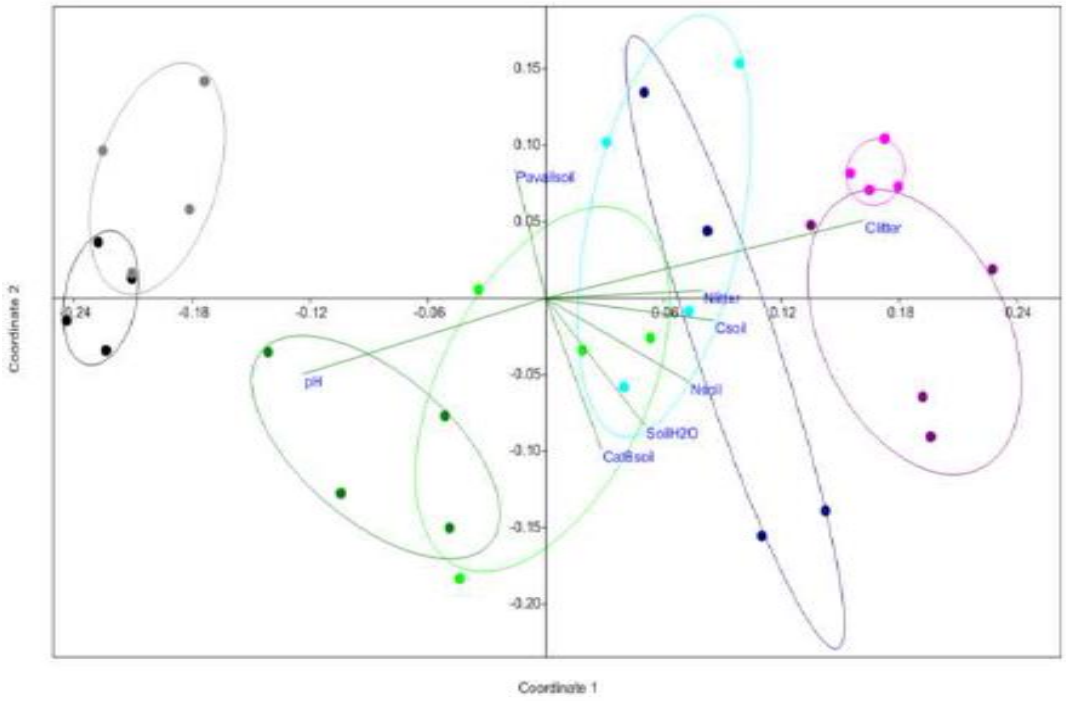
Figure 4. Non metric multidimensional scaling (NMDS) of root community functional parameters (RCFP). RCFP with R>0.5 and R< 0.5 from Table 2 were used for NMDS. The following environmental variables were plotted as explanatory variables: nitrogen and carbon concentrations in soil and litter (Nsoil, Csoil, Clitter, Nlitter), available phosphorus in soil (Pavailsoil), soil water content (H2Osoil) and soil pH (pHsoil). B = Bukit Duabelas, H = Harapan, O = oil palm, R = rubber plantation, J = jungle rubber, F = forest


A
N
Jambi
Bukit 12
Harapan
100km
B
C
Forest
Jungle Rubber
Rubber
Oil Palm
10km
10km


A
Carbon (g g-1)
B
Nitrogen (mg g-1)
C
Phosphorus (mg g-1)
D
Sulfur (mg g-1)
E
Manganese (mg g-1)
F
base cations (mg g-1)
Ca
K
Mg
G
Iron (mg g-1)
H
Aluminium (mg g-1)
BF BJ BR BO HF HJ HR HO
Land use type


A
Fine root mass (g kg-1 soildw)
bc
c
a
ab
bc
a
a
a
B
Distorted root tips (%)
ab
a
a
c
b
a
ab
c
C
Mycorrhizal colonization (%)
EM
AMh
AMa
AMv
b
b
b
b
b
b
b
a
D
AM spores (no in 20 g soildw)
a
bc
f
f
b
c
d
e
BF
BJ
BR
BO
HF
HJ
HR
HO
Land use system


HR4
BR
BR
Feroot
AMves
HJ
AMR
BJ
BJ
BF
BF
BO
BR
Aroot
BR
Mroot
CatBroot
Proot
BO
AMspore
HO
HR
HR
HR
Nroot
FiRdw
Sroot
BO
BO
-4
-3
-2
-1
1
2
3
4
Naroot
BJ
AMarb
HJ
BF
DeadR
EMRT
BJ
BF
HF
HO
Croot
HF
HF
HO
HJ
HO
HJ
3
2
1
-1
-2
-3
-4
PC2 (21.8%)
PC1 (35.1%)


0.15
0.10
Pavailsoil
0.05
Clitter
Nlitter
-0.24
-0.18
-0.12
-0.06
0.06
0.12
0.18
0.24
Csoil
pH
Nlitt
-0.05
SoilH2O
-0.10
CatBsoil
-0.15
-0.20
Coordinate 2
Coordinate 1

LIST OF PUBLICATIONS

Publication

Sahner J, Budi SW, Barus H**, Edy N**, Meyer M, Corré M, Polle A. 2015. Root community traits as indicator for transformation of tropical lowland rain forests into oil palm and rubber plantations. In Press: Plos One.

Poster

Sahner J, **Edy N**, Irawan B, Barus H, Budi SW, Tondok ET, Yelianti U, Polle A. 2015. Functional diversity of mycorrhizal fungi along a tropical land use gradient. Poster International Symposium – Final Workshop CRC 990, 23–24 March, 2015 in Göttingen Germany.

Yelianti U, **Edy N**, Sahner J, Irawan B, Barus H, Budi SW, Polle A. 2015 Long term organic nitrogen uptake in relation to mycorrhiza and forest type. Poster International Symposium – Final Workshop CRC 990, 23–24 March, 2015 in Göttingen Germany.

Declaration of originality and certificate of authorship

I, Nur Edy, hereby declare that I am the sole author of this dissertation entitled “community structure of arbuscular mycorrhizal fungi in temperate grassland and tropical land-use systems”. All references and data sources that were used in the dissertation have been appropriately acknowledged. I furthermore declare that this work has not been submitted elsewhere in any form as part of another dissertation procedure. I certify that the manuscripts presented in chapters 2 and 3 have been written by me as first author.

Göttingen, July 2015