Impact of Rain Forest Transformation on Roots and Functional Diversity of Root-Associated Fungal Communities

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AM	arbuscular mycorrhiza
AMF	arbuscular mycorrhizal fungi
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
EM	ectomycorrhiza
EMF	ectomycorrhizal fungi
GLM	generalized linear model
ITS	internal transcribed spacer
NGS	next generation sequencing
NMDS	non-metric multidimensional scaling
OTU	operational taxonomic unit
PC	principle component
PCA	principle component analysis
PCR	polymerase-chain-reaction
PERMANOVA	permutational multivariate analysis of variance
RCWT	root-community-weighed trait

Summary

Tropical rain forests are representing biodiversity hotspots, but their species richness is threatened by human driven land use changes. Between 1990 and 2015 most of the global deforestation of about 129 million hectares occurred in tropical regions, especially in south-east Asian countries. Among those countries, Indonesia reached the highest deforestation rate with a massive conversion of rain forests into agroforestry plantations of oil palm (*Elaies guineesis*) and rubber (*Hevea brasiliensis*) as major tree crops. The effects of rain forest transformation into tree-species poor systems are currently intensely being studied. The majority of research conducted in the tropical regions has focused on aboveground biodiversity in relation to ecosystem functioning, whereas the immense biodiversity found belowground and its impact on ecosystem functions and services such as tree health or carbon storage have rarely been addressed. Roots and root-associated fungi play an important role in this regard because they supply nutrients and water to the aboveground parts of the plant and anchor the trees in soil. The roots are characterized by different traits. One highly important trait is their fungal assemblage, which can influence root health and decrease productivity (pathogenic fungi) or enhance nutrient supply and increase productivity (mycorrhizal fungi). Furthermore, mycorrhizal fungi can protect their host plants against herbivores and pathogens and act as main pathway of carbon to the soil. The influence of land-use intensification in tropical ecosystems on root traits, fungal diversity and community structure is not well understood. The overarching goal of this thesis was to investigate the influence of tropical low land rain forest transformation into agricultural plantations on root community traits and root-associated fungal communities. The study was conducted in the Jambi Province on Sumatra Island, Indonesia. Sumatra has lost, on average, 550.000 hectares of forest per year over the last 30 years with the majority of land use changes occurring in the low land regions. The sampling sites were, therefore, chosen in two different low land landscapes, i.e. the Harapan and the Bukit Duabelas (Bukit12) landscapes. In each landscape, the sampling sites were located along a land use gradient representing unmanaged rain forests, less-managed jungle rubber agroforests, and intensely managed monoculture rubber and oil palm plantations.

This sampling design was used to investigate (i) root community traits such as colonization by mycorrhizal fungi and root vitality (performance traits) as well as nutrient concentrations (chemical traits). These traits can indicate the impact of land use change on root functions at the community level. (ii) Root-associated fungal communities in terms of diversity and

structural and functional composition. The root-associated fungal community compositions were analyzed by Illumina sequencing, which is a next generation sequencing technique that generates relatively short sequences. This technique has not often been applied for analyzing fungal communities. Therefore, a subset of the samples was additionally analyzed by 454 Pyrosequencing, which generates longer sequences and is the most common next generation sequencing technique applied in fungal research so far.

The present thesis is, therefore, organized in two main chapters in which ecological questions on root communities and root-associated fungal communities were addressed and one technical chapter (iii), in which the results on root-associated fungal communities obtained by Illumina sequencing and 454 Pyrosequencing were compared.

(i) Characterization of root community traits along a transformation gradient from low land rain forests into plantations with tree crops

We hypothesized that root community traits vary with land use system indicating increasing transformation intensity and loss of ecosystem functions.

In tropical rain forests most trees, including the introduced rubber trees and oil palms, are associated with arbuscular mycorrhiza (AM) fungi, but some tree species-rich families, e.g. Dipterocarpaceae, are associated with ectomycorrhiza (EM) fungi. 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. In species-rich tropical rain forests traits of distinct tree taxa are difficult to measure, but instead root traits can be gathered at a community level of the co-occurring species and can then be defined as "root community-weighed traits".

To analyze root community-weighed traits, mixed root samples were collected in different land use systems. The chemical traits (carbon, nitrogen, mineral nutrients, potentially toxic elements (aluminum, iron)), and the performance traits (root mass, vitality, mycorrhizal colonization) of root communities were analyzed. Chemical traits were analyzed by applying a combustion method using an organic element analyzer (carbon and nitrogen) and by inductively coupled plasma optical emission spectrometry (mineral nutrients and potentially toxic elements). Performance traits were analyzed by measuring root biomass, determining root vitality (counting of distorted and vital root tips), and colonization by EM and AM. The influence of land use on root community traits was tested by applying multivariate statistics. Variation of root community traits related to land systems were analyzed by principle

component analysis (PCA) and dissimilarities were visualized by non-metric multidimensional scaling (NMDS).

Roots of oil palm and rubber plantations showed a decrease in nutrient concentrations (carbon, nitrogen, sulfur, manganese, and base cations) compared with those from rain forests. However, the mycorrhizal colonization by AM fungi was stable across land use systems and EM colonization was rare and only found in rain forest and jungle rubber. Furthermore, a degradation of root health in monoculture plantations was evident which was related to an accumulation of plant toxic elements. Concentrations of aluminum and iron were higher in roots from oil palm plantations than those of rain forests, whereas the amount of distorted root tips was, on average, nearly doubled in oil palm plantations in comparison to the other systems. Additionally, root community traits were linked to important ecosystem properties (i.e. soil nitrogen concentrations, soil pH, and litter nitrogen concentrations).

These findings supported the initial hypothesis that root community traits declined with increasing transformation intensity. It was demonstrated that the degradation of root community traits was an indicator for tropical low land rain forest transformation into monoculture plantations. The study revealed a relationship between deteriorating root community traits and a loss of ecosystem functionality and showed that increasing transformation intensity resulted in decreasing root nutrition and health. These findings suggest that land management practices that improve root vitality may enhance the ecological functions of intense tropical production systems.

(ii) Characterization of richness, diversity, and community structure of root-associated fungal communities along a tropical land use gradient

We hypothesized that the fungal diversity of root-associated communities is higher in plant species-rich rain forests than in monoculture plantations because higher plant diversity creates more different habitats for root-colonizing fungi. Consequently, an impact of land use change on the community composition of root-associated fungi was expected. Based on the finding that the roots in oil palm plantations had a distorted appearance, a shift from beneficial functional fungal groups towards pathogens was expected in the highly managed systems compared to natural rain forests.

To examine the impact of rain forest transformation into rubber and oil palm plantations on root-associated fungal communities, mixed root samples were taken in the different land use systems. The composition of root-associated fungal communities was determined by Illumina

sequencing. Fungal operational taxonomic units (OTUs) were characterized by amplifying the internal transcribed spacer (ITS) region 1 of the environmental deoxyribonucleic acid (DNA) samples by using fungal-specific primers. The resulting fungal OTUs were assigned to functional groups: arbuscular mycorrhizal fungi, ectomycorrhizal fungi, plant pathogenic fungi, and saprotrophic fungi. In addition, land use intensity indices were calculated based on data for fertilizer, animal manure, and herbicide applications as well as by soil amendment by liming. Land use intensity indices, data on root community traits, and soil and litter properties were included as explanatory variables for analyses of the community structure. The impact of land use on the community composition was tested with permutational multivariate analysis of variance (PERMANOVA) using distance matrices. The influence of land use on richness and abundances of fungal OTUs was tested by applying generalized linear mixed effects models.
OTU richness and diversity of root-associated fungi did not support the hypothesis that transformation from tree species-rich forests into species-poor plantations led to species reduction. Fungal diversity in the plant species-rich rain forests was not higher than in monoculture plantations but the root-associated fungal community composition was clearly influenced by land use. The fungal communities in oil palm roots showed an increase in the abundance in Ascomycota and a decrease in Basidiomycota compared to those in rain forests. Glomeromycota, on the other hand, were most abundant in fungal communities of rain forests. These findings underpin the expectation that land use changes have massive impact on the fungal community structure in roots. The differences among root-associated fungal communities were mainly explained by chemical root community traits and land use intensity. The results obtained on relative abundances of different fungal functional groups showed an increase of plant pathogenic fungi and a decrease of beneficial EM and AM fungi in oil palm plantations compared to natural forests. This supported the hypothesis that a shift from beneficial toward pathogenic fungi in monoculture plantation compared to natural forests existed.
To conclude, it was demonstrated that rain forest transformation into highly managed plantations impacts the community composition but not the diversity of root-associated fungi. The alterations caused by land use changes led to an accumulation of pathogenic fungi in highly managed monoculture plantations and were mainly explained by land use intensification and root chemical traits. Based on these findings we speculate that land use management at a lower intensity and management practices, which improve root nutrition, may create environmental conditions favorable to beneficial mycorrhizal fungi and unfavorable for plant pathogenic fungi and, thereby, sustain productivity at lower environmental destruction.

(iii) Comparison of 454 Pyrosequencing and Illumina sequencing for root-associated fungal communities

The application of different next generation sequencing techniques may influence the result obtained for microbial communities because of methodology-dependent advantages and disadvantages, e.g., limitations of species annotation due to different sequence lengths obtained by different methods or different numbers of sequence reads that can be generated. To test whether Illumina sequencing and 454 Pyrosequencing methods yielded strongly diverging results or not, the same root samples were analyzed by both methods.

As expected root community samples analyzed by 454 Pyrosequencing recovered a lower sequence and fungal OTU richness than by Illumina sequencing. The taxonomic composition of root-associated fungal communities obtained by both techniques was similar regarding the relative abundance of Ascomycota present. The relative abundance of Basidiomycota was decreased and the one of unidentified fungi was increased in samples analyzed by Illumina sequencing. However, both techniques sampled the same fraction of diversity because the Shannon and Simpson indices for diversity showed no significant differences.

In conclusion, this comparison revealed that both applied next generation sequencing techniques provided comparable results in terms of the recovered diversity of root-associated fungal communities. This finding matters because it indicates that results from differing studies using either 454 Pyrosequencing or Illumina sequencing can be used to compare diversity indices but should be used with caution when comparing the taxonomic composition of samples.

In summary, this thesis demonstrated that the transformation of tropical low land rain forest into agricultural plantations affects root community traits and root-associated fungal communities. The degradation of root community traits can be considered as indicator for rain forest transformation into rubber and oil palm plantations. The diversity of root-associated fungi was not influenced by rain forest transformation. However, root-associated fungal community composition was impacted by land use changes. The dissimilarities of fungal communities were mainly explained by the degradation of chemical root community traits and the intensification of land management practices. The degradation of root traits and the increase of land use intensity led to an increase of pathogenic fungi and a decrease of mycorrhizal fungi in monoculture plantations compared to unmanaged rain forests.

CHAPTER ONE

1 General Introduction

1.1 Anthropogenic Land Use – a Driver for Global Change

Human activities have drastically changed land's surface, especially by forest conversion and habitat degradation (Foley et al., 2005; Newbold et al., 2015). Land use changes in terms of agricultural expansions and land use intensification leads, first of all, to habitat losses which are accompanied by the removal of functionally and structurally complex plant communities. The removal of plant communities also impacts all associated micro- and macro-organisms. These alterations and disturbances of biotic interactions are resulting in multiple ecosystem responses like changes in energy and nutrient fluxes as well as enhanced greenhouse gas emissions or soil degradation (Barnes et al., 2014; Carlson et al., 2012a; Dechert et al., 2004; Wilcove et al., 2013). The most massive agricultural land use changes are currently taking place in the tropical regions (Carrasco et al., 2014; Gibbs et al., 2010; Hansen et al., 2008). The World's growing human population and the related increasing demand for consumer goods will lead to a further agricultural expansion and land use intensification in tropical regions all over the world (Danielsen et al., 2009; Smit et al., 2013; Sodhi et al., 2010).

1.2 Deforestation in The Tropics

Tropical rain forests are representing biodiversity hotspots and their species richness is threatened by human driven land use changes (Ehrlich and Ehrlich, 2013; Hartshorn, 2013; Sodhi et al., 2004). The loss of biodiversity as a consequence of land use change has been shown in several studies (Drescher et al., 2016; Gardner et al., 2009; Gibson et al., 2011; Pimm et al., 2014; Sala, 2000). However, land use transformation is not always leading to a loss in biodiversity. For soil prokaryotes it has been shown that richness and diversity increased with increasing land use intensification (Schneider et al., 2015). Kerfahi et al. (2016) found that the diversity of soil fungi, nematodes, and bacteria was not decreased by forest conversation. The transformation of tropical rain forests into agricultural plantations is rapidly ongoing (Hansen et al., 2008). Lowland rain forests are particularly endangered for conversion and degradation since they are easily to access. In 2012, Indonesia reached the highest deforestation rate worldwide with a loss of 840.000 hectares of forests of which 51 % were categorized as lowland rain forest (Margono et al., 2014). Sumatra, Indonesia, is facing deforestation over decades

(Laumonier et al., 2010). In the past, deforestation was mainly driven by low land rain forest transformation to rubber agroforestry systems and rubber plantations while more recently oil palm plantations are the main driver for deforestation (Villamor et al., 2013). Sumatra has lost on average approximately 550.000 hectares of forest per year over the last 30 years with the majority located in the lowland regions (Laumonier et al., 2010) (Figure 1.2.1). The impact of agricultural expansion and intensification on biodiversity and the consequences on ecosystem functions and services need to be investigated to evaluate future trends for global change.

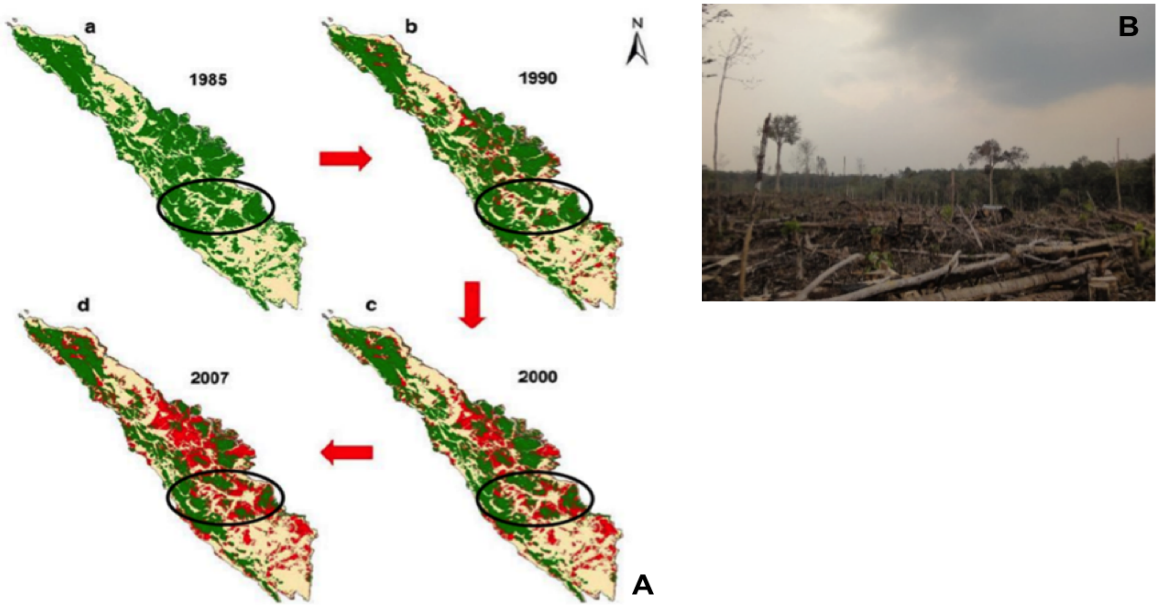

Figure 1.2.1: Changes in Land Coverage with Forest and Deforestation in Sumatra. A) Forest coverage in 1985 **(a)**, 1990 **(b)**, 2000 **(c)** and 2007 **(d)**. **B)** Deforestation in Jambi Province. Black circles are labelling Jambi Province where the research areas were located. Figure 1.2.1 A from Laumonier et al., 2010).

1.3 Rubber Trees and Oil Palms – Main Actors for Land Use Changes in Indonesia

Rubber (*Hevea brasiliensis*) trees (Figure 1.3.1) are native to Brazil and produce rubber which is used for the production of about 50000 different goods, e.g. tires of cars, bicycles and aircrafts (Priyadarshan, 2011). Rubber trees were introduced to Indonesia around 1910 and

farmers started to grow rubber trees within the natural forests resulting in low-input, complex agroforestry systems ("jungle rubber") (Gouyon et al., 1993). However, these agroforestry systems were replaced rapidly by rubber monoculture plantations due to the increasing demand for rubber related products as a consequence of the spectacular development of the automobile industry (Priyadarshan, 2011). World War II and its global consequences on economy interrupted the increase of rubber cultivation. By 1964, 75 % of the rubber market was made up from synthetic rubber, whose development already started during World War I (Priyadarshan, 2011). However, the market for natural rubber stabilized and today, depending on the kind of good, natural rubber has a market share of 50 – 100 % (Priyadarshan, 2011). Indonesia is the second largest rubber producer worldwide (Dove, 1993) and it is estimated that at least two million hectares are under rubber cultivation (Gouyon et al., 1993).

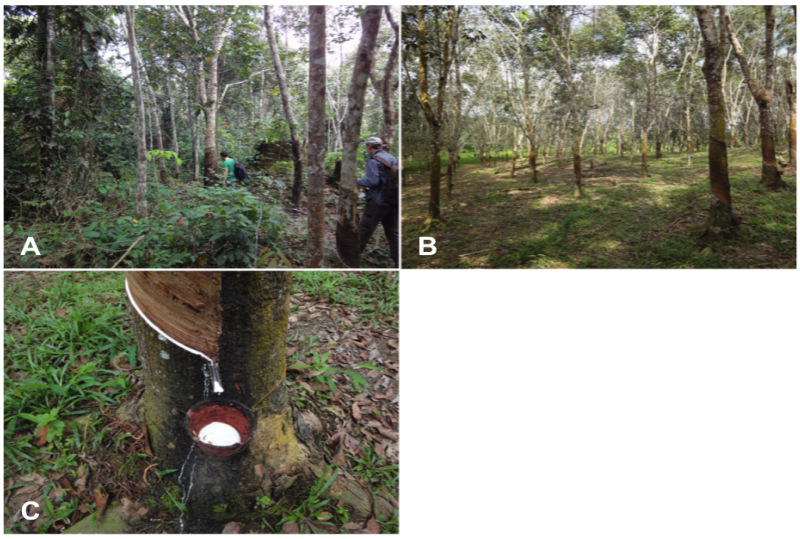

Figure 1.3.1: Extensive and Intensive Rubber Cultivation in Sumatra. A) extensive rubber plantation (jungle rubber) **B)** Rubber monoculture plantation **C)** Rubber extraction.

The oil palm (*Elaies guineesis*) (Figure 1.3.2) has an African origin. The fruits of oil palms are used for the production of oil. The oil yield per hectare from oil palms is the highest compared to all other oil crops (Corley and Tinker, 2015). Palm oil is used mainly as vegetable oil, in biofuel and in the food industry. Oil palms were introduced to Indonesia in 1848 not for commercial use but rather as exhibits in botanical gardens (Corley and Tinker, 2015). The first large oil palm plantation was cultivated in 1911, but the expansion of commercial cultivation

was interrupted by World War II and its consequences for the global economy (Corley and Tinker, 2015). After World War II the oil palm industry was growing slowly in Indonesia until the 1980's but then started to grow rapidly until today (Corley and Tinker, 2015). The oil palm industry isn now one of the world's most rapidly increasing industries of the agricultrual sector (Fitzherbert et al., 2008). The increasing demand for palm oil driven by the Earth's growing population for consumption needs will lead to a further expansion of oil palm plantations in Indonesia and tropical regions all over the world (Danielsen et al., 2009; Smit et al., 2013; Sodhi et al., 2010). In Indonesia, a further expansion of oil palm plantations is supported by the decision of the Indonesian government to double the oil palm production within the next ten years. This will lead to monoculture plantations dominating the landscapes in Indonesia in the future (Carlson et al., 2012 b).

Figure 1.3.2: Oil Palm Cultivation in Sumatra. A) oil palm monoculture plantation **B)** Harvested oil palm fruits **C)** Developing fruits in the leaf axis of an oil palm.

1.4 The Impact of Land Use Changes on Plant Diversity

Changes and losses in biodiversity can occur on the taxonomic, structural or functional level of a community (Duncan et al., 2015). Structural and functional alterations of communities are often having a greater importance for ecosystem functioning than the species richness of a community *per se* (Diaz et al., 2007; Duncan et al., 2015; Lavorel, 2013; Mokany et al., 2008). However, deforestation of tropical rain forests and related land conversions into

agricultural plantations has a major impact on all aspects of biodiversity mentioned. It was reported that tree diversity in a 0.52 km^2 rain forest plot can reach 1175 species in Borneo (Wright, 2002), whereas monoculture rubber and oil palm plantation are dominated by only one tree. And the total plant species richness in rain forests can be up to 6 times higher than in monoculture plantations compared to monoculture plantations (Drescher et al., 2016). These massive plant species are related to massive alterations of species interaction. The species pool present in an ecosystem forms the biotic fundament of the corresponding ecosystem and the complex interactions among its diverse members and the interdependencies of biotic and abiotic ecosystem properties are providing ecosystem functions and finally ecosystem services (Barnes et al., 2014; Drescher et al., 2016; Duncan et al., 2015).

1.5 Plants and their Associated Microorganisms

Plants build the stationary fundament of onshore biomes and are often the first group of organisms directly influenced by land use changes. All plants are associated with microorganisms and they contribute to the adaptation of plants to changing environmental conditions and play an important role for ecosystem functioning (Chen et al., 2014; Peršoh, 2015; Redman et al., 2011). Plants are associated to a broad variety of microorganisms and these associations are present in different parts and tissues of the plant (Quiza et al., 2015) (Figure 1.5.1). The associations between plants and microorganisms have different effects on the partners of the association and can range from mutualism over competition and antagonism (Figure 1.5.1). These differing effects are a result of complex interactions among the different players present in the community. For example, the plant health status can be negatively influenced by infections with pathogens whereas mycorrhizal fungi and beneficial microorganisms can protect their host against these pathogens (Datnoff et al., 1995; Duchesne et al., 1988; Smith and Read, 2008; Yamaji et al., 2005). The majority of research conducted in the tropical regions has focused on aboveground biodiversity in relation to ecosystem functioning whereas the immense biodiversity found belowground and its impact on ecosystem functions and services has rarely been addressed.

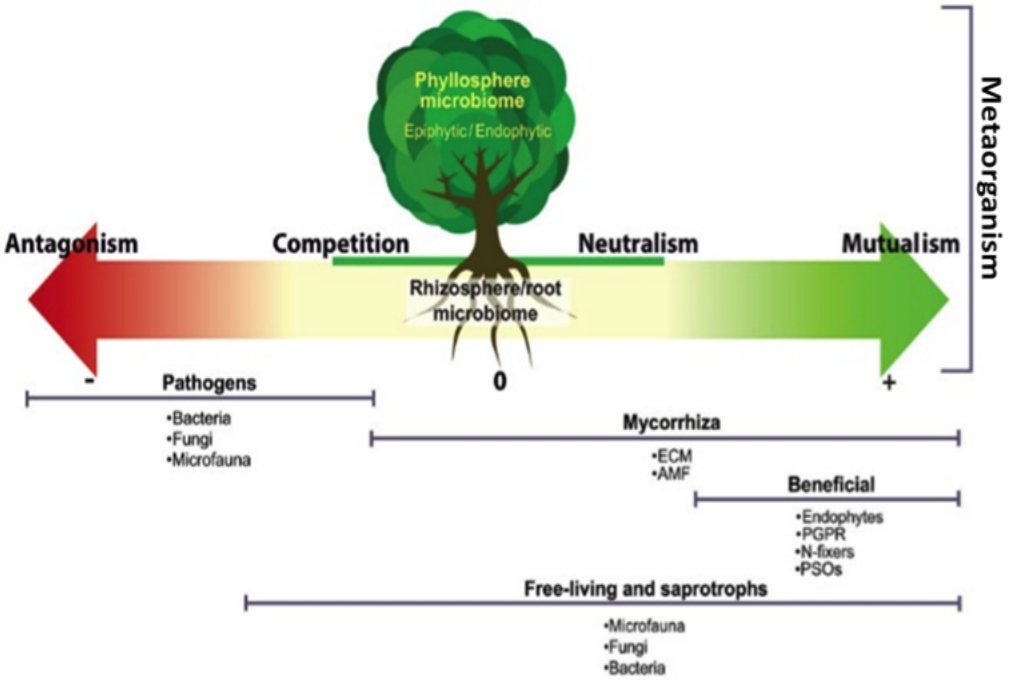

Figure 1.5.1: Plants and their Associated Microorganisms. Figure illustrates the interactions taking place within the plant-microbiome metaorganism. Many microorganisms are involved in these interactions. ECM = ectomycorrhizal, AMF = arbuscular mycorrhizal fungi, PGPR = plant growth promoting rhizobacteria, PSOs = phosphate-solubilizing organisms. Figure from Quiza et al., 2015.

1.6 Plant Root-Associated Fungal Communities

Fungi are a highly diverse group of microorganisms performing multiple ecological functions (Hawksworth, 1991; Peršoh, 2015). Fungi associated with plants can be categorized by their functional role (Figure 1.5.1). Of particular importance are some functional groups, because they control regulatory steps in ecosystems, namely: mutualistic fungi which are including mycorrhizal fungi, pathogenic fungi, and saprotrophic fungi. Only a few studies investigated belowground fungal diversity in tropical rain forests (Kerfahi et al., 2014, 2016; McGuire et al., 2011, 2015; Mueller et al., 2014; Peay et al., 2013; Toju et al., 2014) and with the exception of Toju et al. (2014) all have investigated soil and not root-associated fungal communities. The composition of root-associated fungal communities varies among ecosystems and on different

spatial and temporal scales (Peršoh, 2015; Tedersoo et al., 2014; Toju et al., 2014) and is in many cases related to the host identity and/or phylogenetic affiliation (Dighton and White, 2005; Lang et al., 2011; Smith and Read, 2008; Tedersoo et al., 2008).

1.6.1 Mycorrhizal Fungi

Mycorrhizal fungi from mutualistic interactions with plant roots and supply water and nutrients to their hosts, can protect their host against soil born plant pathogens, and act as main pathway for carbon to the soil (Datnoff et al., 1995; Filion et al., 1999; Hobbie, 2006; Verbruggen et al., 2016; Zhu, 2003). About 90 % of all land plants are forming a mycorrhizal association and the involved fungi are representing the best studied fungal functional group (Peršoh, 2015). The most common mycorrhizal types are the arbuscular mycorrhiza (AM) and the ectomycorrhiza (EM). 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 (Pena and Polle, 2014; Seven and Polle, 2014; Smith and Read, 2008). The large majority of plants in tropical forests are associated with arbuscular mycorrhizal fungi (AMF) but there are some tree species rich families like the Dipterocarpaceae which form ectomycorrhizal symbioses (Tedersoo et al., 2012; Toju et al., 2014). The non-native oil palms and rubber trees are associated with AMF (Bakhtiar et al., 2013; Herrmann et al., 2016; Phosri et al., 2010; Wastie, 1965).
The exchange of nutrients is bidirectional in the mutualistic associations. Mycorrhizal fungi are building hyphal networks to explore the soil and make nutrients available. The host plant receives nutrients via the mobilization and absorbance of nutrients by the fungal mycelia and the host plants supplies photosyntheticaly assimilated carbon to the fungi (Smith and Read, 2008). Estimates suggest thath up to 80 % of the plants phosphorus and nitrogen are acquired via mycorrhiza (van der Heijden et al., 2015) and host plants are allocating up to 20 % of their assimilated carbon to their fungal partners (Jakobsen and Rosendahl, 1990). Mycorrhizal fungi are important for carbon sequestration, because the turnover of mycorrhizal hyphae is a dominant process for carbon input into soil organic matter (Godbold et al., 2006). The sequestration of soil organic carbon is a key process to mitigate the effects of climate change and to conserve soil fertility (Lal, 2004) and converting rain forests into agricultural plantations does lead to losses in soil carbon contents (Don et al., 2011; Guillaume et al., 2015).

Mycorrhizal fungi can protect their host plants against pathogens through the competition for colonization space and the release of antibiotic compounds (Duchesne et al., 1988; Smith and Read, 2008; Yamaji et al., 2005). The mycorrhizal fungal communities are also influenced and can be altered by land use changes and management practices, e.g. through the removal of host plants, logging or fertilizer applications and (Huusko et al., 2015; McGuire et al., 2015; Morris et al., 2013; Oehl et al., 2003). How this in turn influence functioning of mycorrhizal communities in ecosystem processes is not well understood.

1.6.2 Plant Pathogenic Fungi

Plant pathogens fungi represent another important functional group as they influence plant health status and can cause diseases and pests (Li et al., 2014; Maron et al., 2011). The negative effects of plant pathogenic fungi can be species-specific, density-dependent or a combination of both (Bell et al., 2006; Klironomos, 2002; Maron et al., 2011; Van der Putten et al., 1993). Furthermore, land use intensification and consecutive mono-culturing of crops could be one reason for creating a micro-ecological environment promoting pathogens accumulation (Li et al., 2014). An example for cosmopolitan plant pathogens with high agricultural importance are fungi belonging to the genus *Fusarium* (Ma et al., 2013). *Fusarium* diseases include wilts, blights rots and cankers of many agricultural crops and in natural ecosystems (Datnoff et al., 1995; Flood, 2006; Ma et al., 2013). In oil palms and rubber trees *Fusarium* can cause leaf wilt and is thereby influencing health status which might result in reduce of yields (Flood, 2006; Liyanage and Dantanarayana, 1983).

1.6.3 Saprotrophic Fungi

Saprotrophic fungi are the dominate organisms for plant litter decomposition in many ecosystems (Baldrian and Valášková, 2008). They are also important for nutrient distribution in the soil as they are able to translocate carbon, nitrogen or phosphorus via their hyphal networks (Cairney, 2005). Saprotrophic fungi are considered to be the key regulators of soil carbon fluxes between the biosphere and atmosphere as they can contribute up to 90 % to the total heterotrophic respiration in woodland ecosystems and response to grazing by changes in

enzyme production (Crowther et al., 2012; Ingold and Hudson, 1993; Scheu, 1993).

1.7 Metagenomics and Functional Trait-Based Approaches to Investigate Hyperdivers Communities

In many cases, the composition of microbial communities and their link to ecosystem functioning remains a black box for scientists (Shade et al., 2009). Barcoding of DNA extracted from environmental samples (e.g. roots, soil, leaf litter) without prior culturing, defined as metagenomics, increased in order to classify biodiversity (e.g. Amend et al., 2010; Delmont et al., 2011; Luo et al., 2012; Peršoh, 2015; Tedersoo et al., 2014). Next generation sequencing techniques applied for metagenomics make it possible to simultaneously sequence billions of molecules in a nucleic acid extract (Buermans and den Dunnen, 2014). Many technical factors are influencing the results on the observed community composition (Bazzicalupo et al., 2013). One factor beside other is the applied next generation technique for metagenomics (Luo et al., 2012; Tedersoo et al., 2010). To evaluate and compare the effect of differing next generation sequencing techniques on results obtained on community analysis will be helpful to assess to what extent next generation sequencing techniques are comparable.

1.8 Scope of this Thesis

Anthropogenic land use changes have massive effects on biodiversity and related ecosystem functioning and provided ecosystem services. Roots and their associated fungal communities are important as they control regulatory steps in ecosystems. The overarching goal of this thesis was to investigate the influence on tropical low land rain forest transformation into monoculture rubber and oil palm plantations on root-associated fungal communities and root community traits. The aims and hypotheses (H) of this thesis were:

1. The characterization of root community traits in tropical rain forests and transformed land uses systems regarding chemical and performance traits.

2. The characterization of root-associated fungal communities in tropical rain forests and transformed land uses systems in terms of richness, diversity and community structure.

3. Direct comparison of two next generation sequencing techniques from the same root community samples on root-associated fungal communities.

We hypothesized that:

H1: Root community traits vary with forest transformation and are related to transformation intensity

H2: Fungal diversity is higher in plant species rich rain forests than in highly managed monoculture plantations

H3: Land use has an impact on community composition of root-associated fungi

H4: There exists a shift from beneficial functional fungal groups towards pathogens in the highly managed systems compared to natural rain forests

H5: Both next generation techniques generate comparable results on fungal diversity and community structure

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CHAPTER TWO

RESEARCH ARTICLE

Degradation of Root Community Traits as Indicator for Transformation of Tropical Lowland Rain Forests into Oil Palm and Rubber Plantations

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2 Degradation of Root Community Traits as Indicator for Transformation of Tropical Lowland Rain Forests into Oil Palm an Rubber Plantations

2.1 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 million ha) was lost since the countries' independence in 1945 (FAO, 2009). 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 (Gouyon et al., 1993; Tata et al., 2008). Tree species richness is lower but the forest structure of jungle rubber is similar to that of unmanaged lowland rain forests (Gouyon et al., 1993; Tata et al., 2008; Murdiyarso et al., 2002). Pristine lowland rain forests exist only in fragments and most 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; Wilcoves et al., 2013; 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 together with their 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; Fornara, Tilman and Hobbie, 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 fungi, but in lowland tropical forests also a number of native species occur, e.g. dipterocarps that associate with ectomycorrhizal ectomycorrhiza (EM) fungi (Habib, Heller and Polle, 2013).

The ability of tree roots to form mutualistic AM or EM associations is a typical speciesrelated trait that can mediate differences in plant nutrition, especially of phosphorus and nitrogen (Smit 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 (Tata et al., 2008; Murdiyarso et al., 2002) *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 can then be defined as root-community-weighed traits (RCWTs). Only few studies addressed the variation of RCWTs. Prieto et al. (Prieto et al., 2015) found that root morphology, a trait related to resource acquisition and root litter degradability, a trait indicating conservation of resources, co-varied for root communities with land use across tropical, Mediterranean and montane climate. In grassland ecosystems RCWTs were correlated with plant productivity and ecosystem functions (Fornara, Tilman and Hobbie, 2009; Orwin et al., 2010). We, therefore, anticipated that the traits of whole root communities would be useful indicators of land transformation.

Here, we asked whether transformation of tropical rain forest into intensive rubber or oil palm mono-plantations would affect functional traits of the root communities. An important functional trait indicating resource conservation is the chemical composition of roots. In our study we determined the concentrations of nutrients and other elements (C, N, P, N, K, S, Ca, Mg, Mn, Fe, Al, Na) in roots from different land use systems. We further measured traits related to plant performance and life style such as root mass, root vitality and colonization with mycorrhizas (EM colonization, AM colonization including vesicles, arbuscules and spores). All traits were determined in mixtures of roots collected in defined soil volumes and therefore represent RCWTs. Specifically, we hypothesized that chemical and performance parameters of root communities vary with forest transformation and are related to transformation intensity. Because land transformation results in degradation of ecosystem functions, we further

tested whether RCWTs were correlated with ecosystem properties such as soil carbon and nitrogen concentrations. 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 RCWTs and indicators for ecosystems functions (soil carbon and nitrogen concentrations, leaf litter carbon and nitrogen concentrations, soil available phosphorus and base cations concentrations, soil pH).

2.2 Materials and Methods

2.2.1 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 Bukit12 (Figure 2.2.1). In each landscape four land use systems were selected: secondary rain forest, jungle rubber, rubber plantations and oil palm plantations. The study areas were in the lowlands (below 100 m a.s.l.) on deep, well drained, acid soil with low fertility (Murdiyarso et al., 2002). The soils are classified as loam acrisol in the Harapan and clay acrisol in the Bukit12 landscape. The climate is tropical with annual precipitation >2000 mm and only two months with less than 100 mm rain fall. In the Harapan area the annual mean temperature is $26.9\,^{\circ}\mathrm{C}$ and the annual precipitation 2332 mm (location: Dusun Baru, http://en.climate-data.org/location/595657/); in the Bukit12 area the mean annual temperature is $26.8\,^{\circ}\mathrm{C}$ and the precipitation sum is 2860 mm (location: Lubuk Kepayang, http://en.climate-data.org/location/587840/).

2.2.2 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 Pengambilan dan Angkut (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).

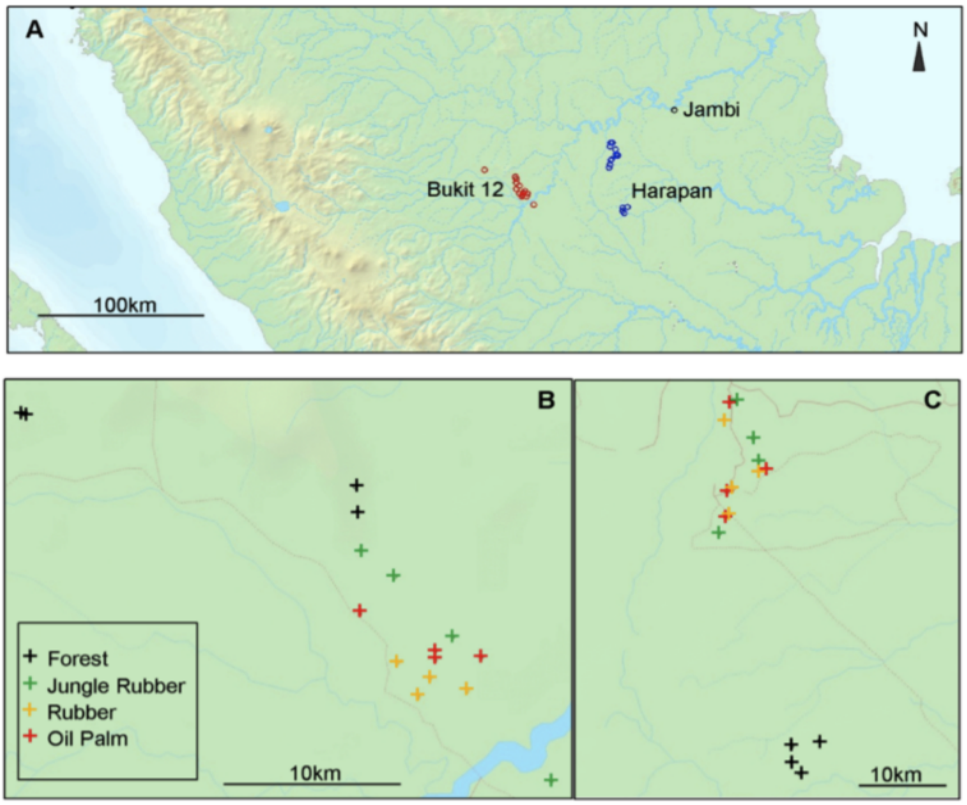

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Figure 2.2.1: Maps of the Province Jambi (A) with the Landscapes Bukit12 (B) and Harapan (C) on Sumatra (Indonesia). The locations of the research plots are indicated.

2.2.3 Sampling Design

In each of the two landscapes and in each forest type four plots (50 m x 50 m) were installed resulting in 32 sampling sites (Table 2.2.1). 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 5 m x 5 m 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 1 m. 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 $4\,^{\circ}\mathrm{C}$ until processing.

	Bukit12				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"	E 103°15'52.0"	71
BO1	S 02°04'26.1"	E 102°48'55.1"	75	HO1	S 01°54'45.6"	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

doi:10.1371/ journal.pone.0138077.t001

Table 2.2.1: 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.

2.2.4 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.
Litter samples were dried in an oven at $80\,^{\circ}\mathrm{C}$ for 48 h. Fresh bulk soil samples (about 20 g) were initially air dried and then oven dried ($105\,^{\circ}\mathrm{C}$ for 48 h) to determine the soil water content according to the following equation:

$$\text{Relative soil water content } (\text{g g}^{-1}\ \text{soil}) = \left(\frac{\text{weight of fresh soil (g) - weight of oven dried soil (g)}}{\text{weight of fresh soil (g)}}\right)$$

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 $\leq$ 2 mm) were weighed, stored in wet tissue paper at $4\,^{\circ}\mathrm{C}$, used for root vitality and mycorrhizal analysis, and were subsequently oven-dried at $60\,^{\circ}\mathrm{C}$ for 48 h. Fine root dry mass was calculated as:

$$\text{Fine root mass } (\text{g kg}^{-1}\ \text{soil}_{\text{dw}}) = \left(\frac{\text{dry weight of fine roots of subplot a + subplot b + subplot c (g)}}{\text{dry weight of soil of subplot a + subplot b + subplot c (kg)}}\right)$$

Dry aliquots of soil, roots and litter were stored in 50 ml reaction tubes (Falcon tube 50 ml, 115 x 28 mm, Sarstedt, Nümbrecht, Germany). Before closing the screw cap, a small reaction tube (Eppendorf micro tube, 1.5 ml, 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), IPB Bogor Agricultural University (Bogor, Indonesia) and Tadulako University (Palu, Indonesia) for further analysis.

2.2.5 Analysis of Root Vitality and Ectomycorrhizal 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 enclosed the root and emanating hyphae (Smith and Read, 2008). Dead, non-EM, and vital EM root tips were documented by photos taken with the microscope camera.

2.2.6 Arbuscular Mycorrhizal 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 2 ml, 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 $90\,^{\circ}\mathrm{C}$. 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 400 x, distance between the intersects 100 μm) 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:

$$\text{AM colonization} = \frac{\text{number of hyphae}}{\text{total number of intersects}} * 100$$

The relative abundance of arbuscules and vesicles was calculated correspondingly.

2.2.7 Determination of Arbuscular Mycorrhizal Spore Abundance

Air dried samples of bulk soil were stored in sealed plastic bags at $4\,^{\circ}\mathrm{C}$. Spores from each soil sample (n = 480) were isolated as described by Gerdemann and Nicolson (Gerdemann and Nicolson, 1963). Twenty gram of soil of each sample was suspended in 500 ml of water, stirred manually for 10 min. 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 min in a 63 μm sieve, filtrated onto a gridded filter paper, then placed in a 90 mm diameter Petri dish. The spores obtained from all sieves were counted under a binocular stereomicroscope with 100 to 400-fold magnification (Olympus SZ61, Osaka, Japan). The number of spores were expressed as spores per 20 g soil sample.

2.2.8 Element Analysis 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 9 mm, 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 the elements Al, Ca, Fe, K, Mg, Mn, Na, P and S (aluminum, calcium, iron, potassium, magnesium, manganese, sodium, phosphorus 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 14 h at 200 °C. 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 640 w, ∅ 90 mm, 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).

$$\text{Element concentration } (\text{mg g}^{-1}) = \frac{\text{element concentration (mg 1}^{-1}) \times \text{ volume (1)}}{\text{mass of dry material (g)}}$$

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 2 mm mesh. Two grams of soil from each sample were mixed with 15 ml of Bray solution containing 0.03 N NH_4 F and 0.025 N HCl and were shaken (Finofors AG, Basel, Switzerland) for 5 min at 180 rpm at room temperature. After shaking, the suspensions were filtered through a phosphorus-free folded filter (filter papers MN 280 $\frac{1}{4}$ 125 mm, Macherey-Nagel, Düren, Germany). Phosphorus concentrations of the filtrates were analysed by ICP OES (iCAP 6300 Series, Thermo Fischer Scientific, Dreieich, Germany).

2.2.9 Determination of Soil pH

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

2.2.10 Maps of the Sampling Site

Maps of plot locations were generated the free software package GPS Visualizer (http://www.gpsvisualizer.com/) (Schneider).

2.2.11 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. The subplot data were used to calculate plot means. All further analyses were based on plot means. Plots means were used as input parameters to construct the data matrices for principle component analysis (PCA). Significant principle components (PCs) were determined by broken stick analysis. Non-metric multidimensional scaling (NMDS) was conducted with Gover as similarity measure. Multivariate analyses were conducted with the PAST free software package 2.17c (http://folk.uio.no/ohammer/past/, (Hammer, Harper, and Ryan, 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. A linear mixed model with landscape a fixed factor and land use system as random factor nested in landscape was used to test the contribution of the variables land use system and landscape to the PCs (Statgraphics, Centurion XV, St Louis, Mo, USA). Variance component analysis revealed no contribution of the factor landscape on

PC 1. Therefore, one-way ANOVA with the only factor land use system was conducted for the PC 1 data (post hoc test: Tukey HSD) and the data were used to develop a general linear model with PC 1 as the dependent variable and soil and litter properties as independent variables. The categorical factors land use system and landscape were not included in the model because they had been used to develop PC 1. Combinations of all eight predictors variables (soil N, soil C, soil pH, soil P, soil cations, soil water content, litter C, litter N) were tested and the model with the lowest Akaike information criterion (AIC = 0.569) containing three variables was chosen. When the data were not-normal distributed the Kruskal Wallis test was conducted and medians and range of the data were indicated.

2.2.12 Data Deposition and Availability

The raw data of this study are deposited and available in the Dryad repository under doi:10.5061/dryad.qf362 .

2.3 Results

2.3.1 Root Community-Weighed Traits are Massively Affected by the Land Use System

Our measurements of the root nutrient elements represent RCWTs because the roots were collected in defined soil volumes representing mixtures of tree species and understory weeds on the plots. Root carbon, nitrogen, sulfur, manganese, and base cations concentrations showed a decline in rubber and oil palm plantations compared with those from forest systems (Figure 2.3.1 A, 2.3.1 B, 2.3.1 D, 2.3.1 E, and 2.3.1 F). In both landscapes, Harapan and Bukit12, the decline in the root nutrient concentrations with land use type was similar. No clear influence of the land use system was observed on the root phosphorus concentrations (Figure 2.3.1 C). The concentrations iron and aluminium, both potentially toxic compounds at high concentrations, showed strong increases in roots of oil palm and rubber plantations compared to jungle rubber and rain forest roots (Figure 2.3.1 G and 2.3.1 H).

We further determined RCWTs that are related to root vitality and mycorrhizal association (fine root mass, colonization by ectomycorrhizal and AM fungi, AM vesicles, AM arbuscles, AM spores in soil, dead root tips) (Figure 2.3.2). Fine root mass was higher in rain forest than in oil palm plots, where also the highest fraction of distorted root tips was found (Figure 2.3.2 A and Figure 2.3.2 B). The fraction of mycorrhizal roots was stable (74.4 $\pm$ 1.7 %) with the exception of the oil plantations in Harapan (51.8 $\pm$ 7.5 %, Figure 2.3.2 C). EM colonization was detected in some plots in Harapan rain forest with a maximum of 6 % in one plot and in jungle rubber in both landscapes, but their overall abundances were rare (Figure 2.3.2 C). AM spore abundance was lowest in the rain forest and highest in oil palm plantations (Figure 2.3.2 D).

2.3.2 Root Community-Weighed Traits Indicate Transformation Intensity

PCA with all sixteen RCWTs shown in Figure 2.3.1 and Figure 2.3.2 revealed that the variables ectomycorrhizal colonization, abundance of AM arbuscules and Na resulted in insignificant loadings with R $<$ 0.5 and the parameters fine root mass and base cations showed collinearity with other root properties and were therefore removed. The reduced PCA was based on eleven RCWTs (Table 2.3.1) and resulted in two significant PCs that explained 42.4 % (PC 1) and 28.3 % (PC 2) of the variation, respectively (Figure 2.3.3). PC 1 separated the land use systems with the rain forests exhibiting the most positive and oil palm plantations the most negative scores (Figure 2.3.3). Positive PC 1 loadings with correlations of R $\geq$ 0.5 were C, N, S, and Mn (Table 2.3.1). Negative PC 1 loadings with R $\leq$ - 0.5 were AM spores, dead root tips, Al and Fe (Table 2.3.1). RCWTs related to mycorrhization (AM colonization, AM vesicles) and to phosphorus were not strongly correlated with PC 1 (Figure 2.3.3, Table 2.3.1), but were significant loadings on PC 2.

To quantify the influence of the factors landscape and land use systems on the variation of the PC 1 and PC 2 scores, the data were analyzed by general linear mixed models. Significant models were obtained for both PCs with $R2_{(\text{adjusted for df})}$ explaining 92.6 % of the variation of the PC 1 scores and 32.9 % of the PC 2 scores (Table 2.3.2). However, the only significant factor was land use system (Table 2.3.2). Analyses of the variance components (in the order of nesting) showed that landscape contributed 0 %, land use system 94.1 % and the error term 5.9 % to the variation of PC 1. For PC 2 the contributions of the components to the total

variation were error term (58.4 %), landscape (23.1 %) and land use system (18.5 %).
Mean values of the PC 1 scores ordered the land use systems according to transformation intensity in the order: forest $>$ rubber jungle $>$ rubber $>$ oil palm (Table 2.3.3).

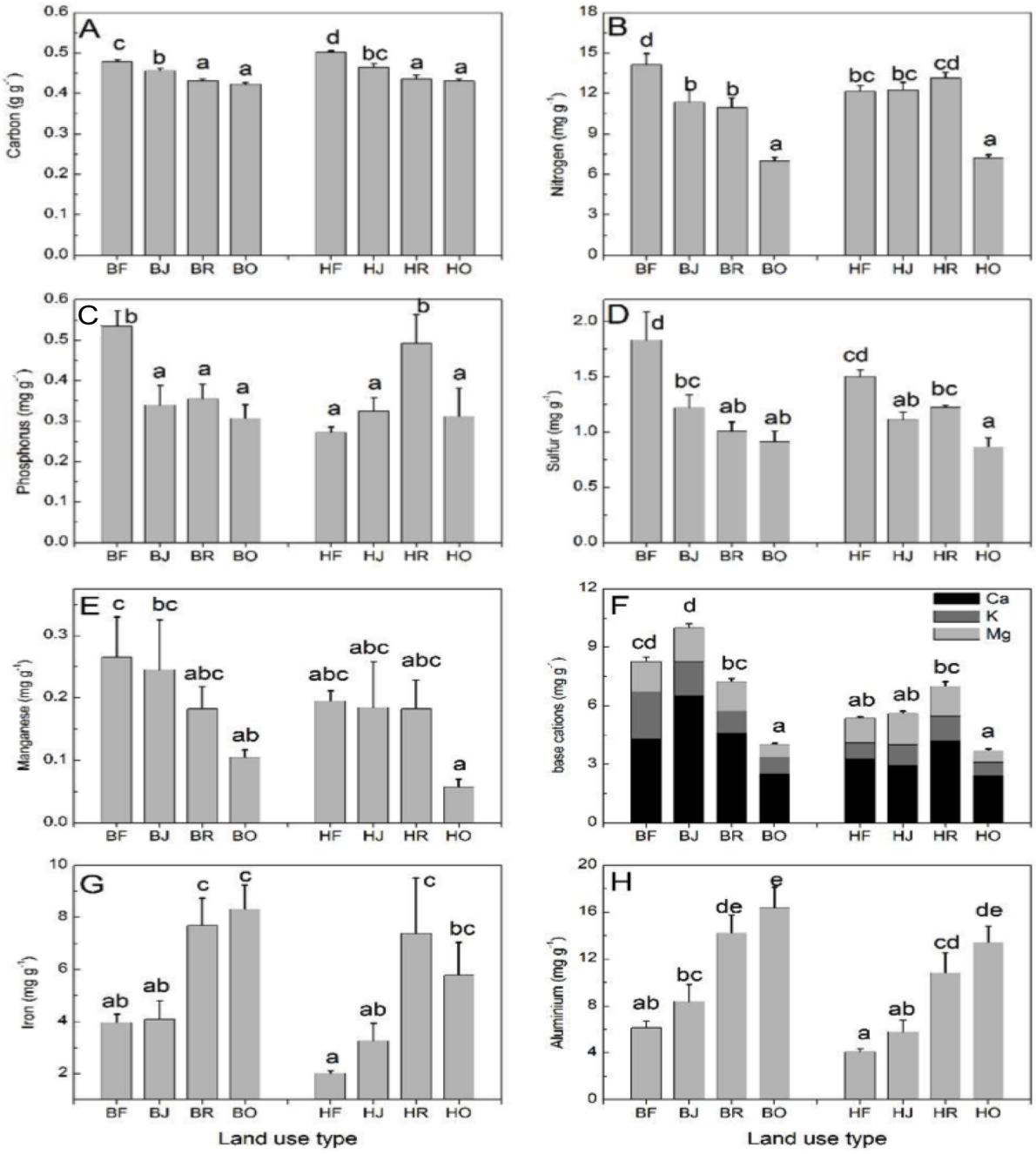

Figure 2.3.1: Chemical Composition of Roots in Different Land Use Systems. Carbon (A), nitrogen (B), phosphorus (C), sulfur (D), manganese (E), base cations (F), iron (G), and aluminium (H) determined as root community-weight traits. Data indicate means (± SE). Different letters indicate significant differences at $P < 0.05$. B = Bukit12, H = Harapan, O = oil palm, R = rubber plantation, J = jungel rubber, F = forest.

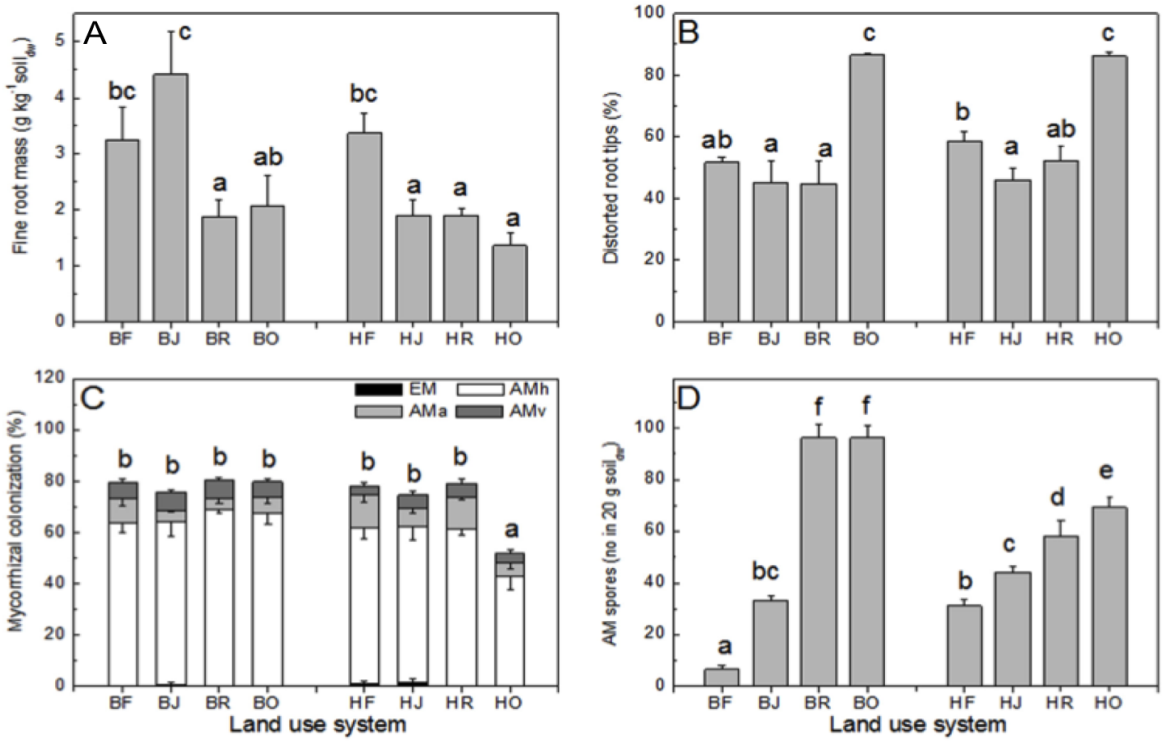

Figure 2.3.2: Performance Parameters of Roots in Different Land Use Systems. (A) Fine root mass to a depth of 0.2 m, (B) Fraction of distorted root tips (100 % is the total number of inspected root tips), (C) Fraction of the inspected root lengths colonized with mycorrhizal hyphae (AMh), arbuscules (AMa), vesicles (AMv) and fraction of vital root tips colonized with EM, (D) Number of arbuscular mycorrhizal spores. Data indicate means (± SE). Different letters indicate significant differences at $P < 0.05$. B = Bukit12, H = Harapan, O = oil palm, R = rubber plantation, J = jungle rubber, F = forest.

Trait name	Abbreviation	PC 1	PC 2
Sulfur	Sroot	0.838	0.180
Nitrogen	Nroot	0.821	0.326
Carbon	Croot	0.786	0.551
Manganese	Mnroot	0.579	0.670
AM root colonization	AMR	0.275	0.709
Phosphorus	Proot	0.306	0.571
AM vesicles	AMves	-0.045	0.773
Iron	Feroot	-0.634	0.665
Dead root tips	DeadR	-0.592	-0.381
Aluminium	Alroot	-0.817	0.414
AM spores in soil	AMspore	-0.866	0.200

doi:10.1371/ journal.pone.0138077.t002

Table 2.3.1: PCA Loadings for Correlations of Root Tratits with PC 1 and PC 2.

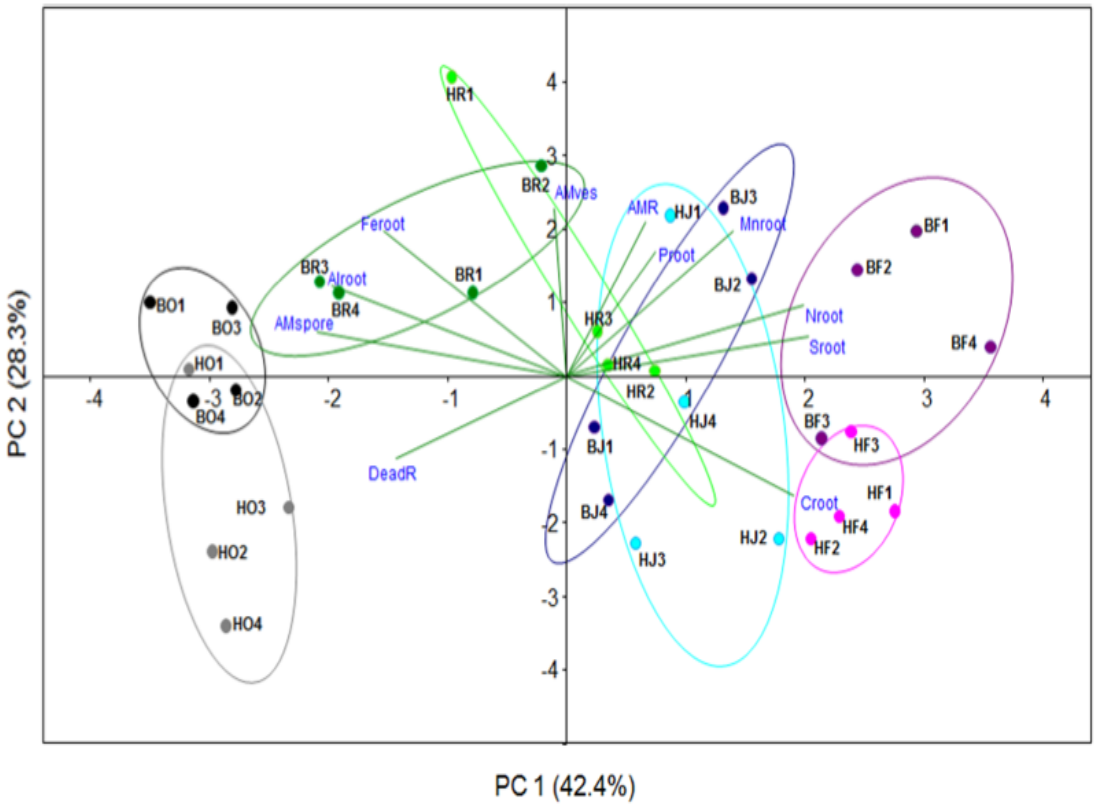

Figure 2.3.3: Principle Component Analysis of Root Community-Weighed Traits. The tratis used for PC A and their abbreviations are listed in Table 1.3.1. B = Bukit12, H = Harapan, O = oil palm, R = rubber plantation, J = jungle rubber, F = forest.

2.3.3 Transformation Intensity is Linked with Ecosystem Properties

In tropical ecosystems loss of forest cover and conversion into agricultural land use systems has often been linked with loss in soil fertility and soil carbon contents (Dechert et al., 2004; Carlson et al., 2012). We, therefore, asked whether the RCWTs that ordered the land use systems according to transformation intensity also corresponded to loss of ecosystem functions indicated by soil properties. Soil (sum of base cations, available phosphorus, pH, water content, carbon, nitrogen) and litter properties (carbon, nitrogen), which we measured as proxies for ecosystem functions showed significant variations among different sites (Table 2.3.4).

Source	Sum of Squares	Df	Sum of Squares	F-Ratio	P-Value
Analysis of Variance for PC 1					
Model	136.46	7	19.49	56.31	<0.001
Residual	115.36	24	0.35		
Landscape	0.92	1	0.92	0.04	0.847
LUS (landscape)	135.54	6	22.59	65.25	<0.001
Residual	8.31	24	0.35		
Total (corrected)	144.77	31			
Analysis of Variance for PC 2					
Model	46.45	7	6.63	3.17	0.016
Residual	50.18	24	2.09		
Landscape	17.97	1	17.97	3.79	0.100
LUS (landscape)	28.47	6	4.74	2.27	0.071
Residual	50.18	24	2.09		
Total (corrected)	96.63	31			

doi:10.1371/ journal.pone.0138077.t003

Table 2.3.2: General Linear Mixed Model for PC 1 and PC 2 as Dependent Variables and Landscape and Land Use Systems (LUS) as Categorical Factors. Landscape was set as fixed and LUS as random factor nested in landscape.

A NMDS conducted with the significant loadings of RCWTs for PC 1 (Table 2.3.1) and the environmental variables (Table 2.3.4) as explanatory vectors indicated that soil pH and soil N were related to the negative scores of oil palm and rubber plantations, while soil C and litter N and C were related to the positive scores of rain forest and jungle rubber (Figure 2.3.4). However, it should be noted that the overall pH differences between the plots were small (Table 2.3.4, mean pH of rain forest plots: 4.25 ± 0.03 and mean pH of the other land use systems: 4.46 ± 0.13, $P = 0.002$).

To find out whether the PC 1 scores which distinguish the land use systems independently from landscape can be quantitatively related to ecosystem functions, we tested general linear models. The PC 1 scores were used as dependent and the environmental properties as independent variables. The categorical factors land use system and landscape were not included in the model, because they had been used to determine the PC 1 components. The model with the lowest AIC contained three significant components: soil nitrogen concentration, soil pH and litter carbon concentration (Table 2.4.1). The model explained 70 % (R^2 adjusted for d. f.) of the variation. 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.

Site	PC 1 ± SE	PC 2 ± SE
BF	2.77 ± 0.31e	0.74 ± 0.62ab
HF	2.34 ± 0.17de	-1.68 ± 0.32ab
BJ	0.86 ± 0.33c	0.30 ± 0.19ab
HJ	1.05 ± 0.26cd	-0.66 ± 1.05ab
BR	-1.25 ± 0.45b	1.60 ± 0.42b
HR	0.09 ± 0.37bc	1.22 ± 0.95ab
BO	-3.06 ± 0.17a	0.35 ± 0.36ab
HO	-2.84 ± 0.18a	-1.87 ± 0.74

doi:10.1371/ journal.pone.0138077.t004

Table 2.3.3: Mean PC Scores of the Land Use Systems. Different letters in colums indicate significant differences at $P < 0.05$ determined with the HSD test. B = Bukit12, H = Harapan, O = oil palm, R = rubber plantation, J = jungle rubber, F = forest.

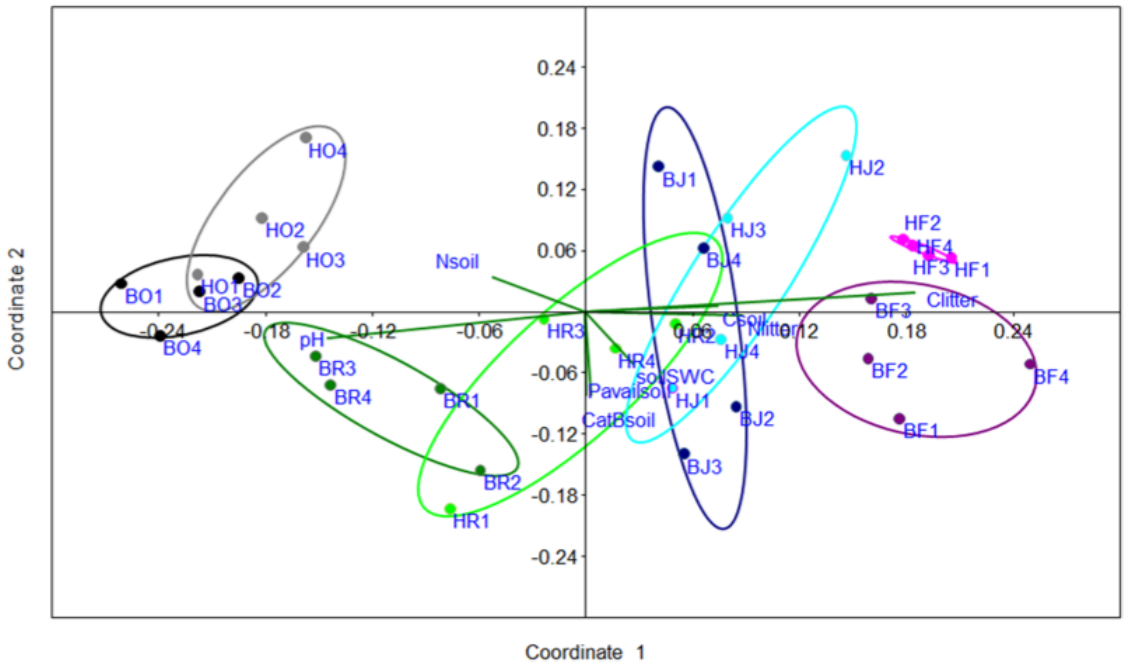

Figure 2.3.4: Non-Metric Multidimensional Scaling (NMDS) of Root Community-Weighed Traits. RCWTs with $R > 0.5$ and $R < 0.5$ from Table 2.2.1 for PC 1 were used for NMDS. The following environmental parameters were plotted as explanatory variables: nitrogen and carbon concentrations in soil and litter (Nsoil, Csoil, Clitter, Nlitter), available phosphorus in soil (Pavailsoil), sum of basic cations in soil (CatBsoil), soil water content (soilSWC) and soil pH (pH). B = Bukit 12, H = Harapan, O = oil palm, R = rubber plantation, J = jungle rubber, F = forest. Stress: 0.106, R^2 for coordinate 1:0.785 and for coordinate 2:0.0735.

Plot	C	Range	N	Range	base cations	Range	P	Range	Water	Range	pH	Range	C	Range	N	Range
	(g g^{-1} $soil_{dw}$)		(mg g^{-1} $soil_{dw}$)		(g^{-1} $soil_{dw}$)		(mg g^{-1} $soil_{dw}$)		(mg g^{-1} $soil_{dw}$)				(mg g^{-1} $soil_{dw}$)		(mg g^{-1} $soil_{dw}$)	
BF	0.29	0.26	2.06	2.06	3.04	10.05	0.0048	0.0067	0.29	0.26	4.2	0.2	424.2	97	14.22	6.06
BJ	0.36	0.18	3.37	0.94	5.96	2.2	0.0019	0.0016	0.36	0.18	4.5	0.2	454	120.8	12.4	3.07
BR	0.25	0.17	1.27	2.18	1.39	2.69	0.0012	0.0006	0.25	0.17	4.5	0.2	377.3	115	11.48	5.77
BO	0.29	0.09	1.84	1.7	2.12	3.41	0.0043	0.0112	0.29	0.09	4.45	0.2	347.3	100.4	12.11	6.54
HF	0.25	0.05	1.47	0.49	1.98	0.72	0.0021	0.0012	0.25	0.05	4.3	0.2	469.8	46.3	13.24	1.37
HJ	0.22	0.09	1.59	1.14	0.79	5.42	0.0022	0.0133	0.22	0.09	4.3	0.2	458.7	38	14.64	3.21
HR	0.24	0.05	1.45	0.56	3.52	3.61	0.0019	0.0032	0.24	0.05	4.45	0.5	435.8	76.9	15.06	3.42
HO	0.2	0.1	1.09	1.06	1.47	4.25	0.038	0.016	0.2	0.1	4.5	0.3	362.2	46.4	12.58	1.4
Test statistic	18.93		14.64		10.88		15.39		12.84		19.8		16.84		10.31	
P value	**0.008**		**0.041**		0.144		**0.031**		0.076		**0.01**		**0.018**		0.172	

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Table 2.3.4: Median and Range of Environmental Properties. P values of the kruskal Wallis rank test for the land use systems are indicated. B = Bukit12, H = Harapan, O = oil palm, R = rubber plantation, J = jungle rubber, F = forest.

2.4 Discussion

2.4.1 Root Community-Weighed Traits and Soil Properties Vary with Forest Transformation

Recent studies highlight the importance of functional structures of communities rather than their biodiversity for ecosystem functioning (Mouillot et al., 2011; Katabuchi et al., 2012; Finegan et al., 2015). Our study clearly demonstrates a decline

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	106.2	3	35.4	25.71	¡0.0001
Residual	38.55	28	1.37		
Nsoil	6.37	1	52.15	37.88	0.0401
pH	26.14	1	26.14	18.99	0.0002
Clitter	52.16	1	52.16	37.88	¡0.0001
Residual	38.55	28	1.37		
Total (corrected)	144.76	31			

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Table 2.4.1: Best General Linear Model for the Relationship of PC 1 with Ecosystem Properties.

of positive RCWTs 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 forest tree species with distinct features. We expected that the impact of dominant trees might have been traced by an effect of the associated EM on RCWTs, because the root nutrient status of forest trees is affected by symbioses with AM or EM fungi and fungal species identities (Lang et al., 2011; Sven and Polle, 2014; Pena and Polle, 2014). However, our data did not reveal an influence of the land use system on the mycorrhizal life traits. 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 links of these agricultural systems with ecologically important life traits.

A negative impact of monoculture oil palms was evident on 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 Noordwijk et al., 1997; Murty et al., 2002; Schroth et al., 2002; Smiley and Kroschel, 2008; Leuschner et al., 2013)[37 – 41]. 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). A beneficial effect of jungle rubber, an extensive agroforest land use system, on soil properties was confirmed in our study because the carbon and nitrogen concentrations in soil of this 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 as biomass production, carbon cycling and carbon sequestration and has been identified as the major regulator of forest carbon balance (Fernández-Martínez et al., 2014).

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) (Melillo, et al., 1989; Prescott, 2010). Therefore, RCWTs and soil properties are to some extent inter-dependent. Our study provides some insights into the nature of these links because the RCWTs that reflected transformation intensity were also linked with soil and litter properties, i.e., soil pH, soil N and litter C concentrations. This finding is 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 (Fernández-Martínez et al., 2014). Our findings, thus, link functional structures of root communities with ecosystem functions, notably with those functions that are more important for carbon sequestration than climate or the rising atmospheric CO_2 concentration (Fernández-Martínez et al., 2014; Cornwell et al., 2008). This finding implies that RCWTs 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 monoculture species with unfavorable root traits could have affected soil properties or management could have altered soil properties with negative consequences for root traits. Regardless the ultimate reason, our results suggest that the loss in ecosystem functions in mono-cultures was

accompanied by complex alterations of root functional traits. Increased transformation intensity was associated with diminished nutrient concentrations and low root mass on the hand and increased concentrations of potentially phytotoxic metals (Al, Fe) and enhanced root tip mortality on the other hand. The transformation intensity was thus indicated by contrasting behavior of distinct RCWTs and not by the loss of traits abundance *per se*. Consequently, we may expect that any management 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.

2.4.2 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. Plant availability of Al is modulated by soil acidity (Brunner and Sperisen, 2013). The soils in the Jambi lowland region are acrisols with pH values of 4.5 and below. In Bukit12 higher concentrations of exchangeable Al were present than in Harapan (0.54 ± 0.18 mg g^{-1} $soil_{dw}$ versus 0.28 ± 0.04 mg g^{-1} $soil_{dw}$), but without showing a clear gradient among the land use systems as found here for the root communities (Allen et al., 2015). In each landscape the exchangeable Fe concentrations were highest in rain forest soil (Allen et al., 2015), where root communities showed the lowest Fe enrichment. Therefore, the Al and Fe enrichments in roots did not simply reflect soil conditions.

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). Indeed, the morphological appearance of the oil palm roots on our plots resembled the symptoms of Al toxicity with stubby root systems lacking fine root branches with many brownish, distorted root tips (Rout et el., 2001). Although oil palms are often cultivated in acid soils (Squire, 2003) injury due to unfavorable soil conditions cannot be excluded. In field studies, a negative correlation between exchangeable Al in soil and root density of oil palms 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 remained unaffected by the land use system. Excess Fe causes oxidative stress leading to cell destruction (Jones, 1998) and may have caused here, together with Al, enhanced root tip 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 a pre-requisite, but was not the immediate reason for the observed decline in root health.

Currently, we can only speculate about the reasons 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 the introduced crop trees were less well-adapted to the prevalent soil conditions than the native tree species and accumulated phytotoxic concentrations of Al and Fe over the years. As a consequence, root health may decline and root soil exploration and root litter input into soil 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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2.6 Author Contributions

Conceived and designed the experiments: AP. Performed the experiments: JS SWB HB NE MMMDC. Analyzed the data: JS SWB HB NEMM MDC AP. Contributed reagents/ materials/ analysis tools: JS SWB HB NE. Wrote the paper: JS SWB HB NE MDC AP.

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CHAPTER THREE

3 The Impact on Roots and Functional Diversity of Root-Associated Fungal Communities

3.1 Introduction

Tropical rain forests are one the ecosystems with the highest species richness on earth (Hartshorn, 2013). Some of the most diverse and carbon rich forests are tropical lowland rain forests in Southeast Asia (Allen et al., 2015). Its biodiversity is increasingly threatened by human driven land use changes and deforestation to extract timber and to make land accessible for agriculture (Sodhi et al., 2004). Lowland rain forests are particularly endangered for conversion and degradation since they are easily to access. In 2012, Indonesia reached the highest deforestation rate worldwide with a loss of 0.84 million hectares forest of which 51 percent was categorized as lowland rain forest (Margono et al., 2014). Sumatra, Indonesia, is facing deforestation over decades and has lost, on average, approximately 550.000 hectares forest per year over the last 30 years with the majority of land use changes occurring in the lowland regions (Laumonier et al., 2010). Rubber (*Hevea brasiliensis*) seeds were introduced to Sumatra around 1910 and farmers started to grow rubber trees within the natural forests resulting in low-input complex agroforestry systems ("jungle rubber") (Gouyon et al., 1993). However, these agroforestry systems were replaced rapidly by rubber monoculture plantations due to the increasing demand for rubber related with a spectacular development of the automobile industry (Priyadarshan, 2011). Oil palms (*Elaies guineesis*) were introduced to Indonesia in 1848 not for commercial use but rather as exhibits in botanical gardens. The first large oil palm plantation was cultivated 1911 in Sumatra (Corley and Tinker, 2015). The expansion of commercial cultivation of oil palms was interrupted by World War II and its consequences for the global economy (Corley and Tinker, 2015). After World War II, the oil palm industry was growing slowly in Indonesia until the 1980's, but then started to grow rapidly until today (Corley and Tinker, 2015). The oil palm industry is now one of the world's most rapidly increasing industries in the agricultural sector (Fitzherbert et al., 2008). The increasing demand for palm oil for biofuel, the food industry, and the cosmetics industry is driven by the economy and the earth's growing population and consumption needs and will lead to a further expansion of oil palm plantations in Indonesia and tropical regions all over the world (Danielsen et al., 2009; Smit et al., 2013; Sodhi et al., 2010). In Indonesia, the prediction of further expansion of oil palm plantations is supported by the decision of the Indonesian government to double the oil palm production within the next ten years, which will lead to monoculture plantations dominating the landscapes in Indonesia in future (Carlson et al., 2012).

It is known that deforestation in the tropics and the expansion of monoculture plantations can lead to losses in biodiversity and, therefore, to a loss in ecosystem functioning and services (Barnes et al., 2014; Drescher et al., 2016; Hooper et al., 2005; Sodhi et al., 2010). The majority of research conducted in the tropical regions has focused on aboveground biodiversity in relation to ecosystem functioning, whereas the immense biodiversity found belowground and its impact on ecosystem functions and services have rarely been addressed. Plants build the stationary fundament of onshore biomes and are the first group of organisms directly influenced by land use changes. This can lead to six-fold decline in plant species richness in converted land use systems comparted to rain forests (Drescher et al., 2016). All plants are associated with microorganisms and they contribute to the adaption of plants to changing environmental conditions and play an important role for ecosystem functioning (Chen et al., 2014; Peršoh, 2015; Redman et al., 2011). However, there is still a lack of knowledge on microbial community composition in different ecosystems and, in particular, tropical and subtropical ecosystems are understudied.

Fungi are a highly diverse group of microorganisms performing multiple ecological functions (Hawksworth, 1991; Peršoh, 2015). Fungal community composition varies among ecosystems as well as on spatial and temporal scales and is in many cases related to the host identity and/or phylogenetic affiliation (Lang et al., 2011; Maron et al., 2011; Pena et al., 2013; Smith and Read, 2008; Tedersoo et al., 2008). Of particular importance are some fungal groups because they control regulatory steps in ecosystems, namely: mutualistic fungi which are including mycorrhizal fungi, pathogenic fungi, and saprotrophic fungi. In this study the term "functional group" is used instead of "guild" to categorize the mentioned fungal groups since the focus is more on the relevance for ecosystem processes and functioning than on similarities in resource sharing (Blondel, 2003). The best studied fungal functional groups are the mycorrhizal fungi. They form mutualistic interactions with plant roots, supply water and nutrients to their hosts, and act as the main pathway for carbon to the soil (Hobbie, 2006; Verbruggen et al., 2016; Zhu, 2003). The large majority of plants in tropical forests are associated with arbuscular mycorrhizal fungi (AMF), but there are some tree species rich families like the Dipterocarpaceae which form ectomycorrhizal symbioses (Tedersoo et al., 2012; Toju et al., 2014). The non-native oil palms and rubber trees are associated with AMF (Bakhtiar et al., 2013; Herrmann et al., 2016; Phosri et al., 2010; Wastie, 1965). The transformation of tropical forests to monoculture oil palm and rubber plantations may lead to changes in community composition of mycorrhizal fungi as the mutualistic interactions can be

species-specific or generalistic (Smith and Read, 2008) and land use intensification can affect mycorrhizal community composition (Bainard et al., 2014; Kerfahi et al., 2014; Oehl et al., 2003). Plant pathogens fungi represent another important functional group as they influence the plant health status and can cause diseases and pests (Li et al., 2014; Maron et al., 2011). The negative effects of plant pathogenic fungi can be species-specific, density-dependent or a combination of both (Bell et al., 2006; Klironomos, 2002; Maron et al., 2011; Van der Putten et al., 1993). Land use intensification and consecutive mono-culturing of crops could be one reason for creating a micro-ecological environment promoting pathogens accumulation (Li et al., 2014). Saprotrophic fungi are important as a decomposer, for nutrient cycling, and nutrient distribution in soil (Baldrian and Valášková, 2008; Cairney, 2005). The impacts of land use changes on saprotrophic fungi will be important to understand feedback mechanisms in terms of nutrition and CO_2 concentrations in ecosystems (Dighton and White, 2005). So far, most studies on fungal communities have focused on the taxonomic and structural aspect of fungal diversity (e.g. McGuire et al., 2011; Mueller et al., 2014; Orgiazzi et al., 2012; Peay et al., 2013). However, there is a need to investigate the functional properties of fungal communities. This would enable us to obtain a more comprehensive understanding of fungal communities and to predict consequences for differing ecosystem functions in response to functional fungal groups.

Studies focusing on the fungal diversity and community composition in tropical ecosystems are still rare (Tedersoo et al., 2014) and most studies carried out in the tropical and subtropical regions focused on fungal diversity in relation to plant diversity (McGuire et al., 2011; Mueller et al., 2014; Peay et al., 2013; Toju et al., 2014). These studied showed a positive correlation between fungal and plant diversity. So far, only few studies investigated the influence of land transformation from tropical forests to agricultural plantations (Kerfahi et al., 2014, 2016; McGuire et al., 2015). Kerfahi et al. (2014) studied the impact of logging and forest clearance for oil palm on soil fungal communities in Borneo, as well as McGuire et al. (2015), they investigated the response of soil fungal communities to logging and oil palm agriculture in Malaysia. In both studies fungal OTU richness showed no significant difference in natural rain forests and oil palm plantations. Kerfahi et al. (2016) investigated the influence of rain forest conversion into rubber plantation on fungal diversity and found no consistent differences in fungal OTU richness among the observed systems. Molecular studies on fungal biodiversity in agroforestry systems like the so called jungle rubber in Indonesia are missing. To our knowledge, there exists no study in Southeast Asia investigating fungal diversity and community

composition including reference rain forest sites, two agricultural land use systems with a high economic value, and an agroforestry system.
The present study was carried out in different land systems in Jambi province, Sumatra (Indonesia) on two different landscapes. The aim of this study was to assess the impact of land use changes and related changes in ecosystem properties from natural forests to oil palm and rubber monoculture plantations on root-associated fungal biodiversity and community structure by metagenomics analysis.
We hypothesized that:

1. Fungal diversity is higher in species rich rain forest sites compared to highly managed monoculture plantations

2. Land use has an impact on community composition of root-associated fungi

3. There exists a shift from beneficial functional fungal groups towards pathogens in the highly managed systems compared to natural rain forests

3.2 Material and Methods

3.2.1 Sites

All sites were located in the Province of Jambi, Central Sumatra, Indonesia. Two landscapes were selected, i.e. the area of Harapan Rainforest and the National Park Bukit12 (Figure 3.2.1, (Sahner et al., 2015)). In both landscapes four land use systems were examined: unmanaged secondary rain forest, less-managed jungle-rubber agroforest and intensively managed monoculture rubber and oil palm plantations. Study sites were in the lowlands on highly weathered soils, which were classified as loam acrisols in Harapan and clay acrisols in Bukit12 landscape (Allen et al., 2015). The sites have a tropical climate with an average temperature of 26.7 $\pm$ 0.2 °C and an annual precipitation of 2235 $\pm$ 381 mm (Drescher et al., 2016).

3.2.2 Sampling

Four core plots (50 m x 50 m) were installed per land use system and landscape resulting in 32 sampling sites (Drescher et al., 2016). In each core plot we extracted samples in three 5 x 5 m subplots. We took five soil cores (0.04 m diameter and 0.20 m depth) in a distance of at least 1 m to each other per subplot. Soil cores were stored in plastic bags and transported in cooling bags to the University of Jambi, where they were immediately stored at 4 °C. Each soil core was weighed, subsequently sieved through two sieves with 10 and 5 mm mesh size, and separated by hand into roots and bulk soil.

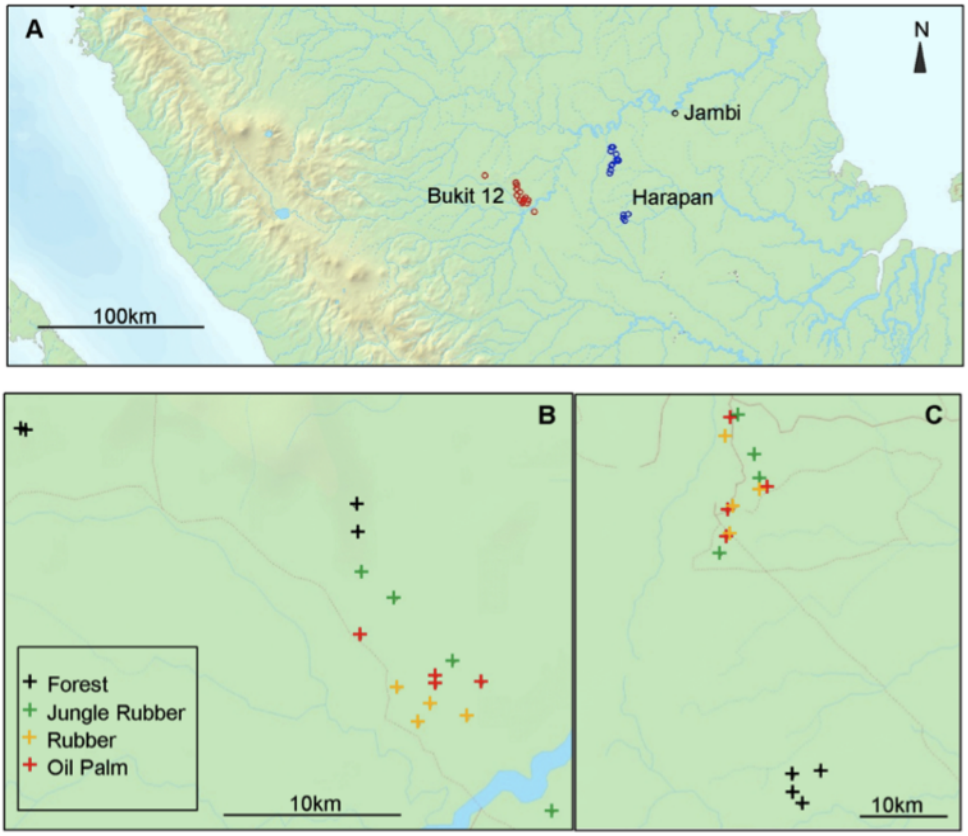

Figure 3.2.1: Maps of Province of Jambi (A) with the Bukit12 (B) and Harapan (C) landscapes on Sumatra (Indonesia). Locations of the research plots are indicated by crosses. Figure from Sahner et al., 2015.

The five samples from the same subplot were pooled and well mixed yielding one root and one

bulk soil sample per subplot. Root samples were washed until visible soil was removed and separated into coarse and fine roots (diameter < 2 mm). Afterwards, fine roots were dried on tissue paper and cut with a scalpel into 10 to 20 mm fragments. Between 100 and 150 fine root fragments from each subplot were stored at -20 °C in three reaction tubes (Eppendorf micro tube 2 ml, Sarstedt, Nümbrecht, Germany). The number of fine root fragments selected for following analysis was depending on the heterogeneity of root morphology in the samples (i.e., less fine root fragments were selected from monoculture oil palm and rubber samples compared to plant species, rich rain forest, and jungle-rubber sites). Two reaction tubes with fine root fragments per subplot were freeze dried. To freeze-dry root samples, the reaction tubes were opened and a 1000 μl pipet tip (Sarstedt, Nümbrecht, Germany) was put into the tube's aperture to avoid loss of root fragments during freeze drying (Figure 3.2.2). Reaction tubes containing fine root fragments were put on a rack and placed in a -80 °C freezer for at least 3 hours before freeze drying to make sure that the root material had a sufficiently low temperature. Freeze drying was performed using a VirTis Bench Top K Freeze Dryer (SP Industries, Warminster, USA) with a dual-stage rotary vane vacuum pump (Trivac E2, Leybold Vakuum GmbH, Köln, Germany) for about 32 hours. Afterwards, reaction tubes were perforated in the upper part with four little holes using the hot copper-bit of a soldering iron (Figure 3.2.2). Three to four of these perforated reaction tubes were placed in a 50 ml reaction tube (Falcon tube 50 ml, 115 x 28 mm, Sarstedt, Nümbrecht, Germany) filled with 5 g of silica gel (desiccant bag silica gel orange (10 g (40 x 90 mm)), Carl Roth, Karlsruhe, Germany). The freeze dried root samples were shipped to the University of Göttingen. Sampling in jungle-rubber sites, oil palm and rubber plantations was performed in October and November 2012 and in the rain forest sites in November and December 2013. Data for root community functional parameters (chemical traits and functional traits of fine roots), soil characteristics and leaf litter chemistry were used from Sahner et al. 2015 (Sahner et al., 2015), data available at the Dryad repository under doi:10.5061/dryad.qf362/.

3.2.3 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

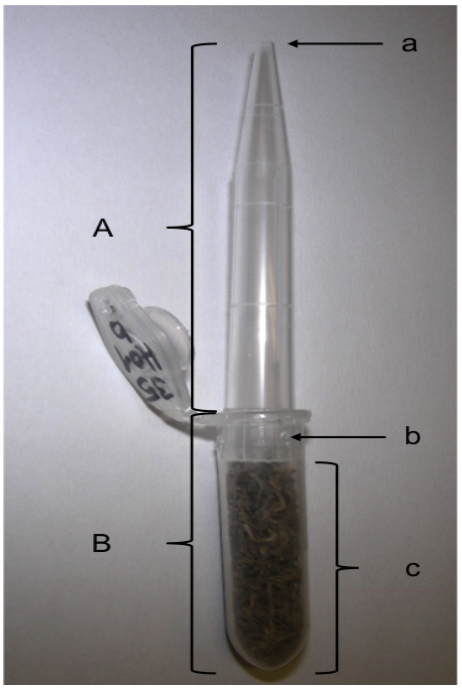

Figure 3.2.2: Setup for Freeze Drying and Storage of Fine Root Material. A 1000 μl pipet tip **(A)** was put into a 2 ml reaction tube **(B)** containing the fine root material **(c)**. The first 3 mm of the tip were cut to enlarge the aperture of the pipet tip **(a)**. After freeze drying the reaction tube was perforated **(b)**.

a sample collection permit (Rekomendasi Ijin Pengambilan dan Angkut (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).

3.2.4 Calculation of Land Use Intensity Index

A land use intensity index was calculated based on the approach by Blüthgen et al. (2012). Land use intensity in our case refers to intensity of management practices in from of levels of substance applications by farmers. The land use intensity index includes the intensity of fertilizer applications, herbicides applications, animal manure (cow compost) inputs, and soil amendments (lime) to the core plots. Across all core plots, seven different types of fertilizer (urea, potassium chloride (KCl), borate, nitrogen-phosphorus-potassium (NPK) fertilizer, triple superphosphate (TSP), 36 % superphosphate (SP 36), and kieserite) and four kinds of herbicides (Gramaxon, Noxone, Roundup, and Ally) were applied. Inputs of fertilizer, herbicides, lime, and cow compost were quantified as kilogram or liter per hectare and year because of missing information on the exact composition of chemicals and substances. The compound land use intensity index adds fertilizer plus herbicides plus soil amendment and animal manure intensity. Each individual component of land use treatment was standardized relative to its mean within the corresponding landscape. Land use intensity index L_i is defined as:

$$L_i = \frac{F2_i}{F2_L} + \frac{F2_i}{F2_L} + \frac{F3_i}{F3_L} + \frac{F4_i}{F4_L} + \frac{F5_i}{F5_L} + \frac{F6_i}{F6_L} + \frac{F7_i}{F7_L} + \frac{H1_i}{H1_L} + \frac{H2_i}{H2_L} + \frac{H3_i}{H3_L} + \frac{S_i}{S_L} + \frac{A_i}{A_L}$$

where $F1_i$ to $F7_i$ is the fertilization level, $H1_i$ to $H3_i$ the level of herbicide input, S_i the level of soil amendment and A_i the level of animal manure of each core plot and $F1_L$ to $F7_L$, $H1_L$ to $H3_L$, S_L, and A_L their respective mean in each landscape L. To reduce the impact of outliers and obtain a more even distribution a square root transformation was applied as $L_i = \sqrt{L_i}$. Data on material and substance inputs applied to the core plots were obtained from interviews with farmers.

3.2.5 DNA Extraction from Root Communities

Freeze dried fine roots were grounded to fine powder in a ball mill (MM 2000, Retsch, Haan, Germany) for three to four minutes with an amplitude of 90. In total, 100 mg of grounded fine root material per sample was weighed into a 2 ml reaction tube and used for DNA extraction. DNA was extracted using the innuPREP Plant DNA kit (Analytik Jena AG, Jena,

Germany), following the manufacturer's instructions (publication number of manual: HB_KS–1060_e_120116). For DNA extraction, lysis of grounded fine root material was performed for 60 min at 50 °C in a thermomixer (Thermomixer comfort, Eppendorf, Hamburg, Germany). Elution of DNA from the Spin Filter was done after an incubation of 15 minutes at room temperature (RT) with 100 μl nuclease-free water (Water for molecular biology, AppliChem GmbH, Darmstadt, Germany). DNA concentrations and the UV absorbance at 260 nm and 280 nm of the extracted DNA were measured by UV-Vis spectrometry using a nanoDrop 2000 (Thermo Fischer Scientific, Dreieich, Germany). The purity of DNA was determined by calculating the ratio of UV absorbance at 260 and 280 nm. Because of the poor quality of isolated DNA with 260/ 280 nm ratios below 1.8, the DNA isolates were purified again using the PowerClean® Pro DNA Clean-Up Kit (MoBio Laboratories Inc., Carlsbad, USA) following the manufacturer's instructions (protocol version 11172015).

3.2.6 Amplicon Library Preparation for Illumina Sequencing

For Illumina sequencing the fungal ITS1 region of environmental DNA was amplified using the ITS1–F_KYO2 (5' TAGAGGAAGTAAAAGTCGTAA 3'; Toju et al., 2012) and the ITS2 (5' GCTGCGTTCTTCATCGATGC 3'; White et al., 1990) primer with specific overhang adapters (adapter sequence 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG 3' of the forward primer and adapter sequence 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG 3' of the reverse primer (Nextera Transposase Adapter sequences, document number 1000000002694 v01, Illumina Inc., San Diego, USA) (Figure 6.1).
The DNA of each sample was amplified separately by polymerase-chain-reaction (PCR). PCR reactions were carried out in 200 μl reaction tubes (Sapphire PCR reaction tubes, 0.2 ml, PP, blue, Greiner Bio-One GmbH, Frickenhausen, Germany) in a total volume of 50 μl.
The reaction mix contained 2 μl DNA template (mean of DNA concentration 16.1 ng μl^{-1}, range 5.0 – 49.1 ng μl^{-1}), 1 μl forward primer, 1 μl reverse primer (primer ordered at Seqlab Sequence Laboratories Göttingen GmbH, Germany), 10 μl Phusion GC buffer, 0.15 μl $MgCl_2$, 0.5 μl Phusion HF DNA Polymerase (2 U μl^{-1}), 2.5 μl 5 % DMSO, 1 μl 10M dNTP mix (GC buffer, $MgCl_2$, Polymerase, DMSO and dNTPs were ordered at Thermo Fischer Scientific, Dreieich, Germany), 2.5 μl BSA (16 mg ml^{-1}, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), adjusted to the final volume of 50 μl with nuclease free water.

Amplification was performed in a PCR cycler (Labcycler, SensoQuest GmbH, Göttingen, Germany) with an initial denaturation for 30 seconds at 98 °C followed by 30 cycles of 1) denaturation at 98 °C for 10 seconds, 2) annealing at 47 °C for 20 seconds and 3) elongation at 72 °C for 20 seconds with a final extension of 5 min at 72 °C. For each PCR run a negative and positive control was performed.

The negative control contained no DNA template to check for possible contaminations of chemicals of the reaction mix. The positive control was performed with DNA extracted from ectomycorrhizal root tips (provided by Thomas Klein and Kristina Schröter, Department of Forest Botany and Tree Physiology, University of Göttingen, Germany) to examine the successful amplification of the fungal ITS1 region during PCR.

After each PCR run 2 μl of each PCR product and 2 μl of the negative and positive control were mixed with 1 μl 10 x DNA loading buffer and analyzed by gel electrophoresis (Power Pac 200, Bio Rad Laboratories Ltd., München, Germany) using a GelRed (0.02 μl ml^{-1}, GelRed™ Nucleic Acid Gel Stain, Biotium Inc., VWR International GmbH, Darmstadt, Germany) stained 1.1 % agarose gel (1.1 g agarose dissolved in 100 ml 1 x TAE buffer (for 100 ml: 0.48 g Tris, 0.11 ml acetic acid (99 – 100 %), 0.2 ml ethylenediaminetetraacetic acid (EDTA) with a pH of 8.0, adjusted to the final volume of 100 ml with ultra-purified water (ultra-pure water system, Arium 611, Sartorius, Göttingen, Germany)).

Afterwards, PCR products were purified using the innuPREP PCRpure Kit (Analytik Jena AG, Jena, Germany) following the manufacturer's instructions (publication number of manual: HB_KS–5010_e_120116). Elution of DNA from the Spin Filter was performed with 35 μl of nuclease free Water after an incubation for 15 min at RT. After purification of PCR products DNA concentrations were quantified using a Qubit™ dsDNA HS assay Kit in a Qubit fluorometer (Thermo Fischer Scientific, Dreieich, Germany).

Up to 6 independent PCRs per sample were carried out to get a sufficient quantity of DNA for pooling. PCR products from one sample were pooled at equimolar concentrations. The resulting pooled solutions were reduced to 30 μl using a concentrator (Eppendorf concentrator 5301, Eppendorf, Hamburg, Germany) at 45 °C and were separated by gel electrophoresis using a GelRed stained 1.1 % agarose gel. The expected length of PCR amplicons ranged between 300 and 500 base pairs (bp) (Toju et al., 2012; White et al., 1990). DNA bands with that length were cut on a UV-light table. The gel fragments were placed into 2 ml reaction tubes (Eppendorf micro tube 2 ml, Sarstedt, Nümbrecht, Germany) and purified using the QIAquick® Gel Extraction Kit (QIAGEN, Hilden, Germany) following the protocol (QIAGEN

Quick-Start Protocol, July 2016). Elution of DNA from the Spin Filter was performed with 34 μl of nuclease free water after incubation for 10 min at RT. After gel purification, DNA concentrations of final amplicons were quantified using a Qubit™ dsDNA HS assay Kit in a Qubit fluorometer.

For Illumina sequencing amplicon DNA concentrations were adjusted to 2 ng μl^{-1} by diluting with nuclease free water or concentrating using a concentrator. Amplicons were submitted to the Göttingen Genomics Laboratory which performed indexing PCR and sequencing. During indexing, PCR unique identifier (indices) and the Illumina adapter were attached to each amplicon (Figure 6.1). Subsequently, DNA concentrations of amplicons were quantified using a Qubit™ dsDNA HS assay Kit in a Qubit fluorometer. Amplicons were then pooled at equimolar concentrations and sequenced on the Illumina's MiSeq platform using the MiSeq Reagent Kit v3 (Illumina Inc., San Diego, USA).

3.2.7 Sequencing Processing

Initial processing and analyzes of the resulting ITS gene sequences from Illumina sequencing was done using the QIIME 1.9 software package (Caporaso et al., 2010) for performing microbiome analysis. For this purpose, sequences that fulfilled at least one of the following criteria were removed with *split_libraries.py*: the average quality score was lower than 20, containing unresolved nucleotides or harboring mismatches longer than 3 bp in the forward or reverse primer. For efficient forward and reverse primer removal we used cutadapt (Martin, 2011) with default settings. Chimeric sequences were removed using UCHIME (Edgar et al., 2011) with the reference dataset for UCHIME from the UNITE database (Abarenkov et al., 2010; Nilsson et al., 2015) available at https://unite.ut.ee/repository.php.

In preparation for operational taxonomic unit (OTU) clustering, we used USEARCH (Edgar et al., 2011; Nilsson et al., 2015) to dereplicate, remove singletons, and sort all quality filtered sequences by length. Subsequently, OTUs were clustered at 97 % sequence similarity among each other using USEARCH. Following, chimeric sequences were removed using UCHIME (Edgar et al., 2011) with the UCHIME reference dataset from the UNITE database (Abarenkov et al., 2010; Nilsson et al., 2015) available at https://unite.ut.ee/repository.php. Finally, all quality filtered sequences were mapped to chimara free OTUs with USEARCH and an OTU table was created using the perl script *uc2otutab.py* (http://drive5.com/python/uc2otutab_py.html).

Taxonomic affiliation of OTUs was performed with *parallel_assign_taxonomy_blast.py* against the same database used for chimera removal. To add the taxonomic information to OTU tables, the add-metadata function from the biom tools (McDonald et al., 2012) was used. Non-fungal OTUs were removed by employing *filter_otu_table.py* in QIIME. Unidentified fungal OTUs were blasted against the National Center for Biotechnology Information (NCBI) database and OTUs not belonging to the kingdom of fungi were removed manually from the OUT table. To assign the fungal OTUs to ecological guilds we used the open annotation tool FUNGuild (Nguyen et al., 2016) available at https://github.com/UMNFuN/FUNGuild by applying the Guilds python script.

3.2.8 Statistical Analysis

Diversity estimates and rarefactions curves were generated by using the *alpha_rarefaction.py* script in QIIME. Total plot level fungal species richness was calculated by rarifying plots to 12.789 sequences (lowest number of sequences across all plots) as described by Peay et al. (2013). To analyze fungal α-diversity among land use systems between the two different landscapes, we applied generalized linear model (GLM) with the *glm* function of the *multcomp* package (Hothorn et al., 2016) in R (R Core Team, 2015). To investigate differences of fungal α-diversity among land use systems only generalized mixed effect models with landscape as random effect with the *glmer* function of the *multcomp* package were applied. Differences of phylogenetic diversity, Shannon and Simpson index among land use systems were analyzed by linear mixed effects models with the *lmer* function of the *multcomp* package because data have a gaussian distribution. To test if there are significant differences among the means of fungal α-diversity from different land use systems analyses of deviance were conducted by applying the *anova* function with the additional option *test* = *"Chisq"*. If the p-value of the analyses of deviance was less or equal 0.05 and we could reject the null hypothesis ($\mu_1 = \mu_2 = \ldots = \mu_x$) the *glht* function was applied to do a multiple comparisons of means (post hoc test).
To test for the influence of different factors (land use and landscape) on fungal OTU composition, PERMANOVA using Bray-Curtis dissimilarity matrices were performed in R using the *adonis* function of the *vegan* packages.
NMDS of fungal communities was done using the *vegan* package (Oksanen et al., 2016) in R. Bray-Curtis dissimilarities matrixes were used for ordination. To test for significance of

explanatory environmental variables (Table 3.2.1) the *envfit* function in R was used and significant variables ($p \leq 0.05$) were plotted onto the NMDS (Schneider et al., 2015). Data on root performance traits, root chemical traits, soil properties, and litter properties were retrieved from Sahner et al. (2015) and Allen et al. (2015).
To analyze overlaps of fungal OTUs between landscapes and among the four different land use systems, Venn diagrams were generated using *draw.pairwise.venn* function and *draw.quad.venn* function of the *VennDiagram* and *limma* package in R. Calculations on percentage of shared fungal OTUs was performed as:

$$\text{Percentage of shared fungal OTUs of x and y} = \frac{\text{Number of shared OTUs between x and y}}{\text{Sum of different fungal OTUs of x and y}} * 100\,\%$$

For analyzing shifts in community structure OTUs assigned to ecological guilds with FUN-Guild were used. Relative abundances in percent were calculated for the ecological guilds of arbuscular mycorrhizal fungi, ectomycorrhizal fungi, plant pathogens, and saprotrophic fungi as:

$$\text{Relative abundance of x} = \frac{\text{Number of x sequence reads}}{\text{Total number of sequence reads}} * 100\,\%$$

Statistical tests on relative abundances of ecological guilds and fungal genera of ecological guilds in the different land use systems were conducted as described above with the *multcomp* package in R. non-metric multidimensional scaling (NMDS) of fungal communities belonging to ecological guilds was done as mentioned above with Bray-Curtis dissimilarities matrixes for ordination. To investigate the average contribution of each genus to the average overall Bray-Curtis dissimilarity of fungal genera, the *simper* function of the *vegan* package in R was used. This function performs a pairwise comparison of groups, in this case between land use systems, and displays the most important genera for each pair of groups.

Category	Variable	Abbreviation
root performance traits	fresh weight of fine roots	fw_fr
	dry weight of fine roots	fw_fr
	fine root water content	dater_fr
	distorted root tips	dead_rt
	vital non-ectomycorrhizal root tips	non_EM_rt
	vital ectomycorrhizal root tips	EM_rt
	total colonization by arbuscular mycorrhizal fungi (AMF)	AMtotal
	colonization by viscles of AMF	AMvis
	colonization by arbuscules of AMF	AMarb
	colonization by hyphae of AMF	AMhyph
	AMF spore number in soil	AMspore
root chemical traits	root carbon concentration	C
	root nitrogen concentration	N
	root carbon to nitrogen ratio	C.N
	root aluminium concentration	Al
	root calcium concentration	Ca
	root iron concentration	Fe
	root potassium concentration	K
	root magnesium concentration	Mg
	root manganese concentration	Mn
	root sodium concentration	S
	root phosphorus concentration	P
	root sulfur concentration	S
soil properties	soil pH value	pH
	gravimetric soil water content	Soil_moisture
	soil carbon concentration	C_soil
	soil nitrogen concentration	N_soil
	soil potassium concentration	K_soil
	soil magnesium concentration	Mg_soil
	soil calcium concentration	Ca_soil
	available phosphorus in soil	avail_P_soil
litter properties	litter carbon concentration	C_litter
	litter nitrogen concentration	N_litter
management	land use intensity	management
diversity indices	phylogenetic diversity	PD
	shannon index	Shannon

Table 3.2.1: Environmental Variables. Categories of environmental variables used to analyze their explanatory character for possible dissimilarities of fungal community compositions of the different land use systems.

3.3 Results

3.3.1 Diversity and Composition of Root-Associated Fungal Communities in Four Different Land Use Systems

It was possible to amplified a sufficient quantity of DNA of 92 from the initial 96 subplots (Table 3.3.1). By Illumina MiSeq sequencing 3.316.276 sequences were generated (Table eS 3.1). The sequence depth of subplots ranged between 89 and 179.248 sequence reads (Table 3.3.1). After quality and taxonomic filtering 2.801.095 fungal sequences remained, representing 4.405 different fungal operational taxonomic units (OTUs) (Table 3.3.1, Table eS 3.2).

The means of observed fungal sequence reads and numbers of fungal OTUs of samples pooled by core plots differed among land use systems (Figure 3.3.1). To compare fungal OTU richness of samples with different sample size (Figure 6.2 – S 3.3.4), fungal sequences of subplots from the same core plot were summed up and rarified. Sequences of core plots were rarified to 12.789 sequences (Table 3.3.1, Figure 3.3.2) representing the lowest sum of sequences reads found in one core plot. After rarefaction about 80 percent of fungal OTUs remained for further analysis (Table 3.3.1).

Land use system and landscape had a significant influence on fungal community composition (PERMANOVA, land use: $R^2 = 0.255$ and $p = 0.0001$; landscape: $R^2 = 0.058$ and $p = 0.0032$). Fungal OTU richness of rarified samples showed different patterns in Bukit12 and Harapan landscape (Figure 3.3.3 A). Rain forest sites of Bukit12 had a significantly higher fungal OTU richness jungle rubber and oil palm sites of Bukit12 landscape and rubber plantations of both landscapes (Figure 3.3.3 A). To investigate the differences of fungal OTU richness among land use systems independent of the landscape of origin, we run generalized linear mixed effect models with landscape as random effects to account for its observed influence on fungal community composition. Fungal OTU richness was highest in rain forest sites and lowest in rubber plantations (Figure 3.3.3 B). Chao 1 and Shannon index showed the same patterns for differences among land use systems as fungal OTU richness (Table 3.3.2). Phylogenetic diversity was significantly higher in rain forest sites compared to the highly managed rubber and oil palm plantations (Table 3.3.2).

Landscapes			Land Use System			Core Plots			Subplots				
Land-scape	Fungal OTU number	Fungal OTU number after rarifying	Land use	Fungal OTU number	Fungal OTU number after rarifying	Core plot	Fungal OTU number	Fungal OTU number after rarifying	Subplot	Sequence number before quality filtering	Sequence number after quality filtering	Sequence number after taxonomy filtering	Fungal OTU number
Bukit 12	3305	2446	**Forest**	2092	1706	**BF 1**	1179	856	**BF 1a**	29713	29583	17827	854
									BF 1b	21749	21719	14258	468
									BF 1c	11300	11127	7705	401
						BF 2	1190	818	**BF 2a**	25147	25069	22784	851
									BF 2b	11161	11137	2334	206
									BF 2c	14248	13810	9205	652
						BF 3	532	516	**BF 3a**	5616	3470	1581	184
									BF 3b	13981	11695	5323	203
									BF 3c	9763	9711	6912	319
						BF 4	517	517	**BF 4a**	6962	6933	6076	242
									BF 4b	4668	4565	4021	245
									BF 4c	3136	3079	2692	205
			Jungle rubber	1594	891	**BJ 1**	858	309	**BJ 1a**	3269	3267	3112	134
									BJ 1b	99124	97790	78650	463
									BJ 1c	179667	179248	164033	662
						BJ 2	472	142	**BJ 2a**	109866	109789	108083	196
									BJ 2b	30656	30631	29907	270
									BJ 2c	29835	29791	29083	237
						BJ 3	587	383	**BJ 3a**	12147	5450	5148	197
									BJ 3b	2732	2710	2560	96
									BJ 3c	29117	29045	26400	463
						BJ 4	649	433	**BJ 4a**	704	693	487	88
									BJ 4b	46327	46126	39682	521
									BJ 4c	24279	19769	5453	198
			Rubber	1120	571	**BR 1**	713	249	**BR 1a**	150445	148868	146201	344
									BR 1b	42142	41830	40196	225
									BR 1c	63127	49284	46756	463
						BR 2	406	272	**BR 2a**	8187	8160	7740	200
									BR 2b	22684	22670	22048	250
									BR 2c	5889	5832	5321	193
						BR 3	477	215	**BR 3a**	13296	13179	12652	49
									BR 3b	86312	86171	82676	401
									BR 3c	67468	42654	41474	207
						BR 4	298	193	**BR 4a**	17018	16973	16533	105
									BR 4b	9829	9742	9480	150
									BR 4c	12036	11980	11432	187
			Oil palm	1270	659	**BO 1**	547	213	**BO 1a**	130539	130360	126505	258
									BO 1b	50791	50658	48948	357
									BO 1c	4458	4454	4368	55
						BO 2	307	272	**BO 2a**	11779	3563	3135	101
									BO 2b	6911	6883	6602	151
									BO 2c	12737	11310	10937	184
						BO 3	558	211	**BO 3a**	7461	7449	7233	135
									BO 3b	124471	124193	121313	366
									BO 3c	124544	124395	118803	336
						BO 4	784	362	**BO 4a**	99175	98700	95572	708
									BO 4b	3789	3431	2605	76
									BO 4c	11690	10492	9956	146
Harapan	3545	2557	**Forest**	1578	1012	**HF 1**	553	368	**HF 1a**	7393	4583	2879	143
									HF 1b	19173	19129	17976	213
									HF 1c	35011	34933	31267	382
						HF 2	874	539	**BO 2a**	5382	5084	3666	205
									HF 2b	88936	88125	59277	742
									HF 2c	1717	1700	1427	177
						HF 3	688	280	**BO 3a**	8512	8492	7891	218
									HF 3b	42503	40261	32282	305
									HF 3c	92571	92463	89653	390

				HF 4	840	465	**BO 4a**	39106	39035	34644	276
							HF 4b	32146	31532	23538	482
							HF 4c	15510	15441	13817	485
	Jungle rubber	1750	1077	**HJ 1**	754	318	**HJ 1a**	9027	8330	7620	190
							HJ 1b	41558	41505	38211	433
							HJ 1c	63701	63454	55049	491
				HJ 2	751	540	**HJ 2a**	15782	12105	11260	267
							HJ 2b	13029	12675	11908	442
							HJ 2c	28613	20995	6935	247
				HJ 3	943	489	**HJ 3a**	22088	22013	18334	413
							HJ 3b	42067	29122	24623	462
							HJ 3c	31009	30964	29805	446
				HJ 4	378	260	**HJ 4a**	NA	NA	NA	NA
							HJ 4b	NA	NA	NA	NA
							HJ 4c	67556	60409	36853	378
	Rubber	1391	901	**HR 1**	483	293	**HR 1a**	NA	NA	NA	NA
							HR 1b	5977	5972	5839	97
							HR 1c	56565	46374	42197	460
				HR 2	374	257	**HR 2a**	NA	NA	NA	NA
							HR 2b	3975	3950	3546	278
							HR 2c	25413	25048	22149	154
				HR 3	852	401	**HR 3a**	55305	54898	52411	391
							HR 3b	22753	22686	21597	537
							HR 3c	26852	26684	23940	382
				HR 4	524	419	**HR 4a**	20418	20391	12504	443
							HR 4b	11741	10491	9032	204
							HR 4c	89	86	70	15
	Oil palm	2014	1105	**HO 1**	715	254	**HO 1a**	46442	46398	437983	364
							HO 1b	6108	6098	5864	99
							HO 1c	151554	147605	140565	559
				HO 2	791	421	**HO 2a**	1393	1366	1313	87
							HO 2b	108173	46410	44833	394
							HO 2c	27364	27318	25909	545
				HO 3	1349	485	**HO 3a**	43305	43221	40647	964
							HO 3b	96779	96580	94412	367
							HO 3c	35427	34999	32828	833
				HO 4	807	606	**HO 4a**	29578	12807	12221	269
							HO 4b	3544	3539	3346	170
							HO 4c	13186	12909	11372	622

Table 3.3.1: Observed Number of Fungal Sequence Reads and Fungal OTUs on Sample Level. The table shows the difference in observed numbers sequences and fungal OTUs. The number of sequence reads represents the sequence depth of each sample (sample equals subplot). B = Bukit12 landscape, H = Harapan landscape, F = rain forest, J = jungle rubber, R = rubber plantations, O = oil palm plantations, 1−4 = number of core plot, a−c = subplot names and NA = not available.

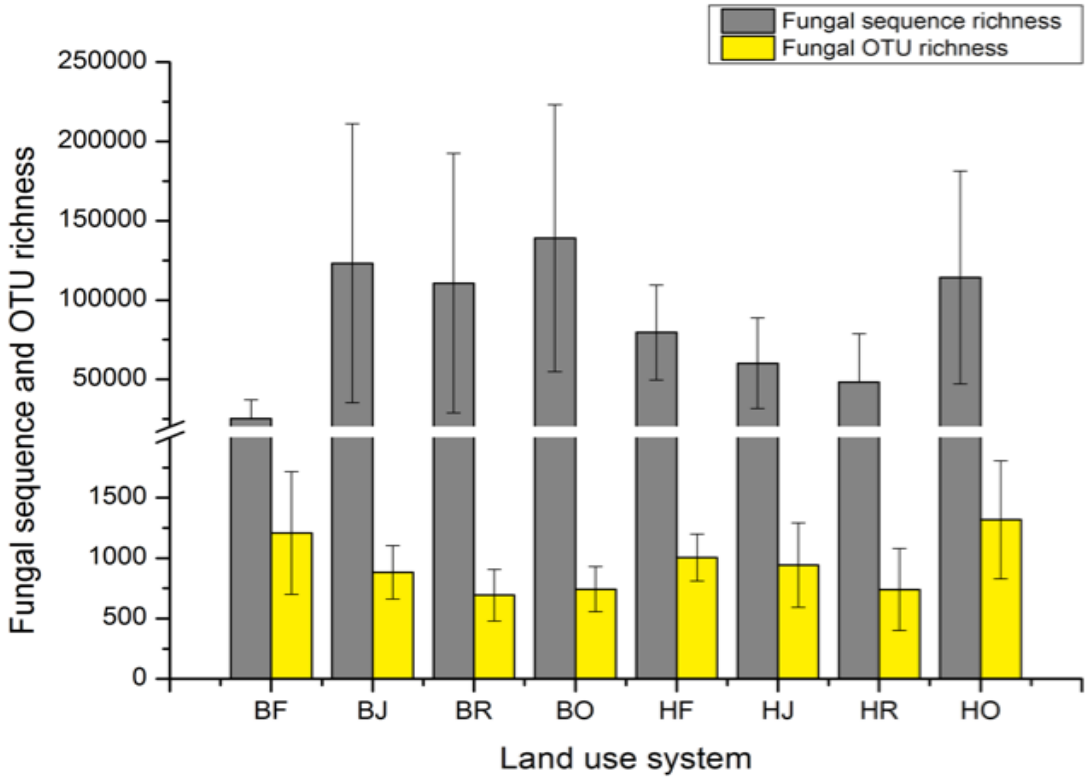

Figure 3.3.1: Richness of Fungal Sequences and OTUs on Land Use Landscape Level. Bars represent the means of sequence reads and number of OTUs of samples on land use landscape level with standard deviation. B = Bukit12 landscape, H = Harapan landscape, F = rain forest, J = jungle rubber, R = rubber plantations and O = oil palm plantations. N = 92.

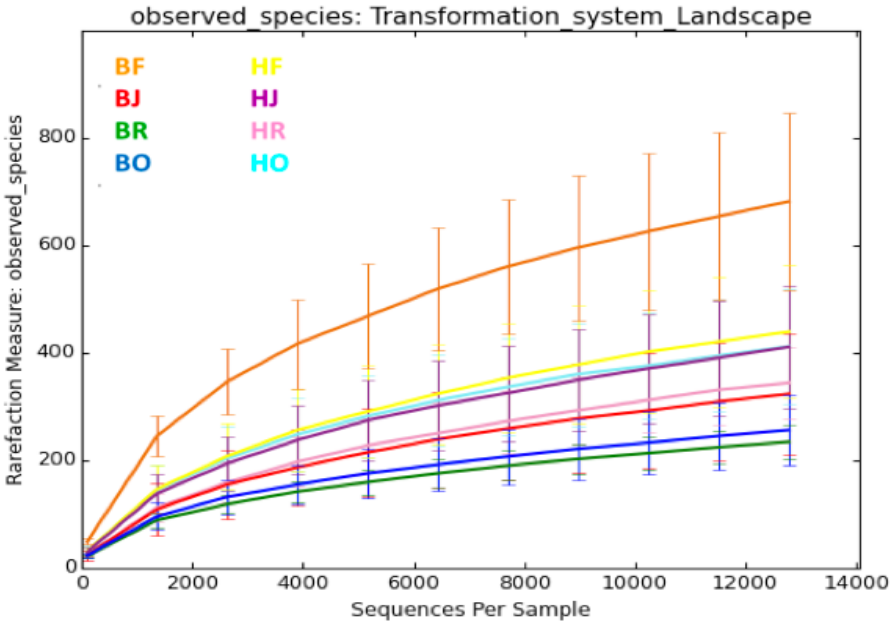

Figure 3.3.2: Rarefaction Curve on Core Plot Level Rarified to 12.789 Sequences in the Two Different Landscapes. Rarefaction curves show the average number of sequence reads of land use system and landscape with standard deviations. B = Bukit12 landscape, H = Harapan landscape, F = rain forest, J = jungle rubber, R = rubber plantations, and O = oil palm plantations. N = 32.

About 10 % of fungal OTUs were shared among the four different land use systems (Figure 3.3.4). Rain forest sites shared the highest number of fungal OTUs with jungle rubber sites (31 %), followed by oil palm plantations (28 %) and rubber plantations (25 %) (Figure 3.3.4). Number of shared fungal OTUs among the other land use systems differed between 28 and 30 percent (Jungle rubber-rubber 30 %, jungle rubber-oil palm 29 %, rubber-oil palm 28 %) (Figure 3.3.4). In Bukit12 landscape 68 % of the remaining fungal OTUs were found and 71 % in Harapan. The two landscapes shared 40 % of different fungal OTUs (Figure 6.6 A). In rain forest sites of both landscapes 63 %of fungal OTUs were present and the forest sites from Harapan and Bukit12 landscape shared 21 %of their fungal OTUs (Figure 6.6 B). Jungle rubber sites in Harapan and Bukit12 landscape included together 44 %of fungal OTUs and shared 24 % of their fungal OTUs (Figure 6.6 C). Rubber plantations of both landscapes contained 32 %of all fungal OTUs and shared 27 % of fungal OTUs (Figure 6.6 D). In oil palm plantations 38 % of fungal OTUs were found in both landscapes and shared 28 % of fungal

OTUs between landscapes (Figure 6.6 E).

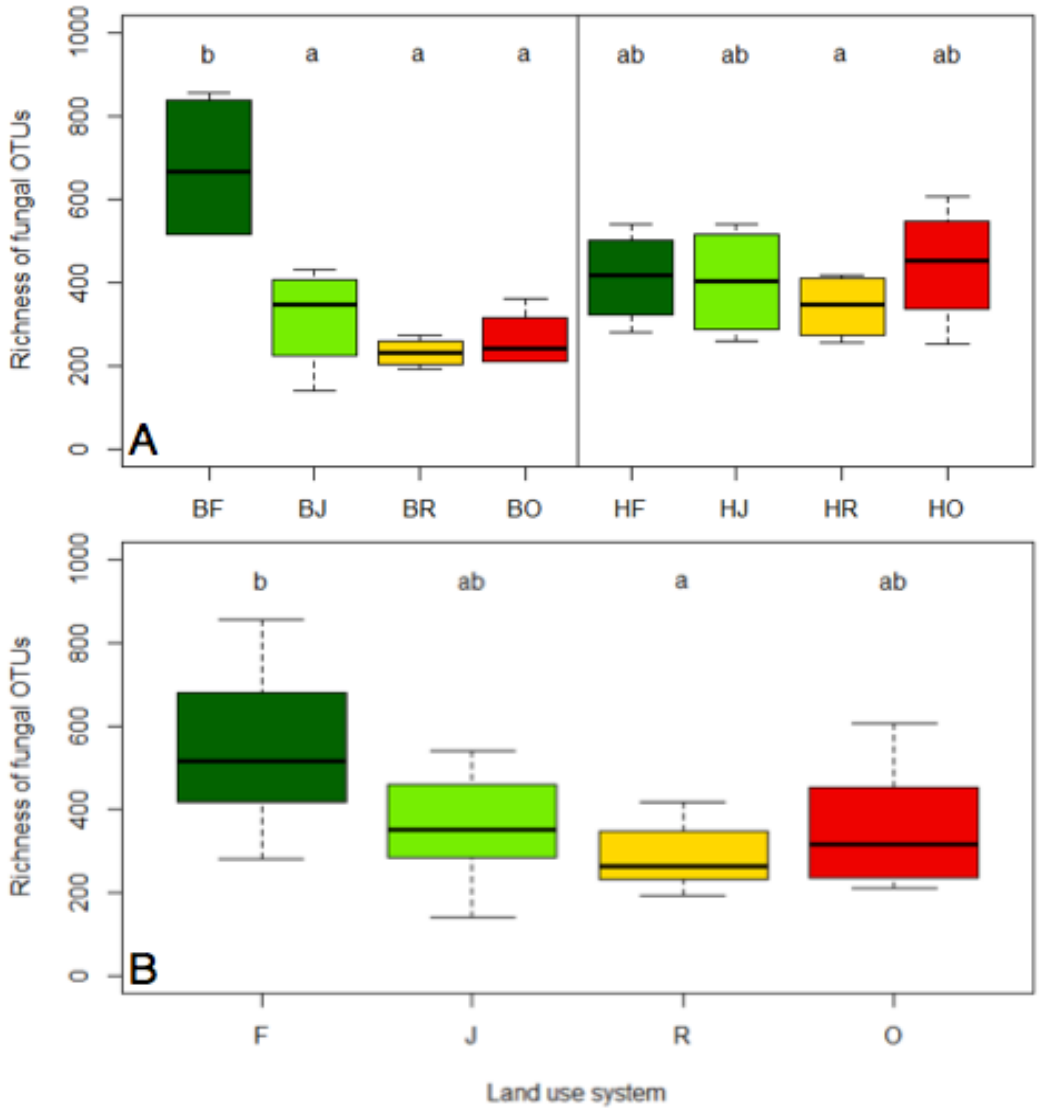

Figure 3.3.3: Fungal OTU Richness of Samples Rarified to 12.789 Sequences. Box-Whisker plots represent the number of OTUs of core plots with standard deviation. **A)** Fungal OTUs richness of different land use systems separated by landscapes. **B)** Fungal species richness in four different land use systems. For statistical analyses, generalized linear models and generalized linear mixed effect models for A and B were performed, respectively. Significant differences between means of groups are indicated by letters with $p \leq 0.05$. B = Bukit12 landscape, H = Harapan landscape, F = rain forest, J = jungle rubber, R = rubber plantations, and O = oil palm plantations. N = 92. N = 32.

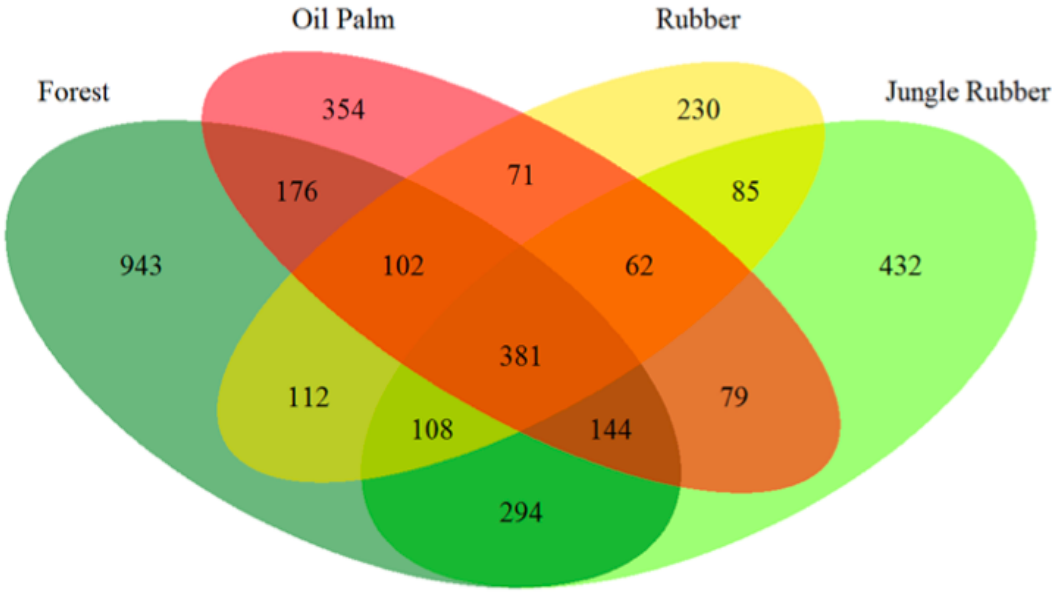

Figure 3.3.4: Venn Diagram of Shared and Unique Shared Fungal OTUs Among the Four Different Land Use Systems. Each colored circle represents a land use system. Numbers in the circles and in overlaps between and among different circles indicate the number of fungal OTUs shared and non-shared between and among land use systems.

Land use system	OTU richness	Michaelis Menten Fit	Km	Phylogenetic diversity	Chao 1	Shannon	Simpson
Forest	545 ± 187**b**	699 ± 251	4327 ± 863	296.04 ± 84.60 **b**	797 ± 265 **b**	5.17 ± 1.26 **b**	0.86 ± 0.13 **a**
Jungle rubber	359 ± 121**ab**	476 ± 160	5403 ± 1647	205.09 ± 62.14 **ab**	560 ± 183 **ab**	3.94 ± 1.28 **ab**	0.78 ± 0.17 **a**
Rubber	287 ± 77**a**	374 ± 106	4873 ± 1108	170.72 ± 39.34 **a**	475 ± 123 **a**	3.54 ± 0.94 **a**	0.73 ± 0.17 **a**
Oil palm	353 ± 133 **ab**	461 ± 186	4620 ± 987	201.76 ± 70.79 **ab**	584 ± 235 **ab**	4.29 ± 0.87 **ab**	0.87 ± 0.05 **a**

Table 3.3.2: Diversity Indices, Estimates for Species Richness and Half Saturation of Rarified Samples on Land Use Level. For statistical analyses, generalized linear and linear mixed effect models were performed. Significant differences between means of groups are indicated by letters with $p \leq 0.05$, $n = 32$. OTU richness = calculation for observed species at a sequence depth of 12.789 sequence reads. Michaelis Menten fit = estimation for maximum species richness. Km = Michaelis Menten constant.

3.3.2 Taxonomic Composition of Root-Associated Fungal Communities

Fungal OTUs belonging to Ascomycota showed a high abundance in all land use systems (Figure 3.3.5 A, Table 3.3.3 A). In oil palm plantations they were significantly more abundant than in rain forest sites (Table 3.3.3 A). The Basidiomycota was the fungal phyla with the second highest abundance across all land use systems (Figure 3.3.5 A, Table 3.3.3 A). Basidiomycota had a significant higher abundance in jungle rubber sites than in oil palm plantations (Table 3.3.3 A). Glomeromycota were significantly more abundant in rain forest sites compared to all other three land use systems (Table 3.3.3 A). The abundances of Rozellomycota showed no significant differences (Table 3.3.3 A). The phylum Zygomycota was significantly most abundant in rain forest sites compared to the other land use systems (Figure 3.3.5 A, table 3.3.3 A). The relative abundance of unidentified fungal OTUs was highest in rain forest sites and lowest in jungle rubber sites (Figure 3.3.5 A, table 3.3.3 A). In total, 106 different fungal orders were found in the four different land use systems (Table S 3.1). Of these orders, 22 showed an abundance above 0.5 % in at least one of the land use systems (Figure 3.3.5 B). The most abundant orders with or more than 5 % mean relative abundance in at least one land use system were Pleosporales, Helotiales, Glomerellales, Hypocreales, Xylariales, Agricales, Tremellales, and Mortierellales (Table 3.3.3 B).

Pleosporales and Glomerellales had the significantly highest abundance in oil palm plantations compared to other land use systems (Table 3.3.3 B). Mortierellales were significantly more abundant in rain forest sites compared to the three other land use systems (Table 3.3.3 B). Helotiales had the significantly lowest abundance in oil palm plantations compared to the other systems (Table 3.3.3 B). The fungal order Xylariales had a significantly higher relative abundance in jungle rubber sites compared to rain forest sites and oil palm plantations (Table 3.3.3 B). Fungal OTUs belonging to Hypocreales showed a significantly higher relative abundance in oil palm plantations than in rain forest and jungle rubber sites (Table 3.3.3 B).

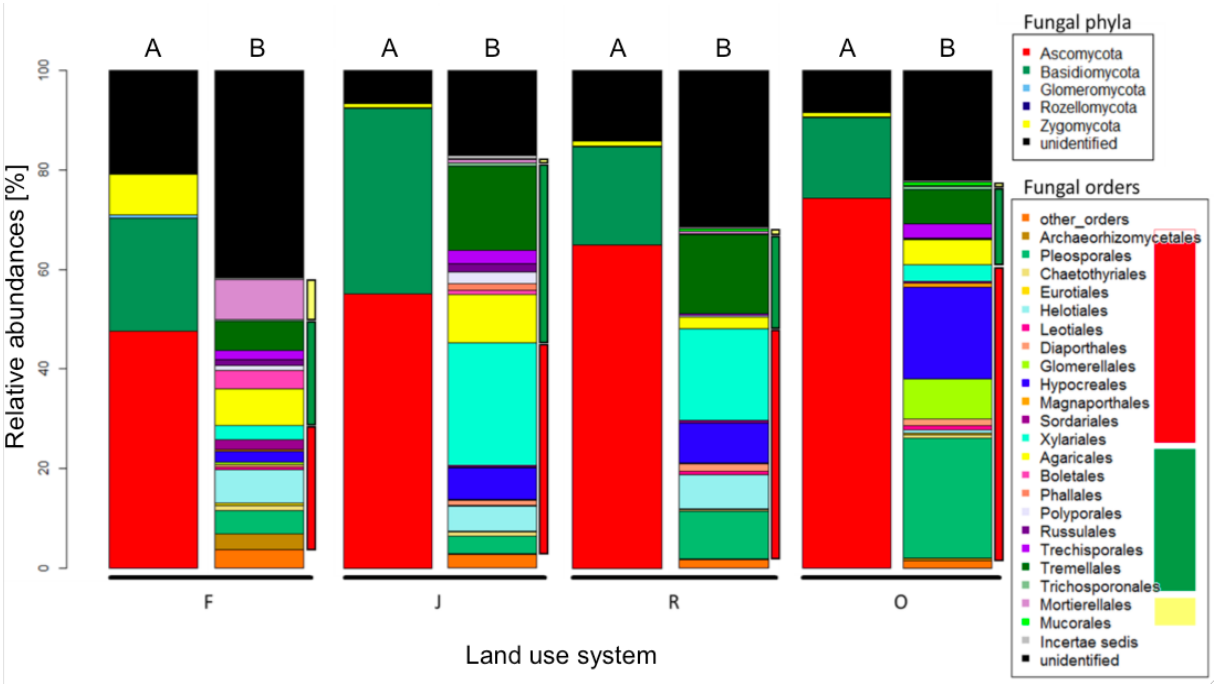

Figure 3.3.5: Relative Abundances of Fungal Phyla (A) and Orders (B) in Four Different Land Use Systems. Bar charts represent the relative abundances of fungal phyla and orders, with the number sequence reads of a taxonomic group in proportion to the total number of sequence reads of each core plot. Small bars close to bars representing the relative abundance of fungal orders (B) are indicating to which fungal phylum the orders belong.F = rain forest, J = jungle rubber, R = rubber plantations and O = oil palm plantations. N = 32.

Phyla	Rain forest	Jungle rubber	Rubber plantation	Oil palm plantations
Acsomycota	47.58 ± 16.66 **a**	55.04 ± 20.12 **ab**	64.90 ± 21.00 **ab**	74.23 ± 15.30 **b**
Basidiomycota	22.71 ± 10.19 **ab**	37.34 ± 16.84 **b**	19.68 ± 16.00 **ab**	16.36 ± 12.85 **a**
Glomeromycota	0.69 ± 0.38 **c**	0.18 ± 0.11 **ab**	0.28 ± 0.18 **b**	0.09 ± 0.06 **a**
Rozellomycota	0.01 ± 0.03 **a**	0.00 ± 0.00 **a**	0.01 ± 0.03 **a**	0.01 ± 0.03 **a**
Zygomycota	8.14 ± 6.91 **b**	0.80 ± 0.92 **a**	0.89 ± 1.13 **a**	0.88 ± 1.57 **a**
Unidentified fungi	20.86 ± 14.02 **b**	6.61 ± 4.81 **a**	14.24 ± 16.54 **ab**	8.40 ± 5.85 **ab**
Orders				
Pleosporales	4.81 ± 4.30 **a**	3.46 ± 1.79 **a**	9.54 ± 5.27 **a**	24.05 ± 9.34 **b**
Heliotales	6.67 ± 5.71 **b**	5.06 ± 4.39 **b**	6.99 ± 5.97 **b**	0.81 ± 0.70 **a**
Glomerellales	0.43 ± 0.40 **a**	0.20 ± 0.13 **a**	0.16 ± 0.09 **a**	7.96 ± 5.46 **b**
Hypocreales	2.16 ± 1.85 **a**	6.38 ± 11.30 **ab**	7.96 ± 4.96 **bc**	18.49 ± 11.28 **c**
Xylariales	2.83 ± 4.06 **a**	24.50 ± 24.85 **c**	18.34 ± 20.89 **bc**	3.36 ± 1.59 **ab**
Agaricales	7.39 ± 4.38 **a**	9.61 ± 14.28 **a**	2.45 ± 1.42 **a**	4.99 ± 5.16 **a**
Tremellales	5.81 ± 8.86 **a**	16.93 ± 16.11 **a**	15.84 ± 15.70 **a**	6.91 ± 5.54 **a**
Mortierellales	8.08 ± 6.92 **b**	0.76 ± 0.92 **a**	0.41 ± 0.80 **a**	0.19 ± 0.15 **a**

Table 3.3.3: Relative Abundances of Fungal Phyla (A) and Orders (B). Comparison of relative abundances of fungal phyla and orders, with the number of sequence reads of a taxonomic group in proportion to the total number of sequence reads of each core plot. For statistical analyses, generalized linear mixed effect models were performed. Significant differences between means of groups are indicated by letters with $p \leq 0.05$, $n = 32$.

3.3.3 Land Use Intensity of the Investigated Core Plots

Land use intensity, based on the calculated land use intensity index, varied among land use systems (Table 3.3.4). In rain forest and jungle rubber sites no land use practices were performed, resulting in the lowest possible land use intensity (Table 3.3.4, Figure 3.3.6). Land use intensity of rubber and oil palm plantations was significantly higher than in rain forest and jungle rubber plots with the highest land use intensity in oil palm plantations (Table 3.3.4, Figure 3.3.6).

Core plot	Calculated land use intensity indices	Land use intensity indices for multivariate statistics
BF 1	0	0
BF 2	0	0
BF 3	0	0
BF 4	0	0
BJ 1	NA	0
BJ 2	NA	0
BJ 3	0	0
BJ 4	NA	0
BO 1	NA	2.16
BO 2	1.27	1.27
BO 3	3.85	3.85
BO 4	1.35	1.35
BR 1	2.61	2.61
BR 2	0	0
BR 3	2.22	2.22
BR 4	2.45	2.45
HF 1	0	0
HF 2	0	0
HF 3	0	0
HF 4	0	0
HJ 1	0	0
HJ 2	NA	0
HJ 3	0	0
HJ 4	NA	0
HO 1	2.91	2.91
HO 2	2.14	2.14
HO 3	5.60	5.60
HO 4	3.93	3.93
HR 1	1.71	1.71
HR 2	0	0
HR 3	1.50	1.50
HR 4	NA	1.07

Table 3.3.4: Land Use Intensity (LUI) indices of Core Plots in the Four Different Land Use Systems. Calculated LUI indices are shown as well as additional LUI indices for core plots where calculation from available data was not possible and used for multivariate statistics. B = Bukit12 landscape, H = Harapan landscape, F = rain forest, J = jungle rubber, R = rubber plantations, and O = oil palm plantations. Numbers 1−4 = core plot ID numbers. NA = not available.

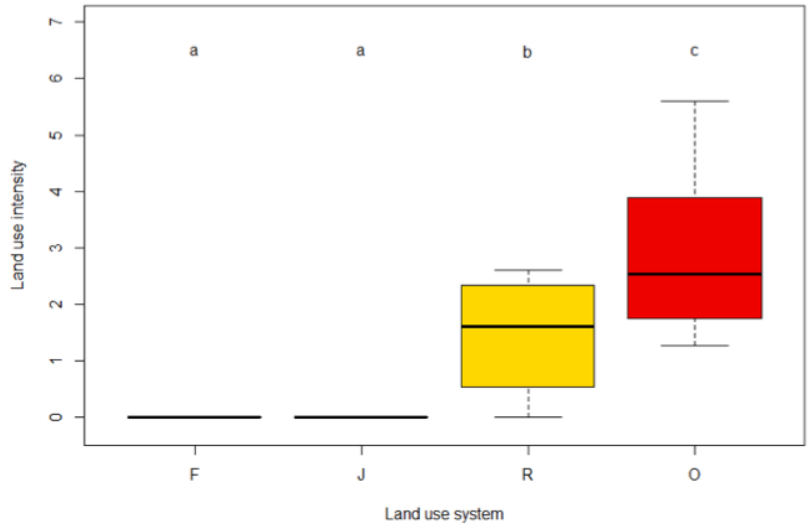

Figure 3.3.6: Land Use Intensity of the Four Investigated Different Land Use Systems. Box-Whisker plots represent the land use intensity of core plots with standard deviation. For statistical analyses, linear models were performed. Significant differences between means of groups are indicated by letters with $p \leq 0.05$. B = Bukit12 landscape, H = Harapan landscape, F = rain forest, J = jungle rubber, R = rubber plantations, and O = oil palm plantations.

3.3.4 Dissimilarities of Root-Associated Fungal Communities Referring to Land Use

Dissimilarities of fungal communities were visualized by non-metric multidimensional scaling (NMDS, Figure 3.3.7). Fungal community composition differed significantly among land use systems (PERMANOVA, $R^2 = 0.255$ and $p = 0.0001$). We tested 36 possible variables in order to explain the dissimilarity and distribution of fungal communities of NMDS. These explanatory variables belonged to six different groups: root performance traits, root chemical traits, soil properties, litter properties, land use intensity, and diversity indices (Table 3.2.1). Variables explaining the distribution along the NMDS 1 and, therefore, the dissimilarities among land use systems are land use intensity, root aluminum, iron, sulfur, nitrogen, and carbon concentrations as well as concentrations of available phosphorus and numbers of arbuscular mycorrhizal spores

in soil. Root-associated fungal communities from rain forests had the highest beta-diversity among sampling sites whereas fungal communities from oil palm plantation had the lowest (Table 3.3.5).

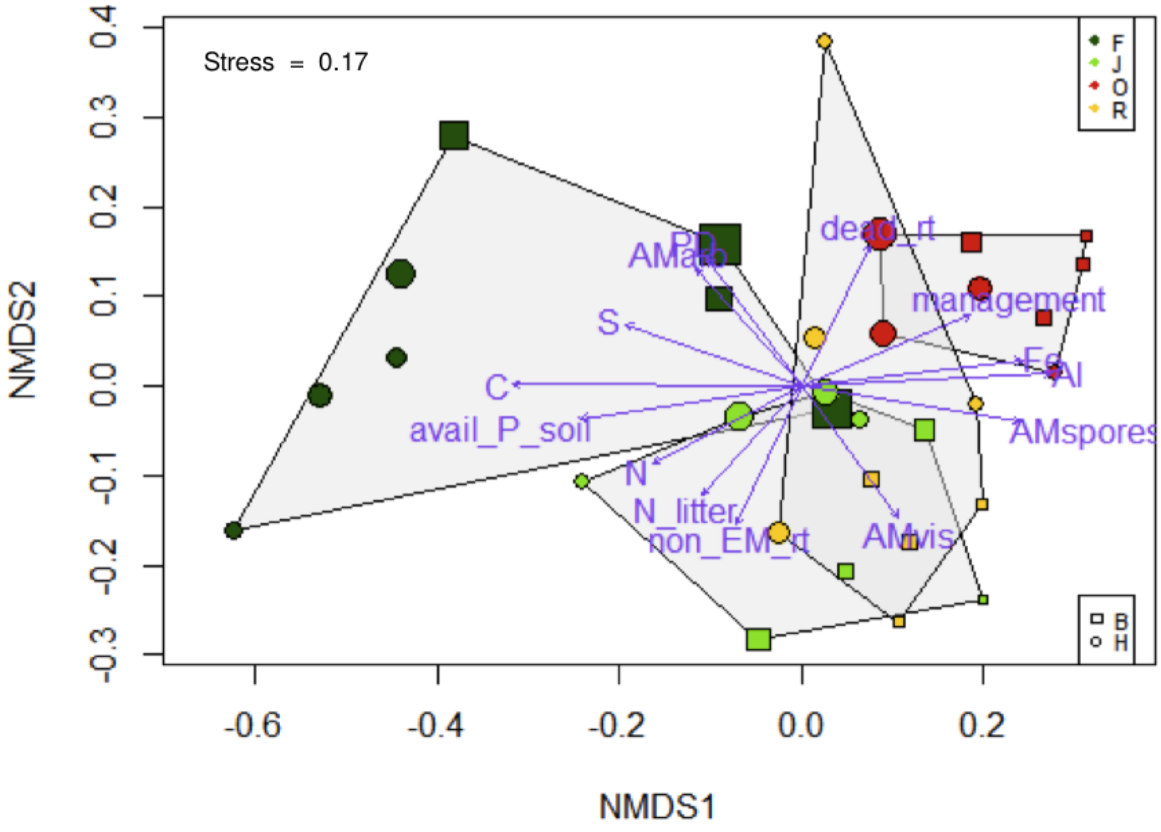

Figure 3.3.7: Non-Metric Multidimensional Scaling (NMDS) of Fungal OTU Communities Based on Bray Curtis Distance Matric. Samples were pooled by core plots and rarified to 12.789 sequences. Significant correlations of environmental parameters and diversity metrics to community composition are shown by purple arrows ($p \leq 0.05$). Sizes of plots (squares and circles) correspond to the phylogenetic diversity (PD). F = forest, J = jungle rubber, R = Rubber plantations, O = oil palm plantations Abbreviations for explanatory variables are shown in table 3.2.1.

Land use system	Total beta-diversity
Forest	0.417
Jungle rubber	0.365
Rubber	0.376
Oil palm	0.284

Table 3.3.5: Total Beta-Diversity of Root-Associated Fungal Communities.

3.3.5 Assignment of Root-Associated Fungal OTUs to Guilds and Functional Groups

About 30 percent of fungal OTUs were assigned to an ecological guild (Table eS 3.3). These guilds were grouped to five functional groups: arbuscular mycorrhizal fungi (AMF), ectomycorrhizal fungi (EMF), plant pathogenic fungi, saprotrophic fungi and fungi of other guilds (Table 3.3.6).

Functional group	Guild	Trophic Mode
Arbuscular mycorrhizal fungi	Arbuscular Mycorrhiza	Symbiotroph
Ectomycorrhizal fungi	Ectomycorrhiza	Symbiotroph
	Ectomycorrhiza - Saprotroph	Saprotroph - Symbiotroph
Plant pathogenic fungi	Plant Pathogen	Pathotroph
	Plant Pathogen - Endophyte	Pathotroph
	Plant Pathogen - Mycoparasite	Pathotroph
	Plant Pathogen - Saprotroph	Pathotroph - Saprotroph
	Plant Pathogen - Wood Saprotroph	Pathotroph - Saprotroph
Saprotrophic fungi	Litter Saprotroph	Saprotroph
	Soil Saprotroph	Saprotroph
	Undefined Saprotroph	Saprotroph
	Wood Saprotroph	Saprotroph
Fungi of other guilds	Animal Pathogen	Pathotroph
	Animal Pathogen - Endophyte - Saprotroph	Pathotroph - Saprotroph
	Animal Pathogen - Saprotroph	Pathotroph - Saprotroph
	Endophyte	Pathotroph
	Foliar Epiphyte	Saprotroph
	Lichenized	Symbiotroph
	Mycoparasite	Pathotroph
	Undefined Root Endophyte	Pathotroph - Symbiotroph

Table 3.3.6: Ecological Fungal Guilds. Table shows ecological fungal guilds found in samples. Categorization = categories of merged guilds assigned by FUNGuild, Guilds = names of ecological guilds assigned by FUNGuild, Trophic Mode = trophic mode of ecological guilds assigned by FUNGuild.

3.3.6 Contribution of Specific Fungal Genera Assigned to an Ecological Guild to Dissimilarities Among Root-associated Fungal Communities From Different Land Use Systems

The relative abundances of fungal genera assigned to ecological guilds and functional groups showed differences among land use systems (Figure 3.3.8). Out of these assigned genera, we identified 11 specific fungal genera across all land use systems which contributed the most to the dissimilarity in community composition between pairs of land use systems (Table 3.3.9, Figure 3.3.9). Whenever comparing oil palm plantations to one of the other land uses, only a few specific fungal genera contributed to the dissimilarity of community composition (Table 3.3.9, Figure 3.3.9). In contrast, in pairwise comparison of rain forests to the other three land use system more specific fungal genera were involved explaining the dissimilarities between fungal communities (Table 3.3.9, Figure 3.3.9). *Fusarium* and *Pyrenochaetopsis* had an influence on the dissimilarity between all pairs of land use systems (Table 3.3.9, Figure 3.3.9). For the dissimilarity between forest sites and the other land use systems *Mortierella* was also important (Table 3.3.9, Figure 3.3.9).

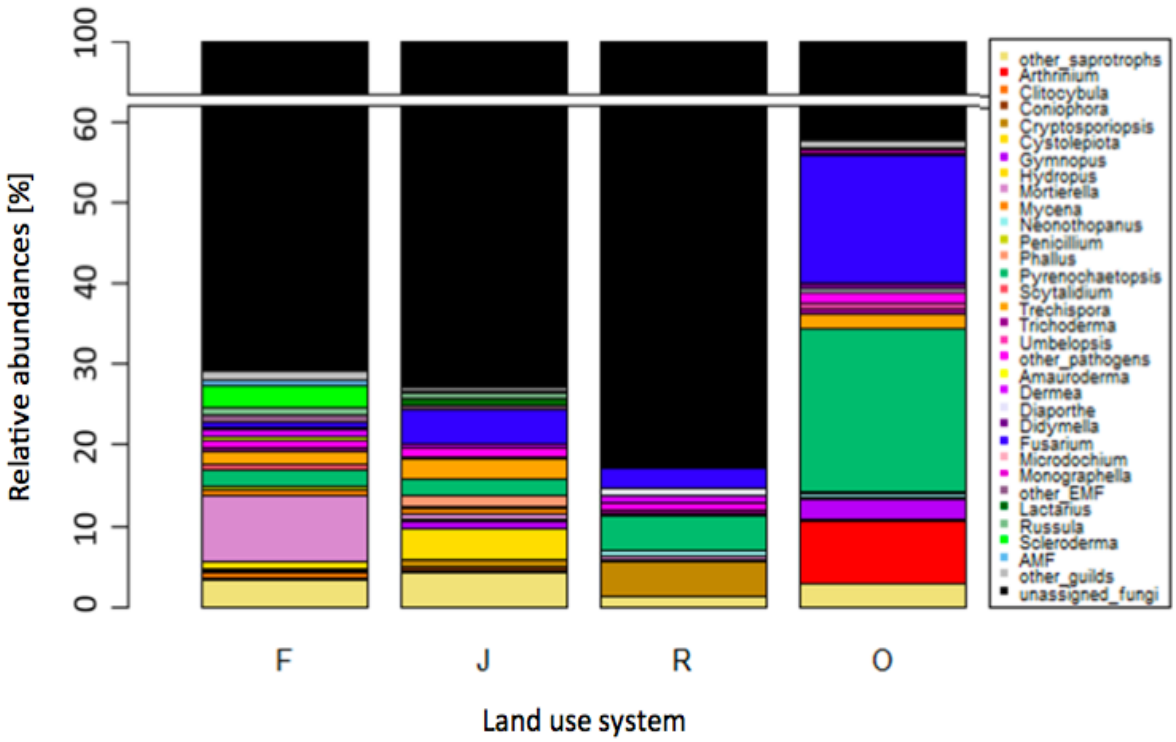

Figure 3.3.8: Relative Abundances of Fungal Genera Assigned to Ecological Guilds in Four Different Land Use Systems. Relative abundances of fungal genera are represented in percentage, with the ofnumber sequence reads of an ecological group in proportion to the total number of sequence reads in each core plot. Genera with abundances of or above 0.05 % are represented by th eir names. Remaining genera were grouped together and are represented as others (e.g. other _saprotrophs). F = rain forest, J = jungle rubber, R = rubber plantations and O = oil palm plantations. N = 32.

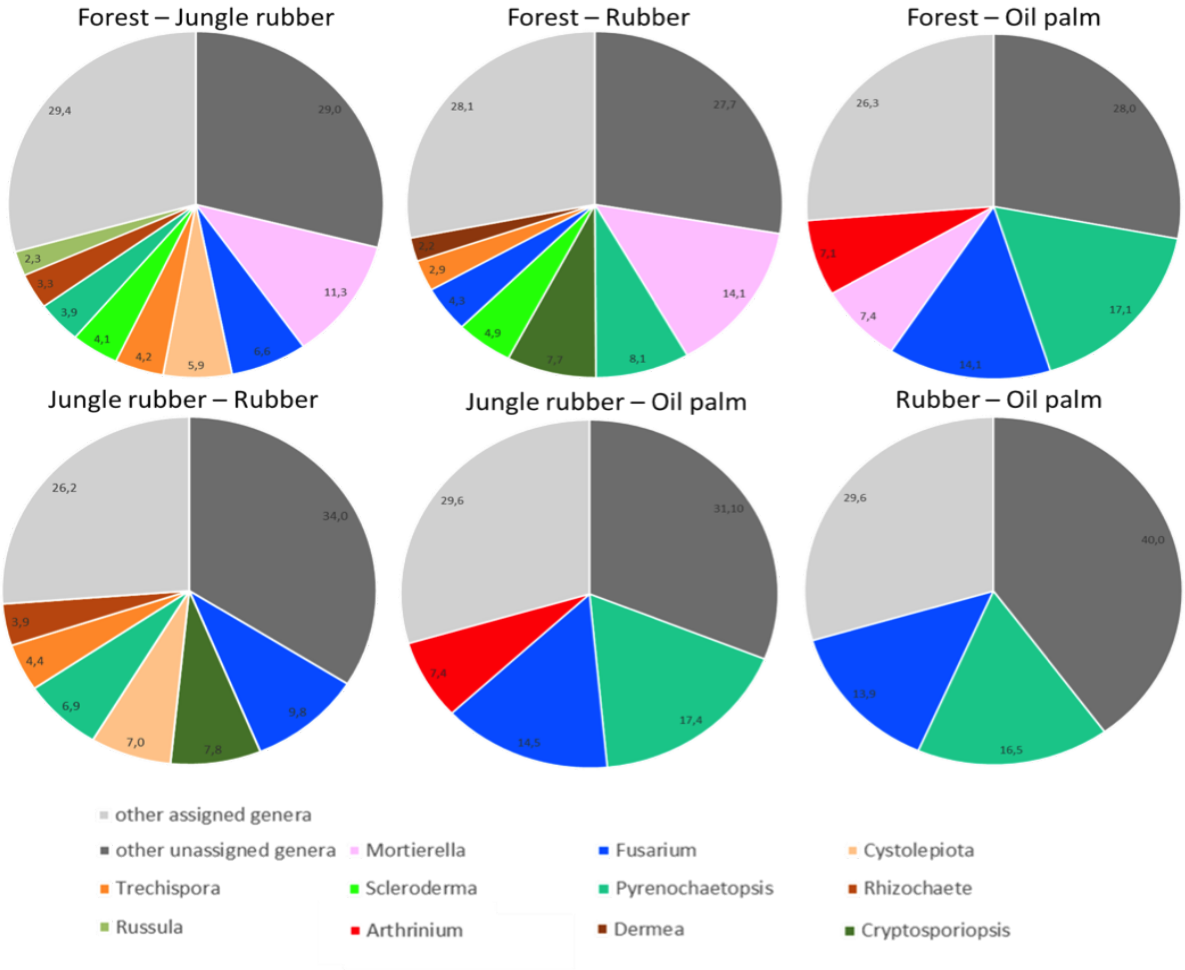

Figure 3.3.9: Contribution of Fungal Genera to the Dissimilarity of Whole Fungal Communities in the Four Different Land Use Systems. Pairwise comparison of land use systems. Genera with the most influence on differences in community composition of land use systems are show. The contribution to dissimilarities in community composition is shown in percentage.

Compared land use systems	Forest – Jungle Rubber		Forest – Rubber		Forest – Oil Palm	
	cumulative contribution [%]		cumulative contribution [%]		cumulative contribution [%]	
Most influential fungal genera explaining the dissimilarity between land use systems	other unassigned genera	29.0	other unassigned genera	27.7	other unassigned genera	28.0
	Martierella	40.3	Mortierella	41.8	Pyrenochaetopsis	45.1
	Fusarium	46.9	Pyrenochaetopsis	49.9	Fusarium	59.2
	Cystolepiota	52.8	Cryptosporiopsis	57.6	Mortierella	66.6
	Trechispora	57.0	Scleroderma	62.5	Arthrinium	73.7
	Scleroderma	61.1	Fusarium	66.8		
	Pyrenochaetopsis	65.0	Trechispora	69.7		
	Rhizochaete	68.3	Dermea	71.9		
	Russula	70.6				

Compared land use systems	Jungle rubber – Rubber		Jungle rubber – Oil palm		Oil palm – Rubber	
	cumulative contribution [%]		cumulative contribution [%]		cumulative contribution [%]	
Most influential fungal genera explaining the dissimilarity between land use systems	other unassigned genera	34.0	other unassigned genera	31.1	other unassigned genera	40.0
	Fusarium	43.8	Pyrenochaetopsis	48.5	Pyrenochaetopsis	56.5
	Cryptosporiopsis	51.6	Fusarium	63.0	Fusarium	70.4
	Cystolepiota	58.6	Arthrinium	70.4		
	Pyrenochaetopsis	65.5				
	Trechispora	69.9				
	Rhizochaete	73.8				

Table 3.3.7: Contribution of Fungal Genera to the Dissimilarity of Whole Fungal Communities in the Four Different Land Use Systems. Pairwise comparison of land use systems. Genera with the most influence on differences in community composition of land use systems are shown. The order of fungal genera shows the cumulative contribution in percentage to dissimilarities in community composition of the respective land use system. The sum of unassigned genera contains fungal genera not assigned to an ecological guild.

A pairwise comparison between land use systems of relative abundances of the 11 identified fungal genera with the most influence on explaining the dissimilarities in fungal composition between groups of the four different land use systems showed that in oil palm plantations the two saprotrophic fungal genera *Arthrinium*, *Pyrenochaetopsis*, and the plant pathogenic fungal genera *Fusarium* were significantly more abundant than in rain forest sites, jungle rubber sites, and rubber plantations (Table 3.3.8). In rain forest sites, the saprotrophic fungal genera *Mortierella* and the ectomycorrhizal fungal genera *Scleroderma* were significantly more abundant than in the other land use systems (Table 3.3.8). Jungle rubber sites and rubber plantations were not characterized by significantly higher abundances of any of the investigated assigned fungal genera (Table 3.3.8).

Land use system	**Arthrinium** saprotrophic fungi	**Cryptosporiopsis** sapotrophic fungi	**Cystolepiota** saprotrophic fungi	**Mortierella** saprotrophic fungi
Forest	0.27 ± 0.34 **b**	0.17 ± 0.15 **a**	0.04 ± 0.05 **a**	8.09 ± 6.89 **b**
Jungle rubber	0.09 ± 0.09 **ab**	1.02 ± 1.28 **a**	3.93 ± 6.39 **a**	0.76 ± 0.92 **a**
Rubber	0.04 ± 0.03 **a**	4.27 ± 4.49 **a**	0.00 ± 0.00 **a**	0.41 ± 0.79 **a**
Oil palm	7.83 ± 5.40 **c**	0.05 ± 0.07 **a**	0.01 ± 0.01 **a**	0.20 ± 0.15 **a**

Land use system	**Pyrenochaetopsis** saprotrophic fungi	**Rhizochaete** saprotrophic fungi	**Trechispora** saprotrophic fungi	**Dermea** plant pathogenic fungi
Forest	2.05 ± 2.96 **a**	0.064 ± 0.141 **ab**	0.20 ± 1.93 **b**	0.91 ± 1.08 **b**
Jungle rubber	2.04 ± 1.61 **a**	2.178 ± 5.597 **b**	1.92 ± 3.56 **b**	0.32 ± 0.39 **ab**
Rubber	4.32 ± 5.05 **a**	0.010 ± 0.023 **ab**	1.78 ± 0.34 **a**	0.89 ± 1.45 **b**
Oil palm	20.13 ± 9.47 **b**	0.003 ± 0.003 **a**	2.04 ± 4.56 **ab**	0.02 ± 0.04 **a**

Land use system	**Fusarium** plant pathogenic fungi	**Russula** ectomycorrhizal fungi	**Scleroderma** ectomycorrhizal fungi
Forest	0.69 ± 1.00 **a**	0.96 ± 1.36 **b**	2.71 ± 3.29 **b**
Jungle rubber	4.34 ± 9.90 **a**	0.80 ± 1.38 **a**	0.03 ± 0.05 **ab**
Rubber	2.46 ± 2.55 **a**	0.01 ± 0.02 **a**	0.01 ± 0.00 **a**
Oil palm	15.70 ± 10.78 **b**	0.04 ± 0.07 **a**	0.02 ± 0.02 **ab**

Table 3.3.8: Relative Abundances of Fungal Genera Assigned to Ecological Guilds with Contribution to Differences in Fungal Community Compositions. Comparison of relative abundances of fungal genera, with the number of sequence reads of a fungal genus in proportion to the total number of sequence reads in each core plot. For statistical analyses, generalized linear mixed effect models were performed. Significant differences between means of groups are indicated by letters with $p \leq 0.05$, $n = 32$.

3.3.7 Shifts Among Functional Groups Referring to Different Land Use Systems

A total of 88 OUTs (2.46 % from a total number (3753) of root-associated fungal OTUs found across land use systems, henceforth referred to as "all OTUs") belonged to AMF. OTUs of AMF were found in all core plots except in one of the jungle rubber plot in Harapan (HJ 4). The relative abundance of AMF OTUs was significantly higher in rain forests and rubber planatations than in oil palm plantations (Figure 3.3.10 A). A total of 108 fungal OTUs (3.02 % of all OTUs) were assigned to ectomycorrhizal fungi ectomycorrhizal fungi (EMF). OTUs of EMF were found in all core plots except BR 4 and HR 1. The significantly highest relative abundance of EMF was found in rain forest sites compared to all other land use systems (Figure 3.3.10 B). A total of 174 OTUs were assigned to plant pathogenic fungi (4.87 % of all OTUs). Plant pathogens were present in all root communities of the different land use systems. The highest relative abundance was found in oil palm plantations (Figure 3.3.10 C). With 573 OTUs, the majority of assigned OTUs belonged to saprotrophic fungi, representing 16.04 % of all OTUs and were found in all root communities of the four land use systems.

Saprotrophic fungi were most abundant in oil palm plantations and had the lowest abundance in jungle rubber sites and rubber plantations (Figure 3.3.10 D). Land use had a significant influence on the community composition of AMF, plant pathogenic fungi, and saprotrophic fungi (Table 3.3.9).

Dissimilarities of AMF, EMF, plant pathogenic and saprotrophic fungal communities were visualized by non-emetric multidimensional scaling (Figure 3.3.11 A – E). Dissimilarities and distribution of fungal communities belonging to different ecological guild were explained by varying environmental variables (Figure 3.3.11 A – E). In total, 34 possible explanatory variables were tested (Table 3.2.1, except for diversity indices). Fungal OTU communities of AMF were distributed corresponding to the different land use systems along the NMDS 2 axis (Figure 3.3.11 A). Significant explanatory variables for the dissimilarities and distribution of AMF OTU communities were land use intensity, root aluminum, iron, carbon, and sulfur concentrations as well as the number of AMF spores found in soil (Figure 3.3.11 A).

Functional group	**Influence of land use on community composition**	
AMF	$r^2 = 0.235$	$p = 0.0001$
EMF	$r^2 = 0.115$	$p = 0.1394$
Pathogens	$r^2 = 0.231$	$p = 0.0002$
Saprothrophs	$r^2 = 0.311$	$p = 0.0001$

Table 3.3.9: Influence of Land Use on the Composition of Root Associated Fungi of Four Functional Groups. Influence was tested by applying PERMANOVA.

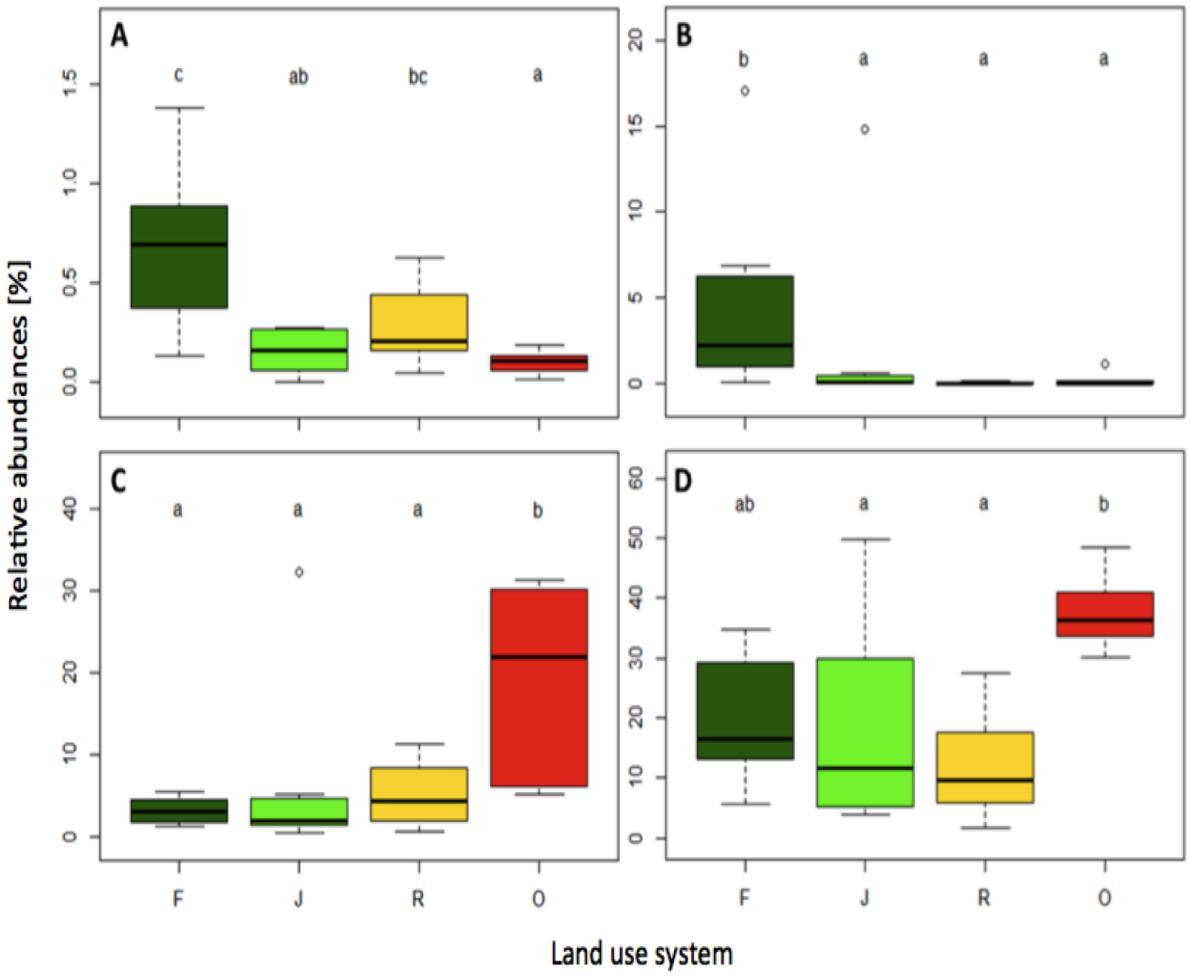

Figure 3.3.10: Relative Abundance of Ecological Fungal Guilds in Four Land use Systems. Comparison of relative abundances of fungal ecological groups, with the number of sequence reads of an ecological group in proportion to the total number of sequence reads in each core plot. **A)** Relative abundance of arbuscular mycorrhizal fungi (AMF). **B)** Relative abundances of ectomycorrhizal fungi (EMF). **C)** Relative abundances of plant pathogenic fungi. **D)** Relative abundances of saprotrophic fungi. For statistical analyzes generalized linear mixed effect models were performed. Significant differences between means of groups are indicated by letters with $p \leq 0.05$, $n = 32$. F = rain forest, J = jungle rubber, R = rubber plantations, and O = oil palm plantations.

3.3.8 Dissimilarities Within the Communities of Different Functional Groups

The OTU communities of EMF showed big overlaps in distributions among communities from different land use systems (Figure 3.3.11 B). A clustering of EMF communities according to land uses is slightly visible along the NMDS 2 axis and the dissimilarities of EMF OTU and their distribution are explained by land use intensity, fine root biomass, root iron concentrations, concentrations of available phosphorus in soil, and the number of AMF spores in soil (Figure 3.3.11 B). The communities of pathogenic fungal OTUs showed a distribution corresponding to different land uses along the NMDS 1 axis with pathogenic fungal communities of oil palm plantations showing only slight overlaps with communities of rubber plantations (Figure 3.3.11 C). The dissimilarities among pathogenic fungal communities and the related clustering by different land uses were explained by land use intensity, amount of non-ectomycorrhizal root tips and distorted root tips of root communities, root iron, aluminum and carbon concentrations, and the concentration of magnesium and available phosphorus in soil (Figure 3.3.11 C). Saprotrophic fungal OTU communities were separated according to land use along the NMDS 1 axis (Figure 3.3.11 D). Saprotroph communities of oil palm plantations were clearly separated from the other land uses and dissimilarities were explained by many diverse environmental explanatory variables with land use intensity being one of them (Figure 3.3.11 D).

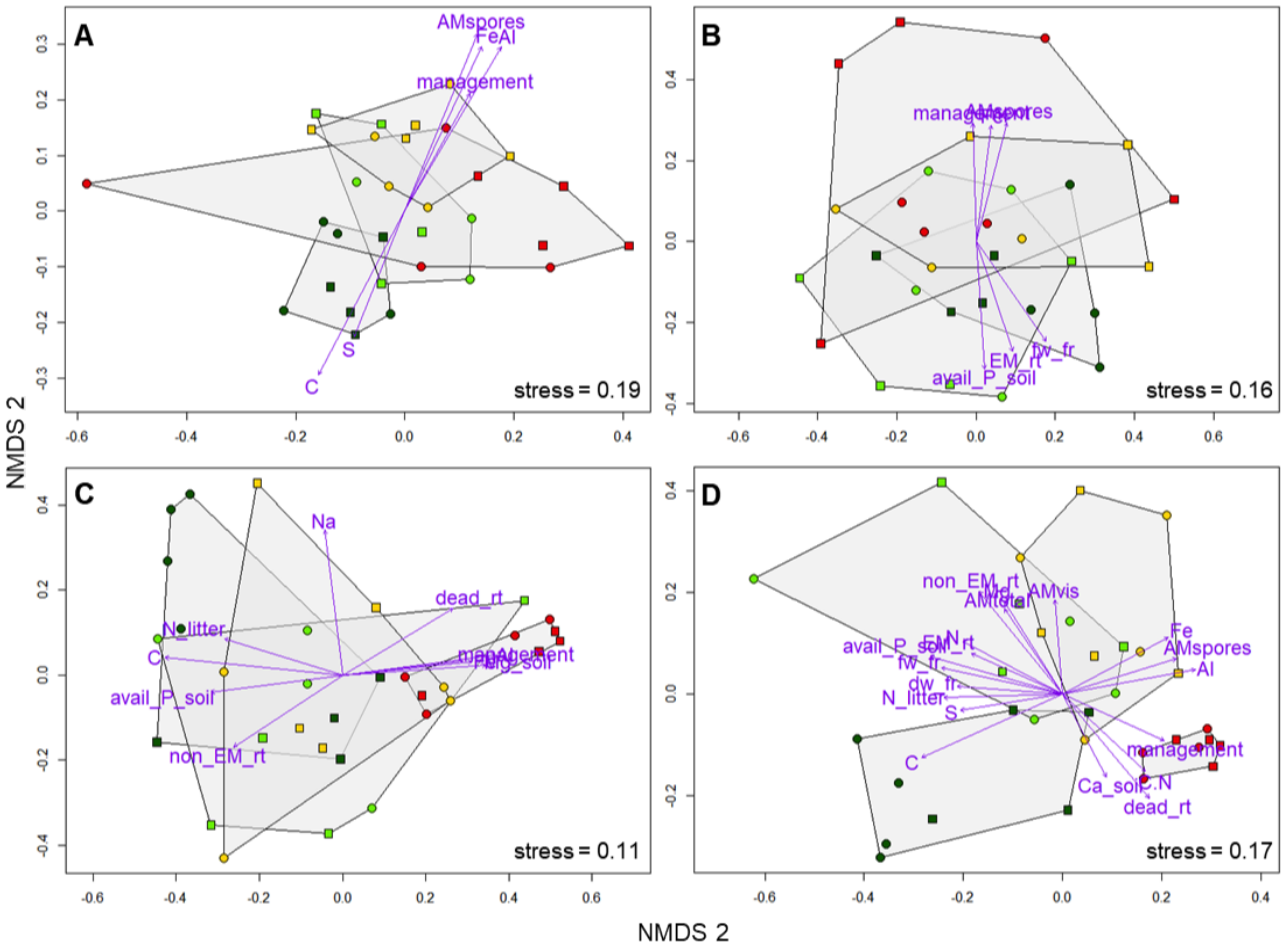

Figure 3.3.11: Non-Metric Multidimensional Scaling (NMDS) of Fungal OTU Communities Based on Bray Curtis Dissimilarity. B) EMF community. **C)** Plant pathogenic fungi. **D)** Saprotrophic fungi. Significant environmental parameters explaining dissimilarities in community composition are shown by purple arrows ($p \leq 0.05$). Circles = core plot in Harapan, squares = core plots in Bukit12, dark green = forest, green = jungle rubber, yellow = rubber, red = oil palm, n = 32.

3.4 Discussion

We investigated root-associated fungal communities from four different land use systems, rain forest sites, jungle rubber sites, rubber monoculture plantations, and monoculture oil palm plantations located in two different landscapes, i.e. Harapan landscape and Bukit Duabelas (Bukit12) landscape in the Province of Jambi, Sumatra (Indonesia).

3.4.1 Research on Fungal Diversity Conducted in Tropical Region

Only few studies investigated belowground fungal diversity in tropical rain forests (Kerfahi et al., 2014, 2016, McGuire et al., 2011, 2015; Mueller et al., 2014; Peay et al., 2013; Toju et al., 2014) (Table 3.4.1) and with the exception of Toju et al. (2014) all have investigated soil and not root-associated fungal communities. In temperate forests, Goldmann et al. (2016) found that root-associated fungi are mainly recruited from the soil. It was shown that 94 % of root-associated fungal OTUs were detected in soil, but in soil 66 % of fungal OTUs were unique. The similarity of communities declines with an increasing distance (Nekola and White, 1999). Goldmann et al. (2016) showed that this distance decay has a greater influence on soil fungal communities than on root-associated fungal communities. In addition, next generation sequencing (NGS) techniques, primer choice and amplified DNA barcode marker, are differing in the abovementioned studies (Table 3.4.1). Studies directly comparing different NGS techniques on the same complex microbial community sample are rare (Luo et al., 2012, Chapter 4). Luo et al. (2012) found a strong positive correlation($r^2 = 0.99$) between observed sequence richness recovered by Illumina sequencing and 454 Pyrosequencing from the same freshwater plankton sample. In the study conducted in this thesis (Chapter 4) we found different patterns for fungal sequence and OTU richness but the applied NGS techniques was not influencing results obtained on the diversity of fungal communities. Studies which compared ITS1 region vs. ITS2 region by analysing fungal diversity and community composition came to the finding that the two ITS regions provide similar results (Amend et al., 2010; Bazzicalupo et al., 2013; Mello et al., 2011). However, primer chosen to amplify the fungal DNA from environmental samples differed between our study and other studies on fungal communities (Kerfahi et al., 2014, 2016; McGuire et al., 2011, 2015; Mueller et al., 2014; Peay et al., 2013; Toju et al., 2014) (Table 3.4.1). A comparison between obtained results on root-associated communities

in this thesis and soil fungal communities in other studies, therefore, should be considered critically.

3.4.2 Differences of Fungal OTU Richness Across Land Use Systems

The diversity of vascular plants in our study area differed significantly among the four land use systems with a six-fold decline in monoculture plantations compared to unmanaged forests (Drescher et al., 2016).

Study	Sample type analyzed	Fungal community investigated	Geographical region	Investigated systems	Study's focus	Sequencing technique	Primer, barcode amplified
Kerfahi et al. 2014	soil	general community composition, **EMF**	Borneo	unlogged rain forest, once and twice logged rain forest, **oil palm plantations**	impact of selective logging for oil palm on fungal communities	454 Pyrosequencing	ITS1F-ITS4, ITS1 and ITS2 region
Kerfahi et al. 2016	soil	general community composition, **EMF**	Malaysia	rain forest sites, **rubber plantations**	impact of forest conversion on fungal diversity	**Illumina sequencing**	ITS1F-ITS2, **ITS1 region**
McGuire et al. 2011	soil and litter	general community composition	Panama	primary rain forest	impact of tree diversity and precipitation on fungal communities	454 Pyrosequencing	SSU 817f-SSU 196r, small ribosomal subunit
McGuire et al. 2015	soil	general community composition, **EMF**	Malaysia	primary rain forest, regenerating rain forest, **oil palm plantations**	response of fungi on logging and oil palm agriculture	**Illumina sequencing**	ITS1F-ITS2, **ITS1 region**
Mueller et al. 2014	soil	general community composition	Brazil	primary rain forest, secondary rain forest, pasture	Links between plant and fungal communities	**Illumina sequencing**	ITS1F-ITS2, **ITS1 region**
Peay et al. 2013	soil	general community composition	Peru	rain forest	Links between plant and fungal communities	454 Pyrosequencing	ITS1F-ITS4, ITS1 and ITS2 region
Toju et al. 2014	**roots**	general community composition, **AMF**, **EMF**	Japan	secondary rain forest	Diversity and spatial structure of plant-fungal symbiosis	454 Pyrosequencing	ITS3_KYO2-LRKYO_1b, ITS2 region

Table 3.4.1: Overview of Studies Conducted in the Tropical Regions Investigating Fungal Communities.

Fungal communities are, in many cases, related to the host identity and/or phylogenetic affiliation (Lang et al., 2011; Maron et al., 2011; Smith and Read, 2008; Tedersoo et al.,

2008). Therefore, it was hypothesized that we would observe a decline in fungal OTU richness in plant species-poor monoculture plantations compared to species-rich rain forests. So far, different patterns have been observed in the tropical regions regarding the link between fungal diversity and plant diversity. Peay et al. (2013) observed a positive correlation between fungal and plant biodiversity in rain forest sites across the western Amazonian region. These findings were supported by Mueller et al. (2014). Their study also investigated a positive link between plant and fungal communities in rain forest sites of the western Amazonian region and also included transformed forest systems. However, McGuire et al. (2011) found no correlation between plant and fungal diversity in rain forests in Panama. Here, we observed no general decline of fungal OTU richness in managed systems compared to rain forest sites. The OTU richnes only decreased significantly in rubber plantations but not in oil palm plantations. This supports the obtained results of Kerfahi et al. (2014). They found no significant difference between the fungal OTU richness in unlogged rain forest sites and oil palm plantations in Borneo, Malaysia. But in contrast to Kerfahi et al. (2016), we found significant differences between fungal OTU richness in rubber plantations and rain forest sites with a decrease in fungal OTU richness in rubber plantations. Further research has to be conducted to obtain more results on the influence of land use change and a related decrease in plant species richness on fungal richness.

3.4.3 Different Patterns in OTU Richness of Root-Associated Fungal Communities in Land Use Systems of the Two Landscapes

The richness of root-associated fungal OTUs among land use systems showed different patterns referring to the landscapes. Fungal communities are spatially structured in response to various biotic and abiotic features and at different scales (Ettema and Wardle, 2002). In Bukit12, richness of root-associated fungi was significantly higher in forest sites compared to jungle rubber, rubber plantations, and oil palm plantations whereas in Harapan no differences were found (Figure 3.3.3). The two landscapes differ in some characteristics. Soils of both landscapes are Acrisol soils, but in Harapan a loam Acrisol can be found with a lower soil fertility than the clay Acrisol in Bukit12 landscape (Allen et al., 2015). The vegetation differed among land use systems with a decline of plant diversity in the managed systems (Drescher et al., 2016). The managed systems from the two landscapes differed not only in plant diversity

but also did the rain forest sites show some differences (personal communication with K. Rembold). In the forests of Harapan the plant communities had a higher diversity than in Bukit12 (personal communication with K.Rembold). Soil type, land use, topography, and vegetation are categories on larger spatial scale which influence the structures of fungal communities (Dighton and White, 2005). However, we decided to focus on the influence of land use and, therefore, only accounted for landscape as a random effect in the applied generalized mixed effect models. For our analysis, we included variables of the spatial microscale to which fungi respond (e.g. root performance and chemical traits, soil and litter properties). Additionally, we calculated land use intensity indices for each sampling location to account for different levels of land use intensity in terms of substance applications (i.e. fertilizer, herbicides, lime and cow compost) (Table 3.3.4). We included these important environmental variables (Table 3.2.1) reflecting the conditions present in each land use system and landscape for analyses done on dissimilarities among fungal communities (Figure 3.3.7, Figure 3.3.11).

3.4.4 Root-Associated Fungal Community Composition was Affected by Land Use Change

An unexpected result was that the diversity of root-associated fungal communities was not higher in rain forests than in highly managed monoculture plantations. However, the composition of root-associated fungal communities was significantly influenced by land use. Two studies investigated the influence of tropical rain forest transformations into oil palm plantations (Kerfahi et al., 2014; McGuire et al., 2015) on soil fungal communities. These studies found an influence of land use on fungal community composition. Both studies report a decrease of fungi belonging to the phylum of Basidiomycota and an increase of Ascomycota in oil palm plantations compared to rain forest which corresponds to our findings. Kerfahi et al. (2016) found a significant decrease of Basidiomycota in the soil of rubber monoculture plantations compared to rain forest. Here, we found no significant decrease of Basidiomycota in root communities from rubber plantations. Kerfahi et al. (2016) also tested which environmental variables are explaining the differences between the soil fungal communities. They included soil pH, total soil carbon, and total soil nitrogen concentrations in their analysis and found that all three soil properties were significant explanatory variables for the dissimilarities among soil fungal communities. Here, we included 36 variables, including root-community-

weighed chemical and performance traits, soil and litter properties, land use intensity as well as diversity indices (Table 3.2.1). Our results on root-associated fungal communities showed that chemical RCWTs explained most of the dissimilarities among different land use systems. A comparison between obtained results on root-associated communities in this thesis and soil fungal communities in other studies should be considered critically (see 3.4.1). We can conclude, however, that root-associated and soil fungal community composition are influenced by land use changes. In addition, both fungal communities showed a decline of Basidiomycota and increase of Ascomycota in managed systems compared to unmanaged rain forest. Kerfahi et al. (2014) as well as McGuire et al. (2015) found a decline in the abundances of ectomycorrhizal fungi (EMF) in soil of oil palm plantations. These results are also in line with those obtained in the present thesis on rot-associated fungal communities. However, results for other fungal functional groups like arbuscular mycorrhizal fungi (AMF) or pathogenic fungi are lacking.

3.4.5 Ecological Fungal Guilds: Abundances in and Shift Between Land Use Systems

To our knowledge, the shifts between functional groups of root-associated fungi among different land use systems in the tropics were reported here for the first time. The relative abundances of arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal fungi (EMF) were significantly lower in monoculture plantations compared to rain forest sites whereas the abundance of plant pathogenic fungi massively increased. The composition of fungal communities varies among ecosystems and on different spatial and temporal scales (Ettema and Wardle, 2002; Hawksworth, 1991; Pena et al., 2013; Peršoh, 2015; Tedersoo et al., 2014; Toju et al., 2014) and is in many cases related to the host identity and/ or phylogenetic affiliation (Lang et al., 2011; Maron et al., 2011; Smith and Read, 2008; Tedersoo et al., 2008). Our findings on a decline of EMF abundance in managed systems corresponds with those of Kerfahi et al. (2014) and McGuire et al. (2015). The low abundance of EMF can be explained by the absence of ectomycorrhiza forming host trees (personal communication with K. Rembold). Oil palms are associated with AMF and it was observed that oil palm roots are well colonized by AMF (Bakhtiar et al., 2013; Phosri et al., 2010; Sahner et al., 2015). By investigating the same samplings sites as in this thesis, Edy (2015) showed that AMF diversity was decreased in oil

palm and rubber monoculture plantations. However, Edy (2015) also found that operational taxonomic units (OTUs) of AMF across land use systems showed no strong host preferences. Therefore, a low abundance of AMF cannot be explained by a lack of plant hosts. Here, we found that community composition of AMF was significantly influenced by land use. Many studies investigated the relation between land use change and intensification on AMF communities and found a decrease in diversity related to land use intensification (e.g. Edy, 2015; Morris et al., 2013; Oehl et al., 2003; Vályi et al., 2015; Xiang et al., 2014). Here, we found that the dissimilarities of AMF communities among land use systems were explained by root sulfur, carbon, iron, and aluminum concentrations as well as land use intensity and AMF spore numbers in the soil. Sahner et a. (2015) showed that higher aluminum and iron and lower carbon and sulfur concentrations of root communities found in oil palm plantations compared to rain forests are important traits indicating forest transformation. Our findings indicate that the degradation of root community traits found by Sahner et al. (2015) is accompanied by the decrease of beneficial mycorrhizal fungi managed plantations compared to rain forests.
The relative massive increase of the abundance of plant pathogenic fungi was mainly induced by fungal OTUs from the genus *Fusarium*. Fungal species of the genus *Fusarium* are able to infect the plant roots and can cause root rot and vascular wilt (Chen et al., 2014b; Flood, 2006; Jiménez-Díaz et al., 2015; Li et al., 2014). In oil palms *Fusarium* can cause vascular wilt. The symptoms of the vascular wilt are drying-out of leaves and a reduction of leave size (Flood, 2006). In oil palm, these symptoms can lead to yield reduction of $20-30\,\%$ and in Africa it is the most destructive fungal disease of oil palm (Flood, 2006). Whether this is also the term in our study sites must be investigated. *Fusarium* also occurs in rain forest but the question arises why it is much more abundant in oil plantations than in unmanaged forests. AMF can protect plants against root-infecting pathogens by high colonization which results in a competition for colonization sites (Smith and Read, 2008). The AMF colonization of root communities in oil palm plantations in Bukit12 landscape was stable (Sahner et al., 2015) but significantly lower in Harapan than in all other land use systems and both landscapes (Sahner et al., 2015). However, abundance of plant pathogens and the colonization by AMF were not correlated ($p = 0.98$). Another, currently speculative possibility is that the decline in EMF enables the increase of pathogenic fungi. EMF are able to produce antifungal compounds (e.g. Duchesne et al., 1988; Yamaji et al., 2005). These compounds can reduce the pathogenicity through the reduction in sporulation of the pathogenic fungi before any root colonization by EMF occurs (Duchesne et al., 1988). Whether oil palms can benefit from the presence of EMF

in their vicinity should be tested in future experiments. Here, oil palm plantations showed a very low abundance EMF in root communities and also in soil (personal communications with N. Brinkmann). The question for the strong accumulation of plant pathogenic fungi in root communities of oil palm plantations is thus still unclear. Fertilization, herbicide, and fungicide applications may have contributed to these shifts.

3.5 References

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CHAPTER FOUR

4 Comparisons of Illumina Sequencing and 454 Pyrosequencing on Fungal Community Samples

4.1 Introduction

The major task for microbiologists is to gain insights into the structure, diversity, and the function of microbial communities. The composition of microbial communities and their link to ecosystem functioning still remains, in most cases, a black box for scientists (Shade et al., 2009). Barcoding of deoxyribonucleic acid (DNA) extracted from environmental samples (e.g. roots, soil, leaf litter) without prior culturing, defined as metagenomics, increased in order to classify biodiversity (e.g. Amend et al., 2010; Delmont et al., 2011; Luo et al., 2012; Peršoh, 2015; Tedersoo et al., 2014). However, many technical factors are influencing the results on the observed community composition (Bazzicalupo et al., 2013). The DNA extraction method used (Delmont et al., 2011), the chosen primer to amplify the DNA region of interest (Bellemain et al., 2010; Ihrmark et al., 2012; Toju et al., 2012), PCR based bias (Acinas et al., 2005), and the sequencing technique applied (Luo et al., 2012; Tedersoo et al., 2010) influence the final results for richness and abundance of operational taxonomic units (OTUs). The most promising molecular methodologies are rapidly developing next generation sequencing (NGS) techniques (Bazzicalupo et al., 2013; Hebert et al., 2003; Taberlet et al., 2012). NGS is also called massive parallel sequencing and allows for the simultaneous sequencing of billions of molecules in a nucleic acid extract from environmental samples (Buermans and den Dunnen, 2014). The two most frequently used NGS techniques to study the diversity and community composition of microbial communities are 454 Pyrosequencing and Illumina sequencing (Luo et al., 2012). Although they are different in their methodology they share some common features. Both are based on the "sequencing by synthesis" principle and are based on fragment libraries, this means that sequence reads are not received by upstream vector cloning or *Escherichia coli*-based amplification stages, but are isolated from DNA fragment libraries directly received from environmental samples (Claesson et al., 2010; Liu et al., 2012). During library preparation target DNA fragments are amplified, linked to specific adapter oligonucleotides and bar code sequences (multiple identifiers (MIDs), indices) by polymerase-chain-reaction (PCR) in order to assign sequences to specific samples (Mardis, 2008). Following the library preparation, a library amplification by PCR (e.g. emulsion PCR, bridge amplification) is required for NGS to ensure that the received signal from the sequencer is strong enough to be detected accurately by respective devices (Claesson et al., 2010; Mardis, 2008).
The so called pyrosequencing with the Roche (454) GS FLX sequencer was first commercially

introduced in 2004 (Mardis, 2008). Pyrosequencing uses the pyrophosphate molecule released during incorporation of a nucleotide by DNA polymerase to promote a set of reactions and finally produces light from the cleavage of oxyluciferin by luciferase (Figure 4.1.1, Mardis, 2008). During the library preparation, DNA fragments are linked to MIDs and specific adapter sequences. Before pyrosequencing, DNA fragments of the prepared library are amplified *en masse* by emulsion PCR on the surfaces of hundreds of thousands of agarose beads (Mardis, 2008). At the surface of these beads millions of oligomers are attached, each of which is complementary to the adapter sequences linked to the target DNA fragment during library preparation (Mardis, 2008). Emulsion PCR uses a mixture of oil and water in which the agarose beads are embedded as micro reactors. Agarose beads are isolated individually, each with a unique DNA fragment hybridized and pipetted into a conventional microtiter plate, were the PCR is performed and up to 100.000 copies of the original DNA fragments are produced on each agarose bead, ready for pyrosequencing (Mardis, 2008). Subsequently, agarose beads are pipetted to a 454 picotiter plate, which is composed of single wells that hold each one bead (Mardis, 2008). Once the 454 picotiter plates are ready they are loaded on the Roche 454 GS FLX sequencer and nucleotides and reagent solutions are delivered sequentially through a sequencing run (e.g. first only cytosine is added and then incorporated in case of being complementary to the base of the target DNA strand etc.) (Mardis, 2008).
A nucleotide complementary to the template DNA strand generates light through luciferase activity during its incorporation. This light signal is recorded with a charge-coupled-device (CCD) camera (Mardis, 2008). Sufficient repetition steps of sequencing runs generate a pyrogram that visualizes the types and amounts of incorporated nucleotides for each DNA strand in the wells of the 454 picotiter plate (Mardis, 2008). The Illumina Genome Analyzer was introduced in 2006 (Mardis, 2008). This NGS technique uses differently labelled fluorescent nucleotides equipped with a terminator to make sure that only one complementary nucleotide is added to the target DNA strand at a time and that the specific fluorescent signal is recorded. During library preparation, DNA fragments are linked to sample specific indices and adapter sequences in two steps. Subsequent to the library preparation, libraries are amplified by bridge amplification (Mardis, 2008). DNA fragments are attached to the surface of a glass flow cell which provides the complementary sequences of the adaptors previously ligated to the DNA fragments (Mardis, 2008). Once the DNA fragments are attached a polymerase creates a complement of the hybridized fragment and the double strand DNA fragment is denatured (Mardis, 2008).

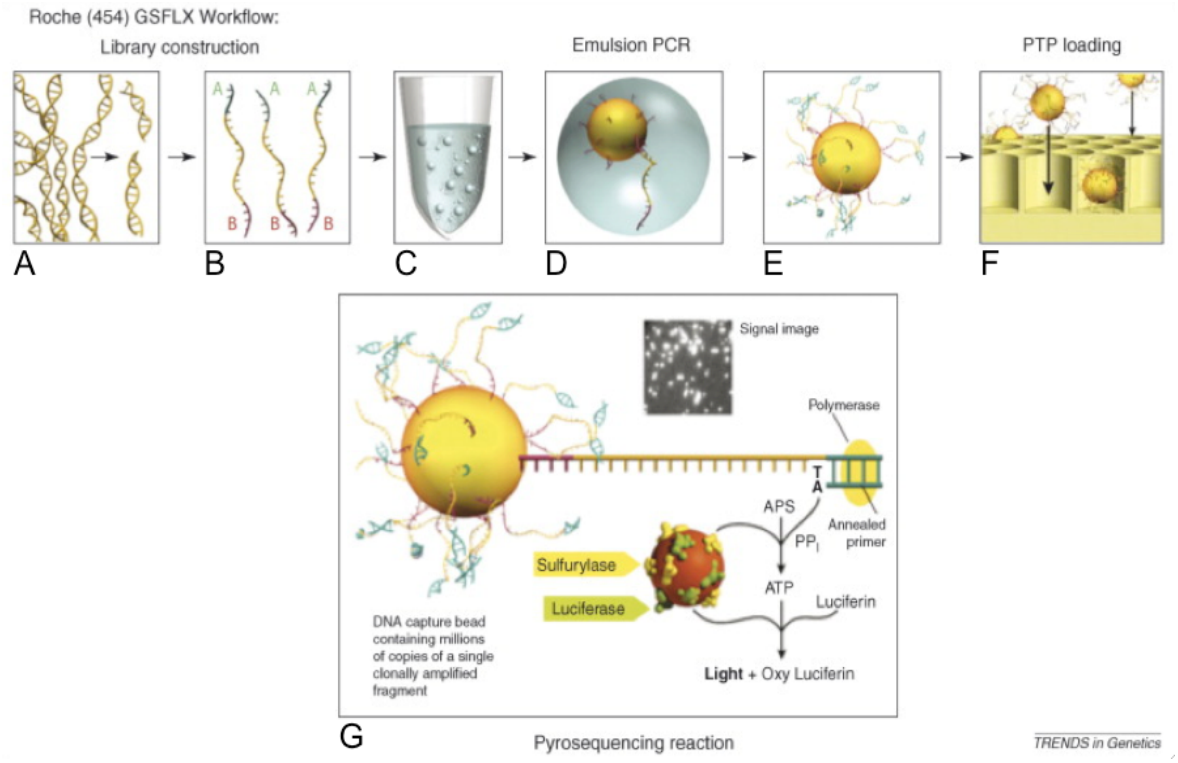

Figure 4.1.1: 454 Pyrosequencing Workflow. **A – B)** Library construction: **A)** Fragmentation of DNA, **B)** Ligation to specific multiple identifiers (MIDs) and adaptor sequences. **C – E)** Emulsion PCR: **C)** Oil-water-mixture with containing agarose beads (micro reactors), **D)** Agarose bead with oligomer complementary to adaptor sequence of a DNA strand. Each bead carries a unique single DNA strand. **E)** Clonally amplification of DNA fragments. **F)** Loading of agarose beads to 454 picotiter plate (PTP). **G)** Pyrosequencing reaction. Graph from Mardis et al. 2008.

The original template is then washed away and the resulting strands are clonally amplified in clusters by bridge amplification. At the end of the bridge amplification, reverse strands of the DNA fragment are removed and the sequencing can begin. The flow cell is loaded into the Illumina analyzer and in order to initialize the first sequencing cycle polymerase and all four differentially labelled fluorescent nucleotides are added (Mardis, 2008). The nucleotides have a chemically inactivated '3 OH (terminator) to ensure that only one is added to the DNA strand at a time/ cycle. Each incorporation cycle is followed by two steps to enable the next incorporation cycle: the identification of the specific base by imaging the fluorescent signal and the chemically removal of the terminator (Mardis, 2008).

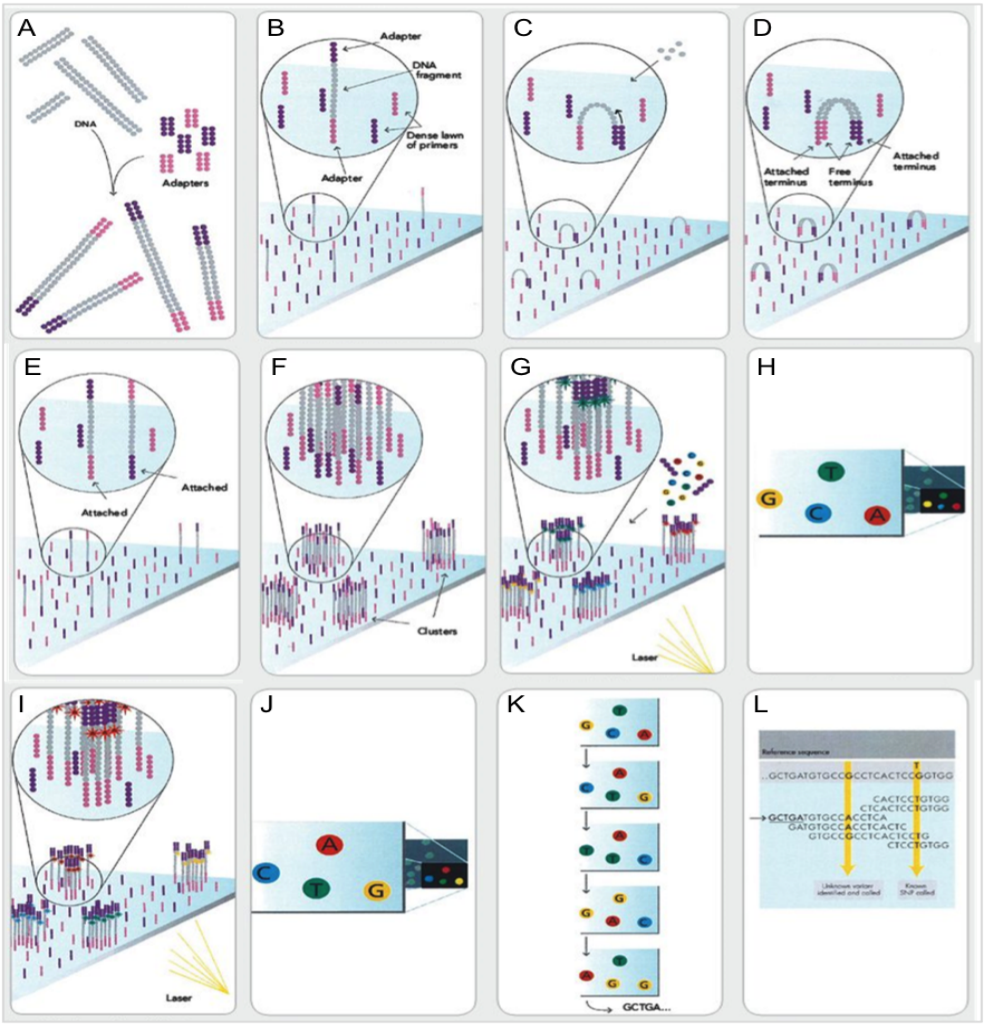

Figure 4.1.2: Illumina Sequencing Workflow. A) Adapter ligation, **B)** Attachment to flow cell, **C–D)** Bridge amplification **E)** Denaturation of double stranded DNA, **F)** Clustering, **G)** Single base extension, first incorporation cycle, **H)** Imaging of fluorescent signal from first incorporation cycle, **I)** Single base extension, second incorporation cycle, **J)** Imaging of fluorescent signal from second incorporation cycle, **K)** Repeated imaging of incorporated bases, **L)** Data alignment. Graph from Mardis et al., 2008.

An advantage of 454 pyrosequencing over Illumina sequencing is that the sequence read length increased with the advancement of this technique (Liu et al., 2012). It has been possible to generate sequence reads of up to 800 base pairs (bp) whereas Illumina sequencing read length is limited to 600 bp (Frey et al., 2014; Liu et al., 2012). Therefore, the quality in terms of ge-

netic information of generated sequence reads based on the sequence read length is potentially higher when using 454 Pyrosequencing. An advantage of Illumina sequencing over 454 Pyrosequencing is the reduction of sequencing costs and the ability to produce up to ten times more reads per run. This higher coverage makes it possible to detect more low-abundant operational taxonomic units (OTUs) by Illumina sequencing than by 454 Pyrosequencing (Liu et al., 2012; Mardis, 2008). Nonetheless, both NGS techniques also have methodology-dependent disadvantages. For example, 454 Pyrosequencing has high error rates for homopolymer regions (Luo et al., 2012; Mackelprang et al., 2011). For Illumina sequencing it has been shown that different sections of the sequencing flow cell produce reads with differing quality (Dolan and Denver, 2008; Schröder et al., 2010).

Technology-dependent sequencing biases have only been determined by investigating relatively simple DNA samples (e.g. single viral genome (Marston et al., 2013)), therefore the relevance for complex community DNA samples still remains unclear. Luo et al. (2012) directly compared 454 Pyrosequencing and Illumina sequencing by analyzing freshwater plankton communities from the same samples with both NGS techniques. They found that, in general, both NGS techniques sampled the same fraction of biodiversity, regarding OTU overlapping, of the plankton communities. To our knowledge, no study exists that compares metagenomics results regarding the biodiversity and structure of root-associated or other fungal communities using Illumina sequencing vs. 454 Pyrosequencing. The internal transcribed spacer (ITS) region has been selected as the universal DNA barcode marker for fungi (Schoch et al., 2012). The ITS region consists of the ITS1 region and ITS2 region. Several studies compared results obtained by 454 Pyrosequencing of the ITS1 vs ITS2 region of differing fungal communities, e.g., from dust samples (Amend et al., 2010), soil samples from truffle grounds (Mello et al., 2011) or leaf samples (Bazzicalupo et al., 2013). Two studies (Bellemain et al., 2010; Toju et al., 2012) also included results on fungal community composition by obtaining not only the ITS1 region vs. the ITS2 region but the whole ITS region using an *in silico* approach. The study of Toju et al. (2012) also embedded a small *in vitro* approach by investigating the coverage of designed primers on seven Ascomycota and seven Basidiomycota species. However, studies comparing the results of a complex fungal community structure from environmental samples obtained by amplifying either the whole ITS region or only the ITS1 or ITS2 region are missing.

In this study we compared results obtained by Illumina sequencing of the ITS1 region vs 454 Pyrosequencing of the whole ITS region from the same root community samples to address

the following research questions:

1. Is it possible to compare the results of observed OTU richness and the sequence of root-associated fungi based on the analyzation of the same root community samples with two different NGS techniques?

2. Do both techniques yield similar results on α - and β-diversity levels of root-associated fungal communities that are analyzed by Illumina sequencing and 454 Pyrosequencing?

3. To what extend do differing NGS techniques generate taxonomic overlaps and differences in root-associated fungal communities?

4.2 Materials and Methods

4.2.1 Study Sites and Sampling

Study sites were located in Jambi Province, in Sumatra (Indonesia), which is one of the key areas for palm oil production in Indonesia. Sampling sites were chosen along a land use gradient representing unmanaged rain forests, less-managed jungle rubber agroforests and intensely managed mono culture rubber (*Hevea basiliensis*) and oil palm (*Elaeis guineensis*) plantations. For this study 24 samples of root-associated fungal communities from the four different land use systems were analyzed by 454 Pyrosequencing and Illumina sequencing. The basis of each sample was a pool of root communities extracted from five soil cores (diameter 0.04 m, depth 0.2 m) that were taken in the subplots of the core plots installed within the framework of the EFForTS project (details on study sites in Chapter 3). The number of root community samples analyzed to assess the root-associated fungal community per land use system varied. From the rain forest sites, we analyzed nine samples, from jungle rubber sites two samples, from rubber monoculture plantations 9 samples, and from oil palm plantation four samples. The varying number of replicates was related to technical problems with the Roche 454 FLX GS sequencer emerged of the stopping support of Roche for the 454 Pyrosequencing technology (personal communication with Dr. Andrea Thürmer).

4.2.2 DNA Extraction

Freeze dried fine roots were ground to fine powder in a ball mill (MM 2000, Retsch, Haan, Germany) for three to four minutes with an amplitude of 90. In total 100 mg of grounded fine root material per sample were weighed into a 2 ml reaction tube and used for DNA extraction. DNA was extracted using the innuPREP Plant DNA kit (Analytik Jena AG, Jena, Germany), following the manufacturer's instructions (publication number of manual: HB_KS–1060_e_120116) (for more details see Chapter 3). DNA concentrations and the UV absorbance at 260 nm and 280 nm of the extracted DNA was measured by UV-Vis spectrometry using a nanoDrop 2000 (Thermo Fischer Scientific, Dreieich, Germany). The purity of DNA was determined by calculating the ratio of UV absorbance at 260 and 280 nm. Because of the poor quality of isolated DNA indicated by 260/280 nm ratios below 1.8 the DNA isolates were purified again using the PowerClean® Pro DNA Clean-Up Kit (MoBio Laboratories, Inc., Carlsbad, USA) following the manufacturer's instructions (protocol version 11172015). Library preparations for both NGS techniques were based on the same DNA extracts.

4.2.3 Primer Choice for 454 Pyrosequencing and Illumina MiSeq Sequencing

For 454 Pyrosequencing the ITS1-F_KYO2 primer (Toju et al., 2012) was selected as forward primer and the ITS4 primer (White et al., 1990) as reverse primer. This primer pair amplifies the ITS1 and ITS2 region of the fungal DNA (Figure 4.2.1). For Illumina sequencing we chose the same forward primer as for 454 Pyrosequencing, but due to the reduced read length ability of the Illumina technology compared to 454 Pyrosequencing, the ITS2 primer (White et al., 1990) was chosen as the reverse primer. The ITS1-F_KYO2 and ITS2 primer pair amplifies the ITS1 region of the fungal DNA (Figure 4.2.1).

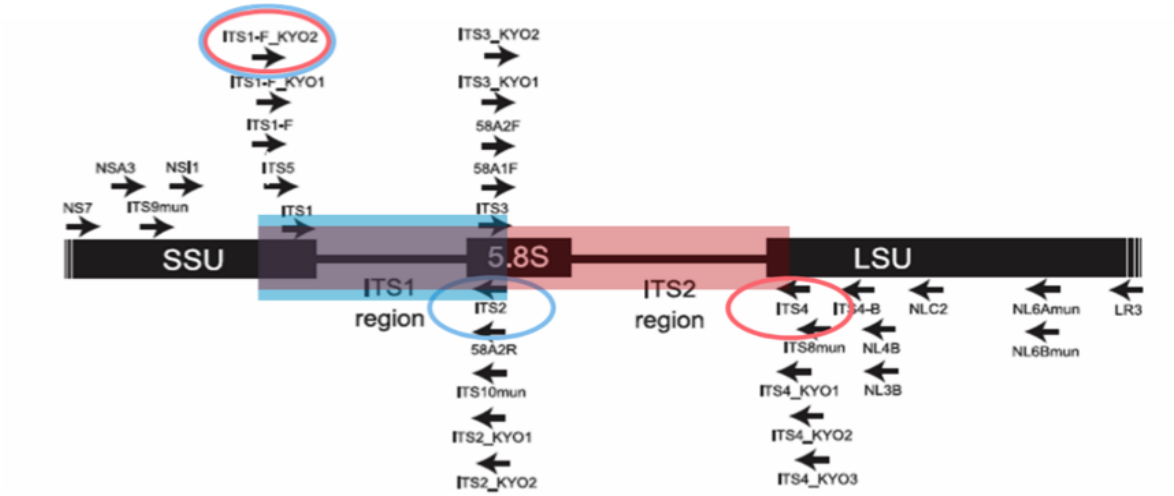

Figure 4.2.1: Map of the Ribosomal RNA Genes and their ITS Regions. The whole ITS region is labelled in red with the corresponding primers to amplify the ITS region labelled by red circles. The ITS1 region is labelled blue with the corresponding primers used for its amplification labelled with blue circles. Graph from Toju et al.,2012.

4.2.4 Amplicon Library Preparation for 454 Pyrosequencing

Fungal ITS rDNA amplicon libraries were produced using fusion primers designed for 454 Pyrosequencing. As forward primer a construct consisting of the 454 pyrosequencing primer B, a four base pair (TCAG) linking sequence, the 10 base pair MID barcode and the fungal specific ITS1-F_KYO2 (Toju et al., 2012) primer (5'–CCTATCCCCTGTGTGCCTTGGCAGTC–TCAG–MID barcode TAGAGGAAGTAAAAGTCGTAA–3') was used.
As reverse primer a construct consisting of the 454 pyrosequencing primer A, a four base pair (TCAG) linking sequence and the ITS4 (White et al., 1990) primer (5'–CCATCTCATCCC TGCGTGTCTCC GAC–TCAG–TCCTCCGCTTATTGATATGC–3') was used.
All 24 DNA samples were amplified separately. For each amplicon of a mixed fine root sample an individual MID bar code was used resulting in 24 different 10 base pair MID barcodes. This allowed the pooling of amplicons for sequencing with sequences assigned to the individual mixed fine root samples. PCR reactions were performed in a total volume of 50 μl. PCR reactions were carried out in 200 μl reaction tubes (Sapphire PCR reaction tubes, 0.2 ml, PP, blue, Greiner Bio-One GmbH, Frickenhausen, Germany). Up to 32 PCR reactions were run

at the same time and for each of this PCR reaction sets a negative and positive control was performed.

The reaction mix contained 50 ng DNA template, 1 μl forward primer, 1 μl reverse primer (primer ordered at Seqlab Sequence Laboratories, Göttingen GmbH, Germany), 10 μl Phusion GC buffer, 0.15 μl $MgCl_2$, 0.5 μl Phusion HF DNA Polymerase (2 U/ μl^{-1}), 2.5 μl 5 % DMSO, 1 μl 10M dNTP mix (GC buffer, $MgCl_2$, Plymerase, DMSO, and dNTPs were ordered at Thermo Fischer Scientific, Dreieich, Germany), 2.5 μl BSA (16 mg/ μml^{-1}, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), adjusted to the final volume of 50 μl with nuclease free water.

Amplification was performed in a PCR (Master Gradient Cycler, Eppendorf, Sarstedt, Nümbrecht, Germany) with an initial denaturation for 1 minute at 98 °C followed by 30 cycles of 1) denaturation at 98 °C for 45 seconds, 2) annealing at 47 °C for 45 seconds, and 3) elongation at 72 °C for 45 seconds with a final extension for 5 min at 72 °C .

To check whether the amplification was successful and if there are any contamination 2 μl of each PCR product and 2 μl the negative and positive control of each PCR reaction set were mixed with 1 μl 10 x DNA loading buffer and analyzed by gel electrophoresis (Power Pac 200, Bio Rad Laboratories Ltd., München, Germany) using a GelRed (GelRed™ Nucleic Acid Gel Stain, Biotium Inc., VWR International GmbH, Darmstadt, Germany) stained 1.2 % agarose gel (1.2 g agarose (LE Agarose, Biozym Scientific GmbH, Oldendorf, Germany) dissolved in 100 ml TBE (Tris/ Borate/ EDTA) buffer.

PCR products were purified using the innuPREP PCRpure Kit (Analytik Jena AG, Jena, Germany) following the manufacturer's instructions. Elution of DNA from the spin filter was performed with 30 μl of nuclease free water after an incubation for 15 min at room temperature. Up to 12 independent PCRs per sample were carried out. PCR products from one DNA sample were pooled and volumes of pooled PCR products were reduced to 30 μl using a concentrator (Eppendorf concentrator 5301, Eppendorf, Hamburg, Germany) at a temperature of 45 °C .

Pooled PCR products then were analyzed by gel electrophoresis using a GelRed stained 1.2 % agarose gel. After gel electrophoresis bands of interest with a length of 600 to 900 base pairs were cut on a UV-light table and put into 2 ml reaction tubes (Eppendorf micro tube 2 ml, Sarstedt, Nümbrecht, Germany). Gel fragments containing DNA were purified using the QIAquick® Gel Extraction Kit (QIAGEN, Hilden, Germany) following the protocol. Elution of DNA from the spin filter was performed with 30 μl of nuclease free water after an incuba-

tion for 5 min at room temperature. After gel purification DNA concentrations of amplicons were quantified twice using a Qubit™ dsDNA HS assay Kit in a Qubit fluorometer (Thermo Fischer Scientific, Dreieich, Germany) and the means were calculated to get the final DNA concentration for each amplicon. The amplicon libraries were pooled in equal amounts for 454-pyrosequencing. Sequencing was performed in the Göttingen Genomics Laboratory using the 454 Genome Sequencer FLX (Roche, Mannheim, Germany).

4.2.5 Amplicon Library Preparation for Illumina MiSeq Sequencing

For Illumina sequencing the fungal ITS1 region of environmental DNA was amplified using the ITS1-F_KYO2 (5' TAGAGGAAGTAAAAGTCGTAA 3'; Toju et al., 2012) and the ITS2 (5' GCTGCGTTCTTCATCGATGC 3'; White et al., 1990) primer with specific overhang adapters (adapter sequence 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG 3' of the forward primer and adapter sequence 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG 3' of the reverse primer (Nextera Transposase Adapter sequences, document number 1000000002694 v01, Illumina Inc., San Diego, USA). Details on PCR conditions and amplicon library preparation are described in Chapter 3 (3.2.6) (amplicon_library_preparation_for_illumina_sequencing).

4.2.6 Sequence Processing

Sequence processing for Illumina sequencing is described in Chapter 3 (3.2.7). Initial processing and analyzes of the resulting ITS sequences from 454 Pyrosequencing was done using the QIIME 1.8 software package (Caporaso et al., 2010) for performing microbiome analysis. For this purpose, sequences that fulfilled at least one of the following criteria were removed with *split_libraries.py*: average quality score was lower than 20, contained unresolved nucleotides, or harbored mismatches longer than 3 bp in the forward or reverse primer. For efficient forward and reverse primer removal we used cutadapt (Martin, 2011) with default settings. Subsequently, pyrosequencing noise was removed by employing Acacia (Bragg et al., 2012) with default settings. Chimeric sequences were removed using UCHIME (Edgar et al., 2011) with the reference dataset for UCHIME from the UNITE database (Abarenkov et al., 2010; Nilsson et al., 2015) available at https://unite.ut.ee/repository.php.

In preparation for operational taxonomic unit (OTU) clustering, we used USEARCH (Edgar et al., 2011; Nilsson et al., 2015) to dereplicate, remove singletons and sort all quality filtered sequences by length. Subsequently, OTU determination was performed with *pick_open_reference_otus.py* using the UNITE database as reference. Taxonomic classication was performed with *parallel_assign_taxonomy_blast.py* against the same database. OTU tables were created using *make_otu_table.py*. Finally, all quality filtered sequences were mapped to an OTU table using with USEARCH and applying the perl script *uc2otutab.py* (http://drive5.com/python/uc2otutab_py.html). Non-fungal OTUs were removed by employing *filter_otu_table.py* in QIIME.

4.2.7 Data Analyses

To visualize sequencing effort of the two different applied NGS techniques raw fungal sequence read-based rarefaction curves were created for each of the analyzed root sample using the *rarefaction* function in R (R Core Team, 2015) of the *vegan* (Oksanen et al., 2016) package. To compare observed fungal sequence and OTU richness between the two different NGS techniques, we applied GLMs with the *glm* function of the *multcomp* package (Hothorn et al., 2016) in R. Where necessary, we accounted for overdispersal of count data with a quasipoisson distribution of the count data in the GLMs (Ver Hoef and Boveng, 2007). To investigate differences in fungal α-diversity between Illumina sequencing and pyrosequencing, Shannon index for diversity and Simpson index for diversity were calculated for each sample using the *diversity* function of the *vegan* package in R. The calculations of diversity indices were based on the following equations:

$$\text{Shannon index (H)} = -\sum_{i=1}^{S} p_i \log\left(p_i\right)$$

$$\text{Simpson index for diversity (D)} = 1 - \sum_{i=1}^{S} p_i^2$$

where S is the number of species in the sample, p_i is the proportion that the i^{th} species contributes to the total abundance of the sample ($p_i = N_i / N$), N_i the number of individuals

of the i^{th} species, and N the number of individuals in the sample. Estimated OTU richness (Chao1) was calculated using the *estimateR* function of the *vegan* package in R. Depending on data distributions (Gaussian or estimates for counts), differences in fungal α-diversity between the two applied NGS techniques were analyzed by linear models with the *lm* function or *glm* function of the *multcomp* package in R. To test whether there are significant differences between the means of variables from the two different NGS techniques, analyses of variance (in case of applied linear models) or analyses of deviance (in case of applied generalized linear models) were conducted by applying the *anova* function with the additional option *test* = *"Chisq"* (in case of applied generalized linear models) . When the p-value of the analyses of deviance was less or equal 0.05 we rejected the null hypothesis ($\mu_1 = \mu_2 = \ldots = \mu_x$) and the *glht* function was applied to conduct a multiple comparisons of means (post hoc test).
As described by Jost (2006) diversity indices are just indices and not representing diversities themselves. As recommended by Jost (2006), Shannon and Simpson indices for diversity were transformed into the number of equally-common OUTs also called "effective number of OTUs" by Jost (2006) using the following equations:

$$\text{Effective number of OTUs associated with Shannon index (H)} = \exp(H)$$

$$\text{Effective number of OTUs associated with Simpson index (D)} = \frac{1}{(1 - D)}$$

This transformation into the effective number of OTUs is making it possible to interpret the measures of diversity expressed by diversity indices more easily as it gives always the same unit in number of OTUs (Jost, 2006). Huge shifts between OTU richness and the effective numbers of OTUs are indicating a high dominance of single OTUs in a community and an uneven distribution of OTUs within a community (Jost, 2006).
Relative abundances in percent were calculated for fungal phyla and selected fungal orders and genera as followed:

$$\text{Relative abundance of x} = \frac{\text{Number of x sequences reads}}{\text{Total number of sequences reads}} * 100\,\%$$

In total, six fungal genera were selected which were assigned to an ecological guild and had an important influence on root fungal community composition regarding the ecological function

of the fungal community (Chapter 3, 3.3.6). Statistical tests on relative abundances of fungal phyla, orders, and genera were conducted as described above with the *multcomp* package in R.

To analyze overlaps of observed fungal genera between the two different NGS techniques, Venn diagrams were generated using *draw.pairwise.venn* function of the *VennDiagram* package in R. Calculations on percentage of shared fungal OTUs was performed as followed:

$$\text{Percentage of shared fungal OTUs of x and y} = \frac{\text{Number of shared OTUs between x and y}}{\text{Sum of different fungal OTUs of x and y}} * 100\,\%$$

non-metric multidimensional scaling (NMDS) of fungal communities was done using the *vegan* package (Oksanen et al., 2016) in R. Bray-Curtis dissimilarities matrixes were used for ordination. To calculate the beta-diversity of root-associated fungal communities, OTU tables containing raw sequencing data (non-rarified data) were Hellinger transformed as recommended by Legendre and de Cáceres (2013). Total beta-diversity of root-associated fungal communities among samples analyzed by the two different NGS techniques was performed by using the *beta.div* function (Legendre and De Cáceres, 2013) in R which is implemented in the *vegan* package.

4.3 Results

4.3.1 Higher OTU Richness and Sequence Richness of Root-Associated Fungal Communities Analyzed by Illumina Sequencing

By Pyrosequencing of the fungal ITS region 138922 quality filtered sequences were generated across all samples where 4563 (3.28 %) sequences were non-fungal (plants and protists) (Table 4.3.1). By Illumina sequencing of the ITS1 region 917312 quality filtered sequences were generated containing 84971 (9.26 %) non-fungal sequences (plants and protists) (Table 4.3.1). Fungal sequence reads of the whole ITS region generated by Pyrosequencing belonged to 1814 different fungal OTUs (Table eS 4.1). The fungal sequences generated by Illumina sequencing of the ITS1 region belonged to 2695 different fungal OTUs (Table eS 4.2). Sequencing performance differed among samples in observed sequence richness and OTU richness with regard

to the applied NGS technique (Table 4.3.1). We obtained significantly more fungal sequence reads by Illumina sequencing than by Pyrosequencing ($p = 0.00000498$) (Figure 4.3.1 and Figure 4.3.2 A). The richness of observed fungal OTUs was significantly higher in samples analyzed by Illumina sequencing than in those obtained by Pyrosequencing ($p = 0.001651$) (Figure 4.3.1 and Figure 4.3.2 B). Estimated OTU richness of root-associated fungal communities was also significantly higher for samples analyzed by Illumina sequencing than by pyrosequencing ($p = 0.000017$) (Figure 4.3.4 A – B).

Sample ID	Observed fungal sequence richness by 454 Pyrosequencing	Observed fungal sequence richness by Illumina sequencing	Observed OTU richness by 454 Pyrosequencing	Observed fungal OTU richness by Illumina sequencing
HF 1a	19767	2879	673	143
HF 1b	11005	17976	218	213
HF 1c	5880	31267	247	382
HF 2a	8784	3666	451	205
HF 2b	943	59277	150	742
HF 2c	335	1427	91	177
HF 3b	1135	32282	99	305
HF 4b	604	23538	95	482
HF 4c	356	13817	75	485
HJ 1a	2474	7619	110	189
HJ 1b	283	38211	40	422
BR 1a	8405	146201	190	344
BR 1b	1161	40195	92	224
BR 1c	98	46754	36	461
BR 2a	17529	7740	424	200
BR 2b	5682	22047	180	249
BR 2c	576	5321	82	193
BR 3b	6378	82675	144	400
BR 3c	1239	41474	45	207
HR 1c	7263	42194	260	459
BO 2b	180	6602	45	151
BO 3a	155	7233	23	135
HO 1c	25985	140555	286	558
HO 4c	8142	11366	405	621

Table 4.3.1: Richness of Observed Root-Associated Fungal OTUs and Sequences Obtained by Analyzing Same Root Community Samples by Illumina Sequencing and 454 Pyrosequencing. Number of root-associated fungal sequences and OTUs are shown separated by applied NGS technique. Sample ID indicates the origin of analyzed root community sample regarding the subplot where the sample was taken. H = Harapan landscape, B = Bukit12 landscape, F = rain forest, J = jungle rubber, R = rubber monoculture, o = Oil palm monoculture, 1 – 4 = core plot ID, a – c = subplot ID. N = 24.

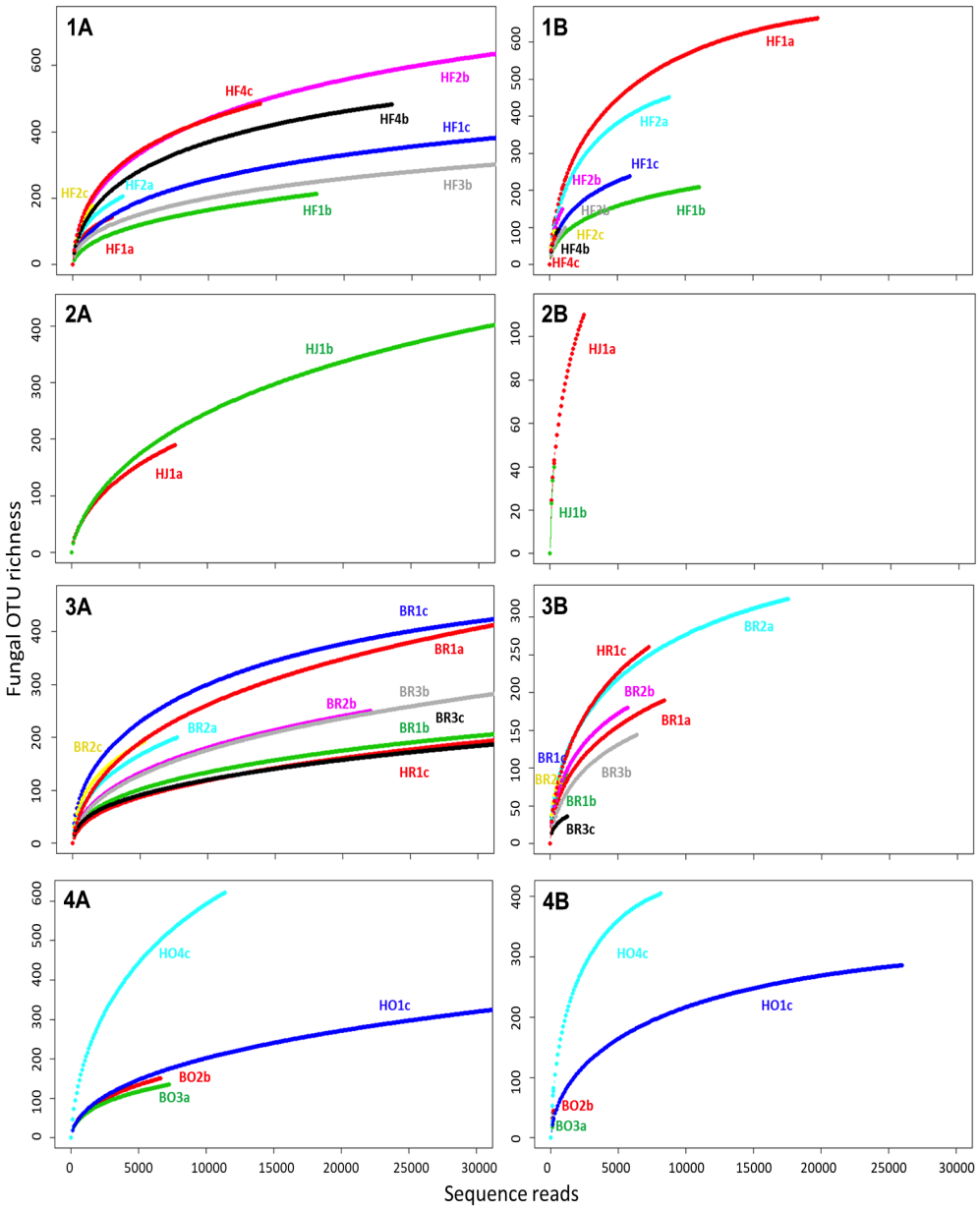

Figure 4.3.1: Saturation Curves of Non-Rarified Sequences Different Land Use Systems. A) Rarefaction curves of non-rarified sequences of subplot samples obtained by Illumina sequencing **B)** Rarefaction curves of non-rarified sequences of subplot samples obtained by 454 Pyrosequencing. **1)** Samples from rain forest sites **2)** Samples from jungle rubber sites **3)** Samples from rubber plantations **4)** Samples from oil palm plantations. B = Bukit12 landscape, H = Harapn landscape, F = rain forest, J = jungle rubber, R = rubber plantations, and O = oil palm plantations. Numbers 1 - 4 = core plot ID numbers, a - c = subplot names.

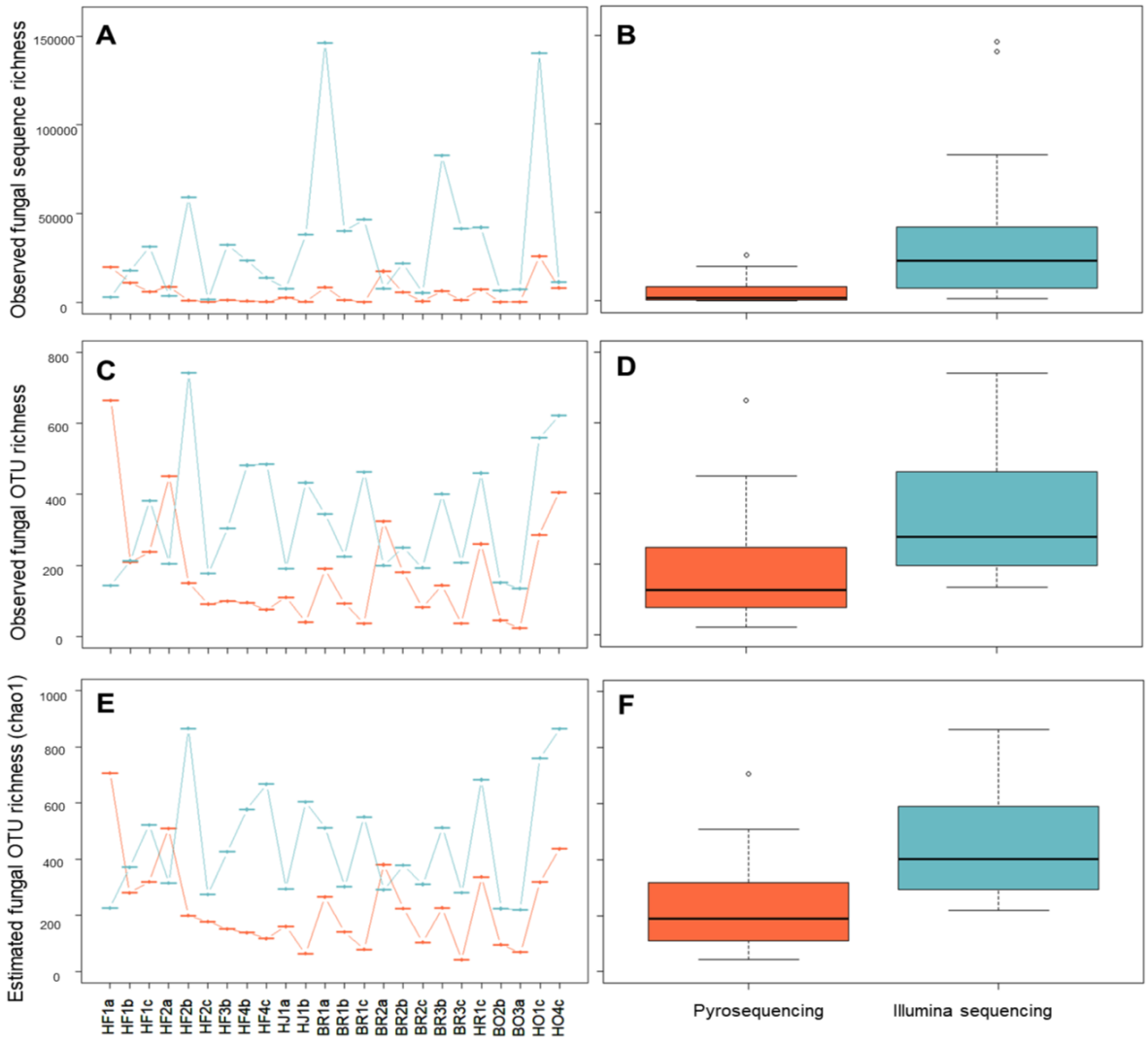

Figure 4.3.2: Observed Fungal Sequence and OTU Richness by Illumina and 454 Pyrosequencing. A) Observed fungal sequence richness of each sample analyzed by Illumina sequencing and 454 Pyrosequencing **B)** Mean observed fungal sequence richness of root-associated fungal communities analyzed by Illumina sequencing and 454 Pyrosequencing **C)** Observed fungal OTU richness of each sample analyzed by Illumina sequencing and 454 Pyrosequencing **D)** Mean observed fungal OTU richness of root-associated fungal communities analyzed by Illumina sequencing and 454 Pyrosequencing Observed fungal OTU richness. **E)** Estimated fungal OTU richness (Chao1 index) of each sample analyzed by Illumina sequencing and 454 Pyrosequencing**D)** Mean of estimated fungal OTU richness (Chao1 index) of root-associated fungal communities analyzed by Illumina sequencing and 454 Pyrosequencing Observed fungal OTU richness. Blue color represents data obtained by Illumina sequencing, orange color represents data obtained by 454 pyrosequencing. Significant differences between means of groups are indicated by letters with $p \leq 0.05$. n = 24. B = Bukit12 landscape, H = Harapan landscape, F = rain forest, J = jungle rubber, R = rubber plantations, and O = oil palm plantations. Numbers 1 - 4 = core plot ID numbers, a - c = subplot names.

There was no correlation between the richness of fungal OTUs in root communities analyzed by Illumina sequencing and pyrosequencing (Figure 4.3.3 A). Fungal sequence richness generated from root community samples analyzed by the two different NGS techniques also showed no correlation (Figure 4.3.3 B). Investigations of correlations between fungal OTU and sequence richness within one of the two NGS techniques separately showed a positive correlation of fungal OTU and sequence richness for root samples analyzed by both methods (Figure 4.3.3 C – D).

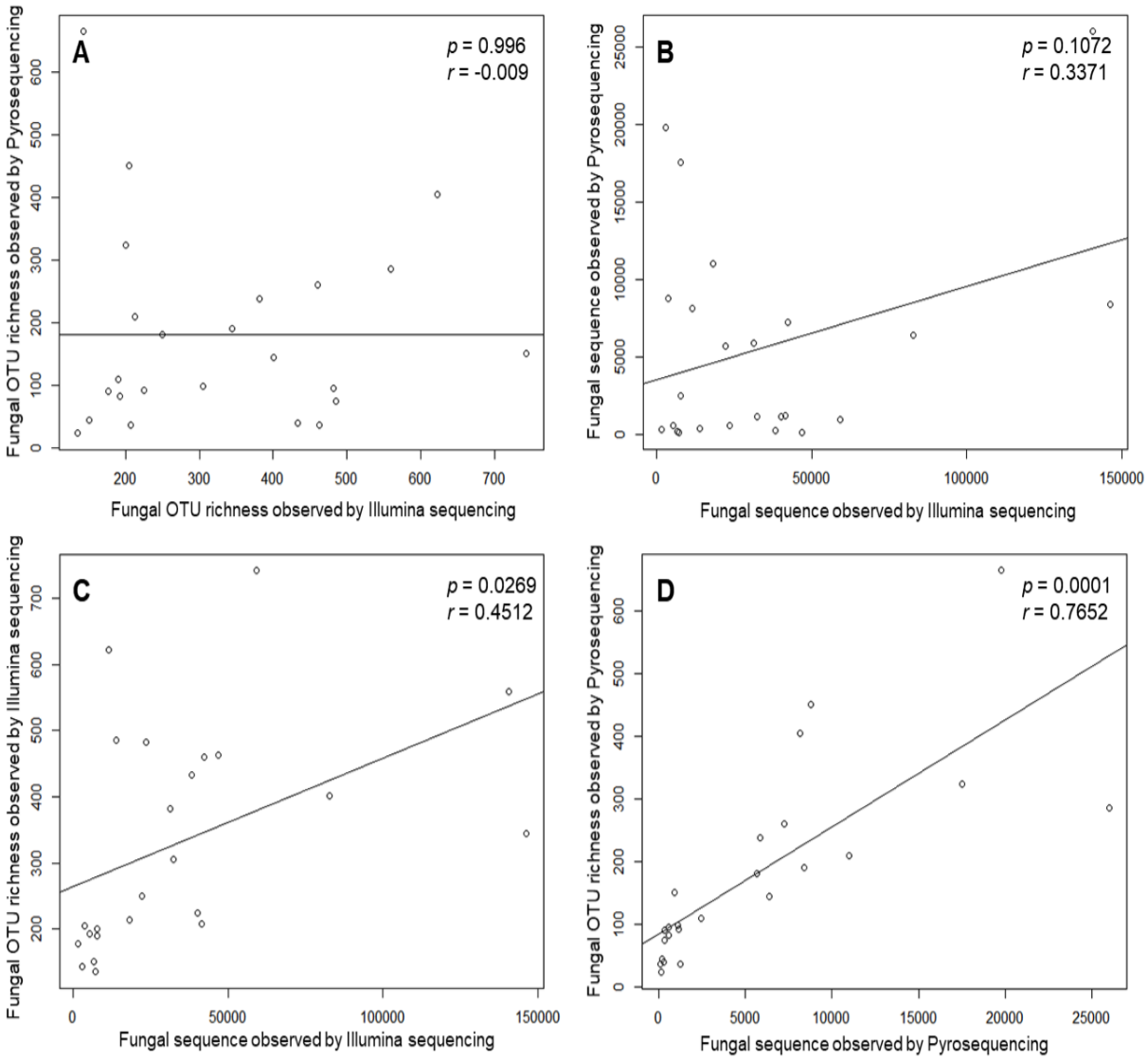

Figure 4.3.3: Relationships of Fungal OTU Richness and Sequence Richness Between and Within the Two Applied NGS Techniques. A) Relation of fungal OTU richness of root community samples analyzed by Illumina sequencing and pyrosequencing. **B)** Relation of fungal sequence richness generated from root community samples analyzed by Illumina sequencing and pyrosequencing. **C)** Relation between fungal OTU richness and sequence richness of root community samples analyzed by Illumina sequencing. **D)** Relation between fungal OTU richness and sequence richness of root community samples analyzed by pyrosequencing. N = 24.

4.3.2 Alpha and Beta-Diversity of Root-Associated Fungal Communities are not Influenced by the Applied NGS Technique and Related Differential Barcoding of Fungal DNA

Alpha-diversity of root-associated fungal communities showed no significant differences between the calculated Shannon and Simpson indices regarding the applied NGS techniques ($p_{Shannon} = 0.118$, $Simpson = 0.05078$) (Figure 4.3.4 C – F). Shannon indices of root-associated fungal communities obtained by Illumina sequencing and Pyrosequencing were correlated ($p = 0.0002$, $r = 0.684$) whereas Simpson indices between root-associated fungal communities were not correlated ($p = 0.0553$, $r = 0.396$).

When examining the effective number of OTUs associated with the Shannon and Simpson indices, we also found no significant differences between data obtained by Illumina sequencing and Pyrosequencing (Table 4.3.2). The means of numbers of observed OTUs and of effective numbers of OTUs are showing huge shifts indicating a high dominance of single OTUs in the communities and an uneven distribution of OTUs within each of the two communities (Table 4.3.2). However, this effect was observed regardless of whether Illumina or Pyrosequencing was applied. Dissimilarities among root-associated fungal communities were visualized separated by the two applied NGS techniques by non-metric multidimensional scaling (NMDS) (Figure **??**). Total beta-diversity among root-associated fungal communities from samples analyzed by 454 Pyrosequencing was slightly greater than that among fungal communities of root samples analyzed by Illumina sequencing (totalBD in Figure 4.3.5).

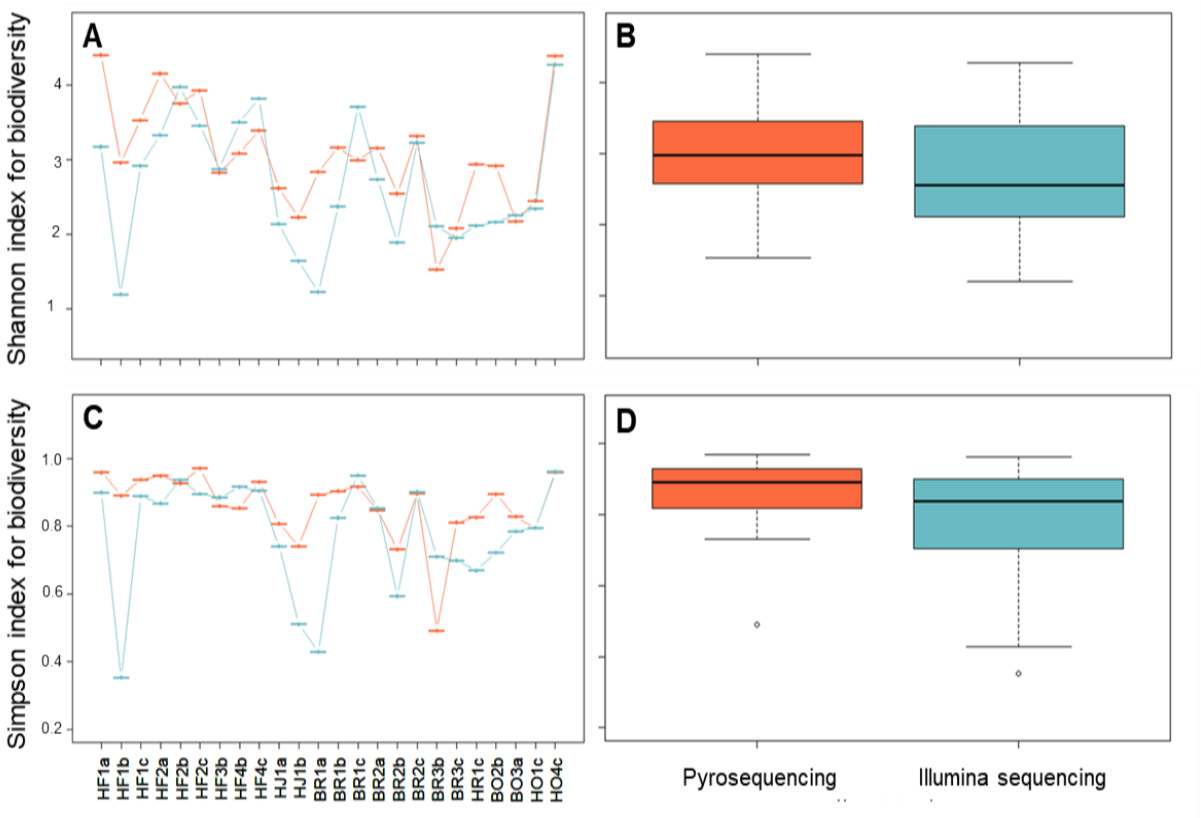

Figure 4.3.4: Comparison of Alpha Diversity of Root-Associated Fungal Communities Obtained by Applying Two Different NGS Techniques. A) Shannon indices of root-associated fungal communities on sample level. **B)** Means of Shannon indices of root-associated fungal communities. **C)** Simpson indices of root-associated fungal communities on sample level. **D)** Means of Simpson indices of root-associated fungal communities. Blue color represents data obtained by Illumina sequencing, orange color represents data obtained by 454 Pyrosequencing. n = 24. B = Bukit12 landscape, H = Harapan landscape, F = rain forest, J = jungle rubber, R = rubber plantations, and O = oil palm plantations. Numbers 1 - 4 = core plot ID numbers, a - c subplot names.

NGS technique	Mean observed fungal OTU richness	Mean effective number of OTUs associated with Shannon indices	Mean effective number of OTUs associated with Simpson indices
Illumina sequencing	332	15	8
454 Pyrosequencing	180	21	11

Table 4.3.2: Means of Observed Fungal Richness and Effective Numbers of OTUs Associated with Shannon and Simpson Indices. n = 24.

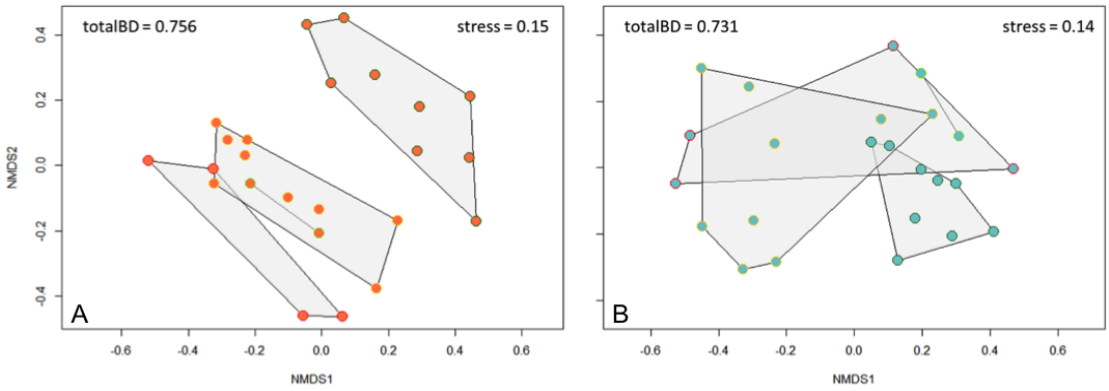

Figure 4.3.5: Non-Metric Multidimensional Scaling (NMDS) of Root- Associated Fungal Communities. A) Root-associated fungal communities generated by 454 Pyrosequencing **B)** Root-associated fungal communities generated by Illumina sequencing. Blue circles represent data obtained by Illumina sequencing, orange circles represent data obtained by 454 pyrosequencing. Color of circle boarders refer to the land use system from which fungal communities are extracted. Dark green = rain forest, green = jungle rubber, orange = rubber monoculture, red = oil palm plantations. Total beta-diversity (totalBD) among different root fungal communities are indicated in the left corner of the NMDS plot. Total BD are calculated by the *beta.div* function in R with previous transformation (Hellinger) of raw count OTU tables. n =24.

4.3.3 Taxonomic Composition of Root-Associated Fungal Communities were Similar Between Root Community Samples Analyzed by Illumina Sequencing and 454 Pyrosequencing

Using the taxonomically resolved groups (e.g. "unidentified" and "Incertae sedis" were not counted) sequence reads were assigned to 6 fungal phyla, 17 classes, 63 orders, 117 families, 240 fungal genera (Table S 4.1), and 1814 fungal OTUs (Table eS 4.1). Sequence reads of the ITS1 region generated by Illumina sequencing belonged to 2694 different fungal OTUs (Table eS 4.2). These sequences were assigned to 6 fungal phyla, 23 classes, 81 orders, 170 families, and 353 fungal genera when counting only taxonomic resolved groups (Table S4.2). Both NGS techniques generated similar results (Figure 4.3.6) regarding to the relative abundances of the fungal phyla of Ascomycota and Chyridiomycota. When applying 454 pyrosequencing and sequencing the whole fungal ITS region of the environmental DNA extracted from root communities, sequence reads of Basidiomycota, Glomeromycota, Rozzelomycota and Zygomycota were more abundant compared to sequencing the ITS1 region with the Illumina MiSeq technique (Figure 4.3.6). Investigations of taxonomic overlap of root-associated fungal communities obtained by the two applied NGS techniques showed that the fungal communities recovered by Illumina sequencing contained more unique fungal orders than the fungal communities recovered by Pyrosequencing (only counting taxonomic resolved orders) (Figure 4.3.7). However, the root-associated fungal community recovered by Illumina sequencing shared 69 % of its fungal orders with the fungal community obtained by 454 Pyrosequencing (Figure 4.3.7). The root-associated fungal community obtained by 454 Pyrosequencing shared 90 % of its fungal orders with the fungal community obtained by Illumina sequencing. Sequence reads of fungal OTUs assigned to 25 unique fungal orders only found by Illumina sequencing had a relative abundance of only 0.09 % (in relation to all fungal sequence reads generated by Illumina sequencing) (Figure 4.3.7). Fungal OTU sequence reads assigned to fungal orders unique in the fungal community recovered by 454 Pyrosequencing had a relative abundance of 0.02 % (Figure 4.3.7).

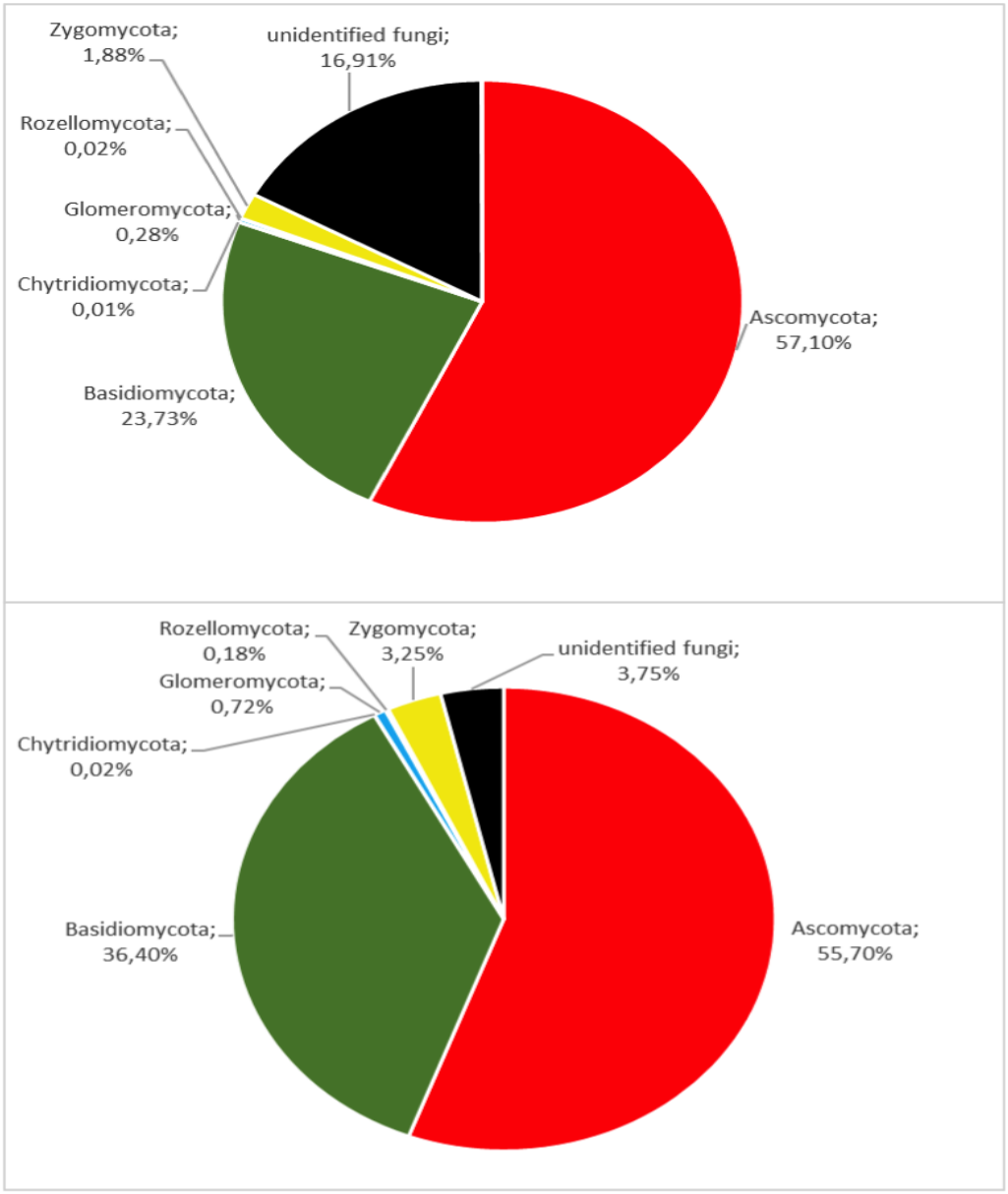

Figure 4.3.6: Abundances of Fungal Phyla. A) Fungal phyla detected by Illumina sequencing **B)** Fungal phyla detected by 454 Pyrosequencing. The means of relative abundances of fungal phyla are indicated in the pie chart. Parts of the pie charts represent the relative abundances of fungal phyla, with the of number sequence reads of each fungal phylum in proportion to the total number of sequence reads obtained in a root community from a subplot. n = 24.

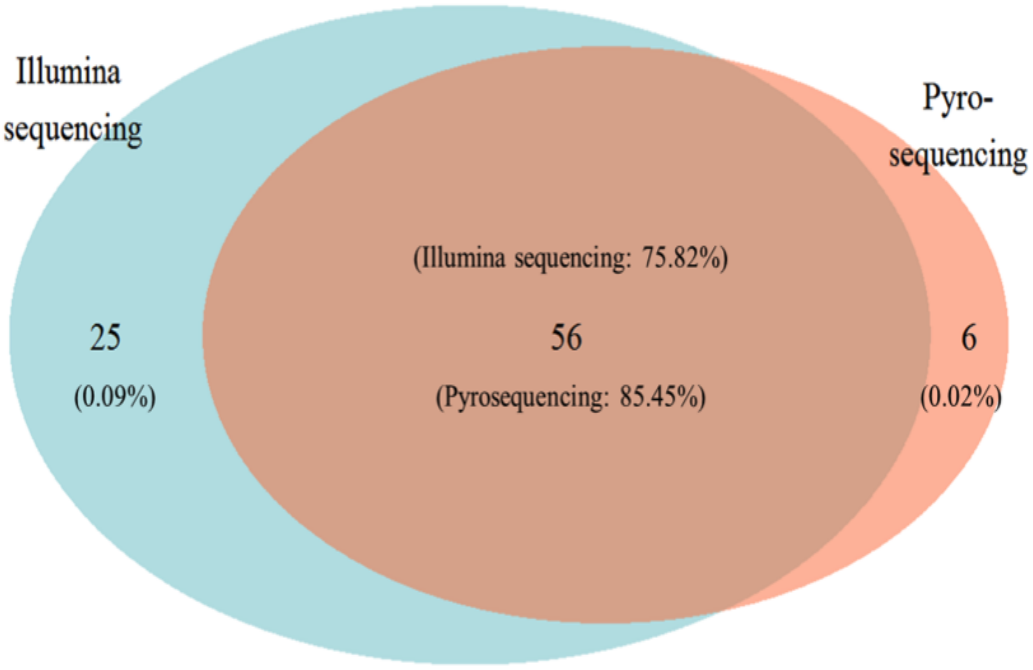

Figure 4.3.7: Venn Diagram of Shared and Non-Shared Fungal Orders Between the Two Applied NGS Techniques. The blue circle represents data from Illumina sequencing and the orange circle data obtained from 454 Pyrosequencing. Numbers in the circles and in area of overlap between circles indicate the number of fungal orders shared and non-shared between the two different NGS techniques. Graph was generated by applying the *draw.pairwise.venn* function of the *VennDiagram* package in R. n =24.

Further analyses of taxonomic overlap of root-associated fungal communities found by Illumina sequencing and Pyrosequencing in the same root samples showed differences among fungal genera (Figure 4.3.8). Fungal communities generated by Illumina sequencing consisted of more different fungal genera than those generated by Prosequencing (Figure 4.3.8). By using taxonomically resolved fungal genera, the fungal community recovered by Illumina sequencing shared 41 % of its fungal genera with the fungal community obtained by 454 Pyrosequencing. The root-associated fungal community obtained by 454 Pyrosequencing shared 60 % of its fungal genera with the fungal community obtained by Illumina sequencing. Both fungal communities were composed of a high number of unique fungal genera only present in one of the two root-associated fungal communities obtained by the two different NGS techniques (Figure

4.3.8). However, fungal OTUs assigned to these unique fungal genera were only present at low abundances in relation to the whole fungal OTU community composition. Fungal OTUs of unique fungal genera generated by Illumina sequencing had a relative abundance of only 0.8 % and those recovered by pyrosequencing had a relative abundance of 2.89 %.

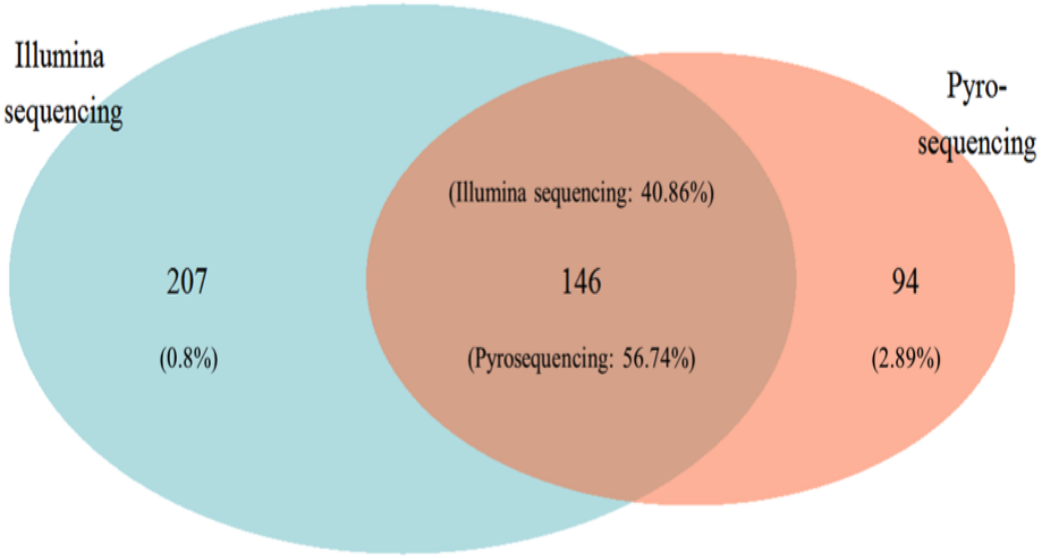

Figure 4.3.8: Venn Diagram of Shared and Non-Shared Fungal Genera Between the Two Applied NGS Techniques. The blue circle represents data from Illumina sequencing and the orange circle data obtained from 454 Pyrosequencing. Numbers in the circles and in area of overlap between circles indicate the number of fungal genera shared and non-shared between the two different NGS techniques. Relative abundances (related to the total number of sequence reads of fungal OTUs observed in each community) of fungal genera in root-associated fungal communities are indicated in percentage. Graph was generated by applying the *draw.pairwise.venn* function of the *VennDiagram* package in R. N = 24.

4.3.4 The Applied NGS Technique had no Influence on the Relative Abundance of Selected Fungal Orders and Genera

Comparisons of the relative abundance of specific fungal genera in the fungal communities found in root community samples analyzed by Illumina sequencing and Pyrosequencing showed

similar results for both NGS techniques (Figure 4.3.9). There were no significant differences found in relative abundances for the fungal genera of *Arthrinium*, *Pyrenochaetopsis*, *Fusarium*, *Mortierella*, *Russula* and *Scleroderma* between root samples analyzed by the two different NGS techniques ($p_{Arthrinium} = 0.882$, $p_{Pyrenochaetopsis} = 0.693$, $p_{Fusarium} = 0.794$, $p_{Mortierella} = 0.564$, $p_{Russula} = 0.0.072$ $p_{Scleroderma} = 0.867$). In addition, the applied NGS technique had no significant influence on the relative abundance of the six selected genera within one land use system (Table 4.3.3).

Land use system	**Arthrinium**	**Pyreno – chaetopsis**	**Fusarium**	**Mortierella**	**Russula**	**Scleroderma**
F_pyro - F_illu	$p = 0.99$	$p = 1$	$p = 1$	$p = 0.99$	$p = 0.26$	$p = 1$
J_pyro - J_illu	$p = 1$	$p = 0.99$	$p = 1$	$p = 1$	$p = 1$	$p = 1$
R_pyro - R_illu	$p = 1$	$p = 0.99$	$p = 0.99$	$p = 1$	$p = 1$	$p = 1$
O_pyro - O_illu	$p = 1$	$p = 1$	$p = 0.99$	$p = 1$	$p = 1$	$p = 1$

Table 4.3.3: Statistical Differences of Relative Abundances of Selected Fungal Genera in Root-Associated Fungal Communities Observed in Each Land Use System Related to the Applied NGS Technique. Table is showing p-values obtained by first testing for differences in relative abundances by applying generalized linear models to evaluate the influence of land use and then investigating differences between groups by applying a post hoc test.

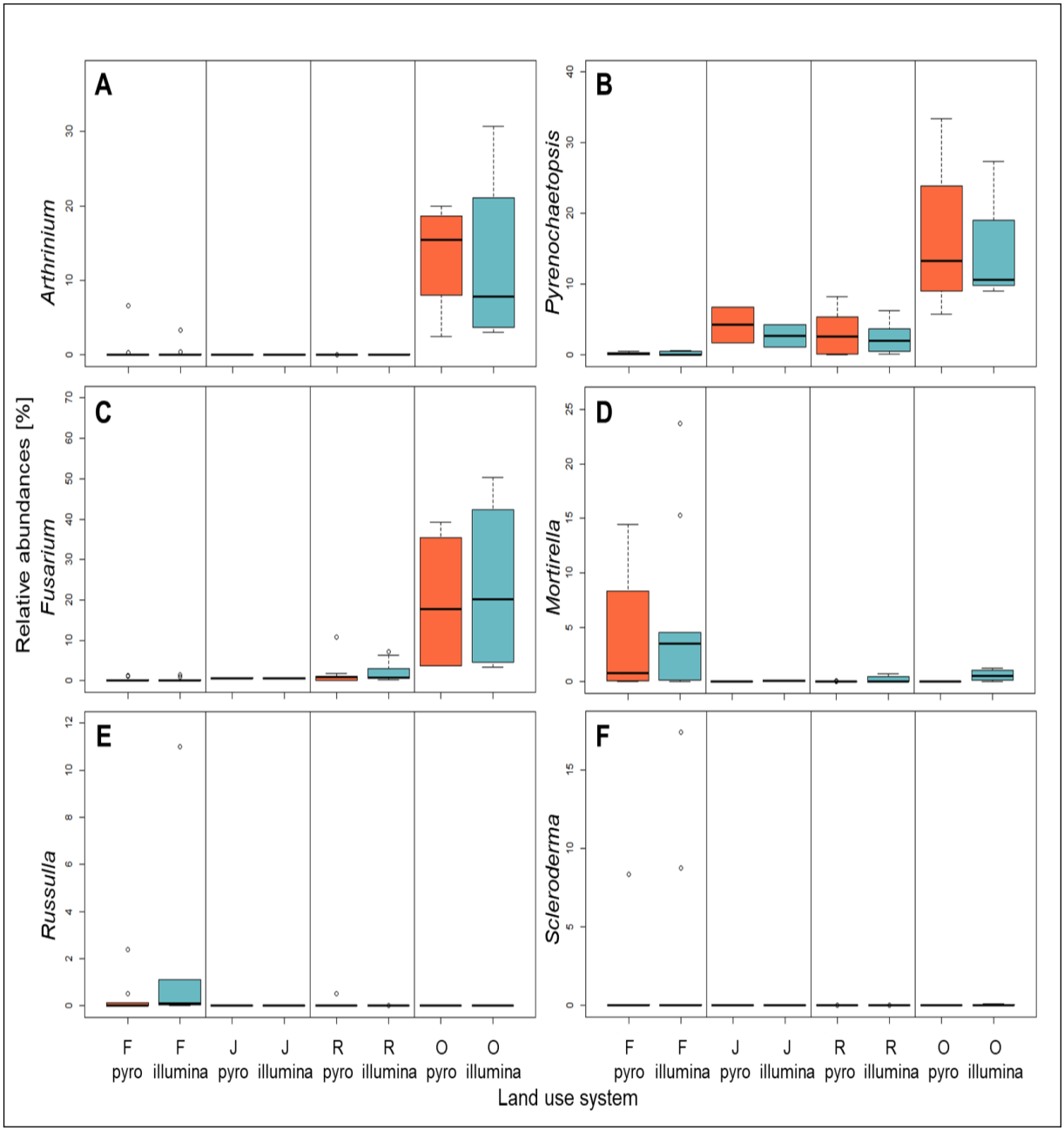

Figure 4.3.9: Relative Abundances of Six Selected Fungal Genera Observed by Applying Two Different NGS Techniques. A) Arthrinium **B)** Pyrenochaetopsis **C)** Fusarium **D)** Mortierella **E)** Russula **F)** Scleroderma. Relative abundances correspond to the total number of fungal sequence reads. n= 24. B = Bukit12 landscape, H = Harapan landscape, F = rain forest, J = jungle rubber, R = rubber plantations, and O = oil palm plantations, pyro = 454 pyrosequencing, illumina = Illumina sequencing.

4.4 Discussion

4.4.1 Effect of Applied NGS Technique and Related Sequenced Fungal Barcode on Obtained Results on Fungal OTU and Sequence Richness

We found that OTU and sequence richness of root-associated fungi was higher in root community samples analyzed by Illumina sequencing of the amplified ITS1 region than in those analyzed by 454 Pyrosequencing the amplified whole ITS region (Figure 4.3.1 and Figure 4.3.2). These results were as expected because of the ability of the Illumina sequencing technology to generate more sequence reads compared to the technology of 454 Pyrosequencing. This was reported in several studies comparing these two NGS techniques (Claesson et al., 2010; Frey et al., 2014; Liu et al., 2012; Luo et al., 2012). We found no correlation between the richness of observed root-associated fungal sequences found in the same root community samples that were analyzed by the two different NGS techniques (Figure 4.3.3). This means that the sequencing performance of the applied NGS technique was not sample-related (Table 4.3.1). Luo et al. (2012) found a strong positive correlation ($r^2 = 0.99$) between observed sequence richness recovered by Illumina sequencing and 454 Pyrosequencing from the same freshwater plankton sample. Here, the richness of observed root-associated fungal OTUs was 1.7 times higher in samples of root communities analyzed Illumina sequencing than in those analyzed by 454 Pyrosequencing (Figure 4.3.3). When investigating the relation between fungal OTU richness generated by Illumina sequencing and 454 Pyrosequencing also no correlation was found. Our results cannot be explained by different DNA extraction methods because the root-associated fungal communities analyzed by the two different NGS techniques were detected in same DNA extract. Primer choice may have had an influence on observed sequence and OTU richness differences, but it is more likely that primer choice influenced the taxonomic community structure (Bazzicalupo et al., 2013; Claesson et al., 2010) than sequence read or OTU numbers in samples. It is known that PCR-based errors can occur (Acinas et al., 2005). They may have played a role for sample-dependent differences in sequencing performances observed by the two NGS techniques. However, here the PCR conditions in sample preparation were identical for the two different NGS techniques applied, with regard to the cycle number (30 cycles), annealing temperature 47 °C, and extension time (5 minutes). However, examining both NGS techniques individually, root-associated fungal OTU and sequence richness

were positively correlated (Figure 4.3.3 C – D), which simply means that with an increasing sequence read number the number of detected OTUs increases regardless of the respective NGs technique (Figure 4.3.1). The significantly higher richness of observed root-associated fungal sequences generated by Illumina sequencing than by 454 Pyrosequencing (Figure 4.3.2 B) and the fact that sequences richness and OTU richness are positively correlated explains higher fungal OTU richness of root community samples detected by Illumina sequencing than by 454 Pyrosequencing.

4.4.2 Effects of the Applied NGS Techniques and Related Differing Sequenced Fungal Barcode Regions on Alpha- and Beta-Diversity

Mean alpha-diversity was not significantly different between root-associated fungal communities analyzed by Illumina sequencing and 454 Pyrosequencing (Figure 4.3.4). A slight decrease in mean alpha-diversity regarding Shannon and Simpson index for diversity in root-associated fungal communities analyzed by Illumina sequencing was detected compared to those analyzed by 454 Pyrosequencing, although the mean OTU richness of root-associated fungal communities was higher in root community samples analyzed by Illumina sequencing (Figure 4.3.4 D and B). This observation could be explained by a higher number of unique fungal OTUs in root-associated fungal communities obtained by Illumina compared to those in communities analyzed by 454 Pyrosequencing sequencing and in contrast their low relative abundances in the communities (Figure 4.3.7 and Figure 4.3.8). These results indicate that the distribution of fungal OTUs in root-associated fungal communities found by Illumina sequencing is more uneven than by those found by 454 Pyrosequencing. Total beta-diversity was slightly higher in root-associated fungal communities obtained by 454 Pyrosequencing than in communities obtained by Illumina sequencing (Figure 4.3.5) which means that differences in the diversity of root-associated communities among samples were greater in root-associated fungal communities obtained by 454 Pyrosequencing than those by Illumina sequencing.

4.4.3 The Detection of the Taxonomic Composition of Root-Associated Fungal Communities is Affected by the Applied NGS Techniques and Different DNA Barcode Regions

Taxonomic community composition of root-associated fungi on the phylum level showed relatively similar results for fungal communities obtained by whether analyzing the same root community samples by Illumina sequencing or by 454 Pyrosequencing. Similar proportions of the detected fungal OTUs belonged to the phylum of Ascomycota (Figure 4.3.6). Fungal OTUs belonging to the phyla of Chyridiomycota, Glomeromycota, Rozellomycota, and Zygomycota were rare in abundance in root-associated fungal communities obtained by both NGS techniques, but all of these fungal phyla were represented in higher proportions in the communities obtained by 454 Pyrosequencing than in those identified by Illumina sequencing (Figure 4.3.6). Huge differences were found regarding fungal OTUs belonging to the Basidiomycota and unidentified fungi (Figure 4.3.6). The proportion of fungal OTUs belonging to unidentified fungi was nearly 5 times (17 %) higher compared to those found in root-associated communities obtained by 454 Pyrosequencing (Figure 4.3.6). The higher proportion of fungal OTUs with unidentified taxonomy in root-associated communities obtained by Illumina sequencing might be partly due to the different DNA regions amplified for taxonomic analyses. For Illumina sequencing the ITS1 region (including a part of the ribosomal small subunit (SSU) and a part of the conserved 5.8 S) of the environmental DNA was amplified and for 454 Pyrosequencing the whole ITS region (including a small part the SSU, the ITS1 region, the 5.8 S, the ITS2 region and a part of the ribosomal large subunit (LSU)) was amplified. Compared to the whole ITS region with a length varying between 450 and 800 bp (Bellemain et al., 2010; Gardes and Bruns, 1993) the ITS1 region is much shorter with a varying length of 100 to 380 bp (Bellemain et al., 2010). Longer fragments of the ITS region yield better taxonomic resolution. Therefore, Pyrosequencing with a fragment length of 450 to 800 bp might have resulted in a lower proportion of fungal OTUs with an unidentified taxonomy. In root-associated fungal communities obtained by Illumina sequencing 23.7 % of OTUs belonged to Basidiomycota compared to 36.4 % of fungal OTUs in communities obtained by 454 Pyrosequencing (Figure 4.3.6). These findings do not support the results of Bellemain et al. (2010) on their *in silico* approach on the comparison of the taxonomic resolution of the whole ITS region vs ITS1 and ITS2 region. They found that targeting the whole ITS region will

lead to bias towards a higher proportion of Ascomycota relative to Basidiomycota. A possible explanation for the divergent results of our study and that of Bellemain et al. (2010) may be related to the further development of fungal specific primers with a high coverage of both Ascomycota and Basidiomycota (Toju et al., 2012). We used the ITS1F_KYO2 primer (Toju et al., 2012) as forward primer for the analyses of root-associated fungal communities for both applied NGS techniques. The ITS1F_KYO2 primer has a higher and a more balanced coverage (Toju et al., 2012) of fungal OTUs belonging to the Ascomycota and Basidiomycota compared to the ITS1 and ITS1F primer employed by Bellemain et al. (2010). In addition, our results are supported by the study of Toju et al. (2012). They showed that the ITS2 reverse primer (White et al., 1990), which was also used in our study to amplify the ITS1 region, amplifies a relatively lower proportion of Basidiomycota than the ITS4 reverse primer (White et al., 1990). Here we used the ITS4 primer to amplify the whole ITS region and applied for the resulting fragments 454 Pyrosequencing.

4.4.4 Taxonomic Overlap and Distinctness of Root-Associated Fungal Communities Investigated by Two Different NGS Techniques

The root-associated fungal communities obtained by Illumina sequencing and 454 Pyrosequencing showed an overlap and distinctness on different taxonomic levels (Figure 4.3.7 and Figure 4.3.8). The high relative abundance of fungal OTUs belonging to shared orders and genera in root-associated fungal communities obtained by Illumina sequencing or 454 Pyrosequencing indicate that both NGS techniques and the related difference in amplified DNA regions sampled a similar fraction of the fungal diversity present in root communities of the investigated samples. These findings agree with those of Luo et al. (2012) who compared Illumina sequencing vs. 454 Pyrosequencing using freshwater plankton communities. Unique fungal orders and genera only observed in root-associated fungal communities obtained by one of the two applied NGS techniques were high in numbers, but low in abundances (Figure 4.3.7 and Figure 4.3.8). We observed higher numbers of unique fungal orders and genera in those communities obtained by Illumina sequencing which is probably the result of a lager sample size in terms of sequence richness (Figure 4.3.1, Table 4.3.1). It has been shown in several studies that sample size and recovered richness of taxonomic groups are positively correlated (e.g. Bazzicalupo et al., 2013; Claesson et al., 2010; Porras-Alfaro et al., 2011). The fact that

unique fungal orders and genera were also found in root-associated fungal communities obtained by 454 Pyrosequencing is maybe a result of the better taxonomic resolution of the DNA region amplified that was used as the fungal barcode. This assumption is supported by the lower abundance of unidentified fungi found in root-associated fungal communities analyzed by 454 Pyrosequencing (Figure 4.3.6).

4.4.5 Validation of Data on Relative Abundances of Fungal OTUs Belonging to Selected Fungal Genera with a Proven Ecological Function

Our previous results (Chapter 3, 3.3.5 – 3.3.8) showed that root-associated fungal communities from four different land use systems showed a shift in the relative abundance of distinct functional groups. To cross-check the data obtained by Illumina sequencing with Pyrosequencing, six fungal genera were selected and their abundances in the Illumina sequencing and Pyrosequencing approach were compared. These six fungal genera were chosen because they were specifically affected by rain forest transformation into rubber and oil palm plantations. We found no difference in relative abundance of these fungal genera regardless of the applied NGS technique (Figure 4.3.9). Furthermore, no significant differences were found when each land use system was compared regarding the relative abundance of the six fungal genera related to the applied NGS technique (Table 4.3.3). These results are strongly supporting and verifying conclusions made on observed shift in root-associated communities in relation to land use transformation (Chapter 3, 3.4.5).

4.5 References

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CHAPTER FIVE

5 Synthesis

5.1 The Broader Frame of this Thesis

Tropical rain forests are one of the most species-rich ecosystems on earth (Hartshorn, 2013). A major threat for biodiversity of tropical forests are human driven land use changes which are leading to deforestation because of timber extraction and the need to increase agricultural areas (Ehrlich and Ehrlich, 2013; Sodhi et al., 2004). The cultivation of oil palm (*Elaies guineesis*) is a major driver for forest transformation in the tropics (Carrasco et al., 2014; Fitzherbert et al., 2008). Indonesia reached the highest deforestation rate worldwide with a loss of 840.000 hectare per year ft in 2012 (Margono et al., 2014). World's growing human population and the related increasing demand for palm oil as a biofuel, and as a feedstock for food and cosmetics will lead to a further expansion of oil palm plantations in Indonesia and tropical regions all over the world (Danielsen et al., 2009; Smit et al., 2013; Sodhi et al., 2010).

The loss of biodiversity as a consequence of global environmental changes and of land use change in particular has been a major concern because of the impact on ecosystem functions and services (Drescher et al., 2016; Gardner et al., 2009; Gibson et al., 2011; Pimm et al., 2014; Sala, 2000). However, land use transformation is not always leading to a loss in biodiversity. For soil prokaryotes it has been shown that richness and diversity increased with increasing land use intensification (Schneider et al., 2015). Kerfahi et al. (2016) found that the diversity of soil fungi, nematodes, and bacteria was not decreased by forest conversation. But changes and losses in biodiversity can occur on the taxonomic, structural or functional level of a community (Duncan et al., 2015). Structural and functional alterations of communities are often having a greater importance for ecosystem functioning than the species richness of a community *per se* (Diaz et al., 2007; Duncan et al., 2015; Lavorel, 2013; Mokany et al., 2008). The species pool present in an ecosystem forms the biotic fundament of the corresponding ecosystem, the complex interactions among its diverse members, and the interdependencies of biotic and abiotic ecosystem properties are providing ecosystem functions and finally ecosystem services. To understand the impact of anthropogenic driven land use changes on biodiversity, different levels of biodiversity have to be included and related to the functional and structural aspects. The majority of research conducted in the tropical regions has focused on aboveground biodiversity in relation to ecosystem functioning, whereas the immense biodiversity found belowground and its impact on ecosystem functions and services have rarely been addressed. Plants

build the stationary fundament of terrestrial biomes and are often the first group of organisms directly influenced by land use changes. This can lead to a six-fold decline of plant species richness in converted land use systems comparted to rain forests (Drescher et al., 2016). All plants are associated with microorganisms which contribute to the adaption of plants to changing environmental conditions and play an important role for the ecosystem functioning (Chen et al., 2014 a; Peršoh, 2015; Redman et al., 2011). Fungi are a highly diverse group of microorganisms and the composition of fungal communities varies among ecosystems and on different spatial and temporal scales (Hawksworth, 1991; Peršoh, 2015; Tedersoo et al., 2014; Toju et al., 2014) and is in many cases related to the host identity and/ or phylogenetic affiliation (Lang et al., 2011; Maron et al., 2011; Smith and Read, 2008; Tedersoo et al., 2008). Of particular importance are these fungal groups which are controlling regulatory steps in ecosystems (Hawksworth, 1991; Peršoh, 2015). One important functional group is represented by mycorrhizal fungi which form mutualistic interactions with plant roots and supply water and nutrients to their hosts and act as a main pathway for carbon to the soil (Hobbie, 2006; Verbruggen et al., 2016; Zhu, 2003). Plant pathogenic fungi are of functional importance because they influence plant health status and can cause diseases and pests (Li et al., 2014; Maron et al., 2011). Saprotrophic fungi are important decomposer for nutrient cycling and nutrient distribution in soil (Baldrian and Valášková, 2008; Cairney, 2005). So far, most studies on fungal communities have focused on the taxonomic and structural aspect of fungal diversity (e.g. McGuire et al., 2011; Mueller et al., 2014; Orgiazzi et al., 2012; Peay et al., 2013). However, there is a need to investigate the functional properties of fungal communities. This would enable us to obtain a more comprehensive understanding of fungal communities and to predict consequences for differing ecosystem functions in response to functional fungal groups.

5.2 Relationship Between Root Community Traits, Fungal OTU Richness and Ecological Functions

The present thesis showed that transformation of tropical rain forest into intensive rubber and oil palm mono-plantations affected functional traits of root communities and that root community traits were correlated with ecosystem properties (Chapter 2). The findings are

demonstrating that the degradation of root community traits is an indicator for tropical low land rain forest transformation into monoculture plantations, because a decline of positive traits and the degradation of root health in monoculture plantations was related to an accumulation of plant toxic elements. As a result, root community traits were linked to ecosystem properties such as soil carbon (Sahner et al., 2015)

Analyses of root-associated fungal diversity in terms of taxonomic, structural, and functional community composition did not reveal clear patterns of a fungal operational taxonomic unit (OTU) richness decline in monoculture plantations, but land use systems had a strong influence on the community composition of root-associated fungi. Most importantly, land use had an influence on the abundances of different functional fungal groups, led to a decrease in the abundance of beneficial functional fungal groups (i.e. arbuscular mycorrhizal fungi and ectomycorrhizal fungi), and an increase of the functional group of plant pathogenic fungi.

The analysis on fungal diversity was cross-checked by a second next generation sequencing technique which supported our obtained results on fungal OTU diversity. An unexpected result was, that the diversity of root-associated fungal communities was not higher in rain forests than in highly managed monoculture plantations. However, the composition of root-associated fungal communities was significantly influenced by land use and the variables explaining most of the dissimilarities among land use systems were root-community-weighed traits (RCWTs). To evaluate whether RCWTs can be related to root-associated fungal OTU richness, the scores of PC1 (Sahner et al., 2015) which reflected the status of degradation of each root sample, were related to the fungal OTU richness of the same root sample 5.2.1 (Figure 5.1). Applying a generalized linear model (with quasipoisson distribution) showed that the richness of root-associated fungal OTUs is related to the RCWTs.

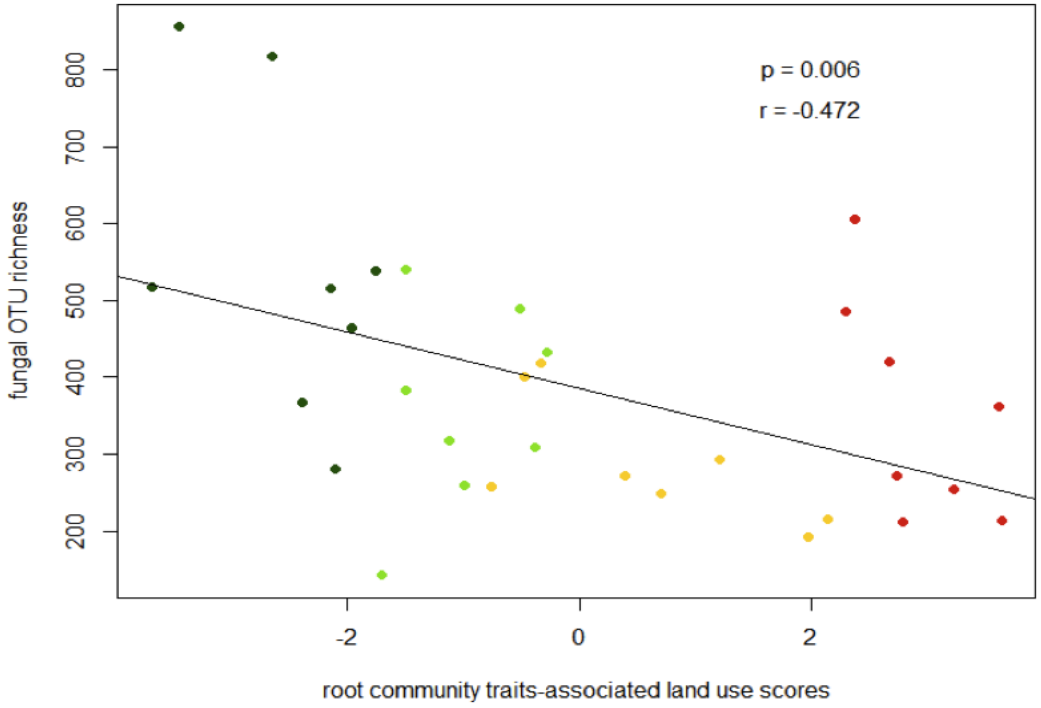

Figure 5.2.1: Relation Between Root Community Traits and Richness of Root-Associated Fungal OTUs. Richness of fungal OTUs is related to the rarified data.

Only few studies investigated belowground fungal diversity in tropical rain forests (Kerfahi et al., 2014, 2016; McGuire et al., 2011, 2015; Mueller et al., 2014; Peay et al., 2013; Toju et al., 2014) and with the exception of Toju et al. (2014), all have investigated soil not root-associated fungal communities. In temperate forests Goldmann et al. (2016) found that root-associated fungi are mainly recruited from the soil. It was shown that 94 % of root-associated fungal OTUs were detected in soil, but in soil 66 % of fungal OTUs were unique. With an increasing distance the similarity of communities declines (Nekola and White, 1999). Goldmann et al. (2016) showed that this distance decay has a greater influence on soil fungal communities than on root-associated fungal communities. A comparison between obtained results on root-associated communities in this thesis and soil fungal communities in other studies should, therefore, be considered critically. Two studies investigated the influence of

tropical rain forest transformations into oil palm plantations (Kerfahi et al., 2014; McGuire et al., 2015) on soil fungal communities. These studies found an influence of land use on fungal community composition but not on OTU richness. Both studies report a decrease of fungi belonging to the phylum of Basidiomycota and an increase of Ascomycota in oil palm plantations compared to rain forest which corresponds to our findings. Kerfahi et al. (2014) as well as McGuire et al. (2015) found a decline in abundances of ectomycorrhizal fungi (EMF) in oil palm plantations. These results also agree with those obtained in the present thesis. However, results for other fungal functional groups, like arbuscular mycorrhizal fungi (AMF) or pathogenic fungi are lacking.

To our knowledge, the shifts between functional groups of root-associated fungi among different land use systems in the tropics were reported here for the first time. The relative abundances of AMF and EMF were significantly lower in monoculture plantations compared to rain forest sites whereas the abundance of plant pathogenic fungi massively increased. The low abundance of EMF can be explained by the absence of ectomycorrhiza forming host trees (personal communication with Dr. Katja Rembold). The low abundance of AMF in root communities in oil palm plantations compared to those in rain forest cannot be explained by a lack of plant hosts. Oil palms are associated with AMF and it was observed that oil palm roots are well colonized by AMF (Bakhtiar et al., 2013; Phosri et al., 2010; Sahner et al., 2015). However, Edy (2015) showed that AMF diversity was decreased in oil palm and rubber monoculture plantations.

The relative massive increase of the abundance of plant pathogenic fungi was mainly induced by fungal OTUs from the genus *Fusarium*. Fungal species of the genus *Fusarium* are able to infect the plant roots and can cause root rot and vascular wilt (Chen et al., 2014b; Flood, 2006; Jiménez-Díaz et al., 2015; Li et al., 2014). In oil palms *Fusarium* can cause vascular wilt. The symptoms of the vascular wilt are drying-out of leaves and a reduction of leave size (Flood, 2006). In oil palm, these symptoms can lead to yield reduction of 20 – 30 % and in Africa it is the most destructive fungal disease of oil palm (Flood, 2006). Whether this is also the term case in our study sites must be investigated.

Fusarium also occurs in rain forest but the question arises why it is much more abundant in oil plantations than in unmanaged forests. AMF can protect plants against root-infecting pathogens by high colonization, which results in a competition for colonization sites (Smith and Read, 2008). The AMF colonization of root communities in oil palm plantations in Bukit 12 landscape was stable (Sahner et al., 2015) but significantly lower in Harapan than in

all other land use systems and both landscapes (Sahner et al., 2015) (Figure 5.1 A). Results on relative abundances of functional groups of root-associated fungi were presented by land use systems only, not separated by landscapes. Therefore, data on the relative abundances of plant pathogenic fungi in root communities in oil palm plantations were reanalyzed to check whether differences exist between the landscapes. The relative abundances of pathogenic fungi present in root communities from oil palm plantations showed no differences between the landscapes (Figure 5.1 B). Additionally, it was tested by generalized linear models whether the AMF colonization impacts the abundance of pathogens in the roots in oil palm plantations and in general across all land use systems. No impact was found ($p_{oil\_palm} = 0.12$, $p_{across\text{-}land\_uses} = 0.98$). The decrease of colonization by AMF in oil palm plantations, therefore, did not result in an increase of the relative abundances of plant pathogenic fungi.

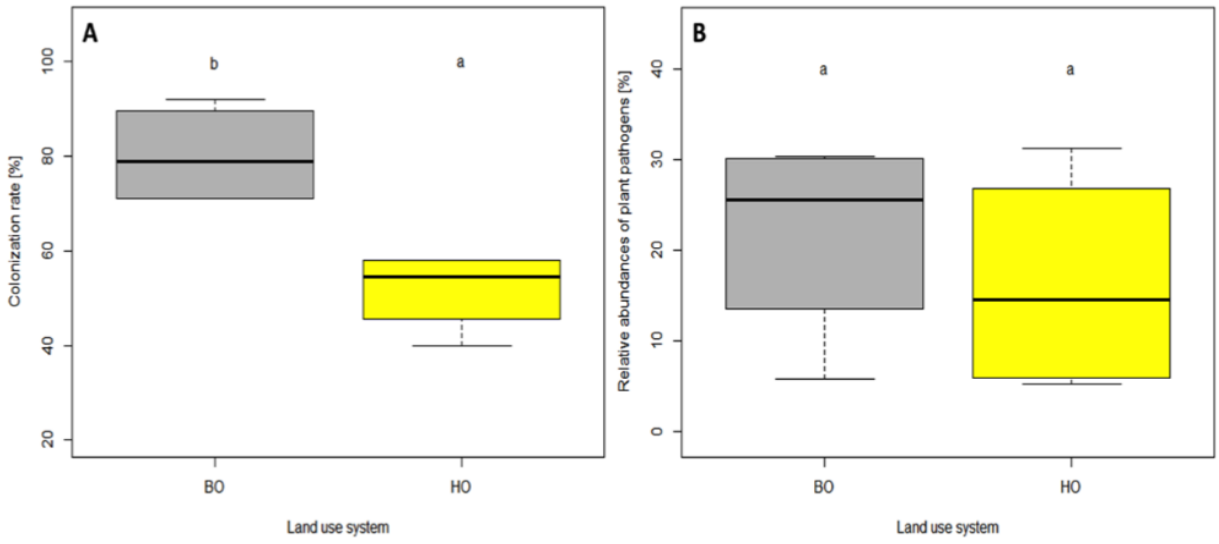

Figure 5.2.2: AMF Colonization of Root Communities (A) and Relative Abundances of Plant Pathogenic Fungi (B) in Oil Palm Plantations of Harapan and Bukit12 Landscape. B = Bukit12 landscape, H = Harapan landscape, and O = oil palm plantations.

Another currently speculative possibility is that the decline in EM enable the increase of pathogenic fungi. EMF are able to produce antifungal compounds (e.g. Duchesne et al., 1988; Yamaji et al., 2005). These compounds can reduce the pathogenicity through the reduction in sporulation of the pathogenic fungi before any root colonization by EMF occurs (Duchesne et al., 1988). Whether oil palms can benefit from the presents of EMF in their vicinity should be tested in future experiments. Here, oil palm plantations showed very low abundance EMF in root communities and also in soil (personal communications with N. Brinkmann).
The question for the strong accumulation of plant pathogenic fungi in root communities of oil palm plantations are thus still unclear. Fertilization, herbicide, and fungicide applications may have contributed to these shifts.

5.3 Conclusion and Outlook

To summarize, it was shown that the degradation of root community traits can be considered as an indicator for rain forest transformation into rubber and oil palm plantations. This degradation of root community traits, along with land use intensification, was correlated with the changes in the community structure of root-associated fungi. Obviously, land use changes led to an increase of pathogenic fungi and a decrease of myccorhizal fungi in monoculture plantations compared to unmanaged rain forests. These findings are representing the first insights into a complex topic and further research has to be conducted to gain more knowledge on the interdependencies and mechanisms shaping fungal community structures in relation to changing environmental conditions. As fungal community composition can differ on spatial and temporal scales, a resampling of root-associated fungal communities would be helpful to evaluate the obtained results of the present thesis. Furthermore, it would be of great importance to investigate fungal communities in agricultural plantations with differing defined levels of land use intensities to evaluate which management practices and intensity are leading to a negative shift in community compositions. In addition, management practices should be tested which enhance root vitality to antagonize the proliferation of pathogens and, therefore, may enhance ecosystem functioning.

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6 Supplements

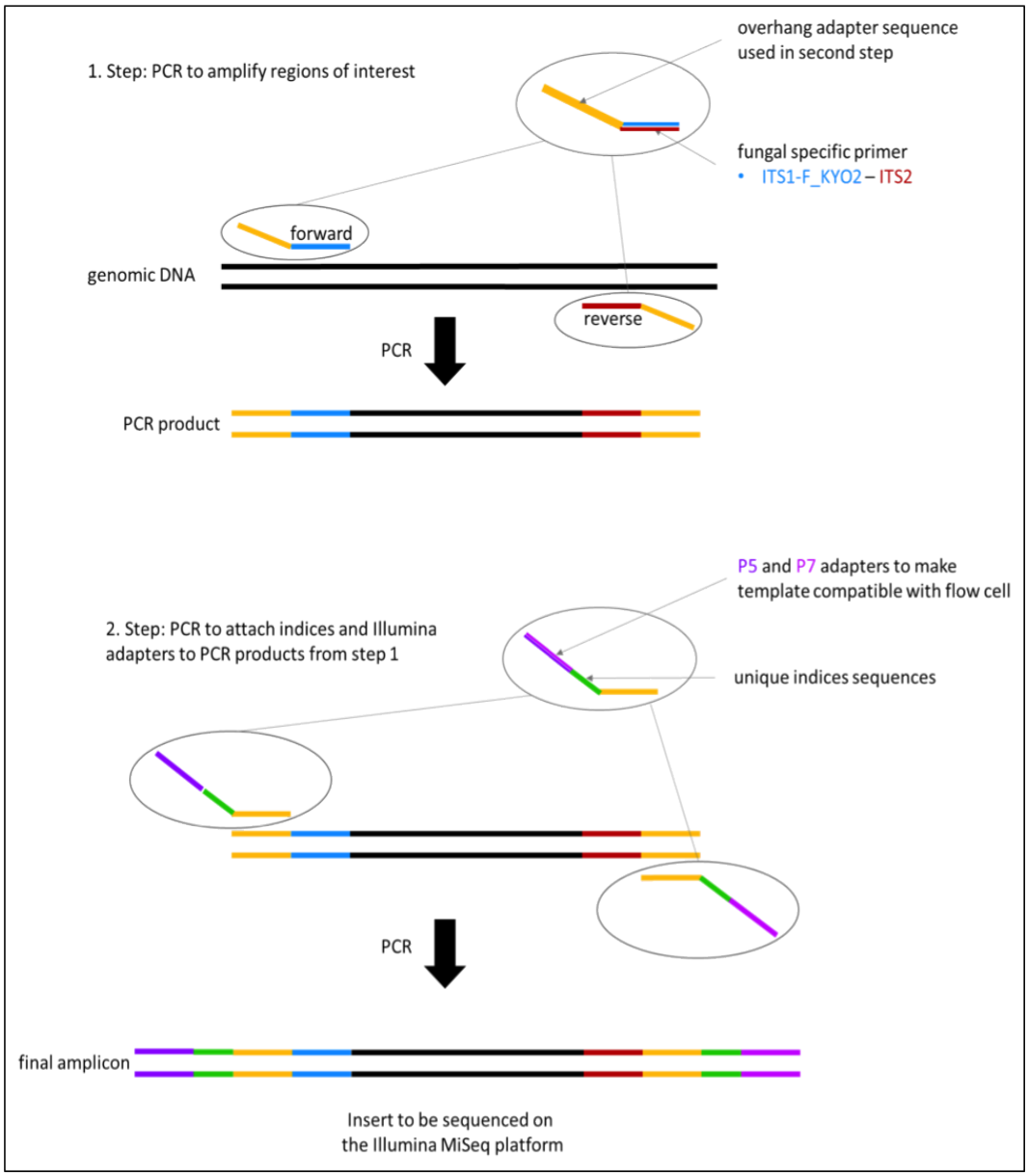

Figure S 3.2.1: Two Step PCR for Sample Preparation.

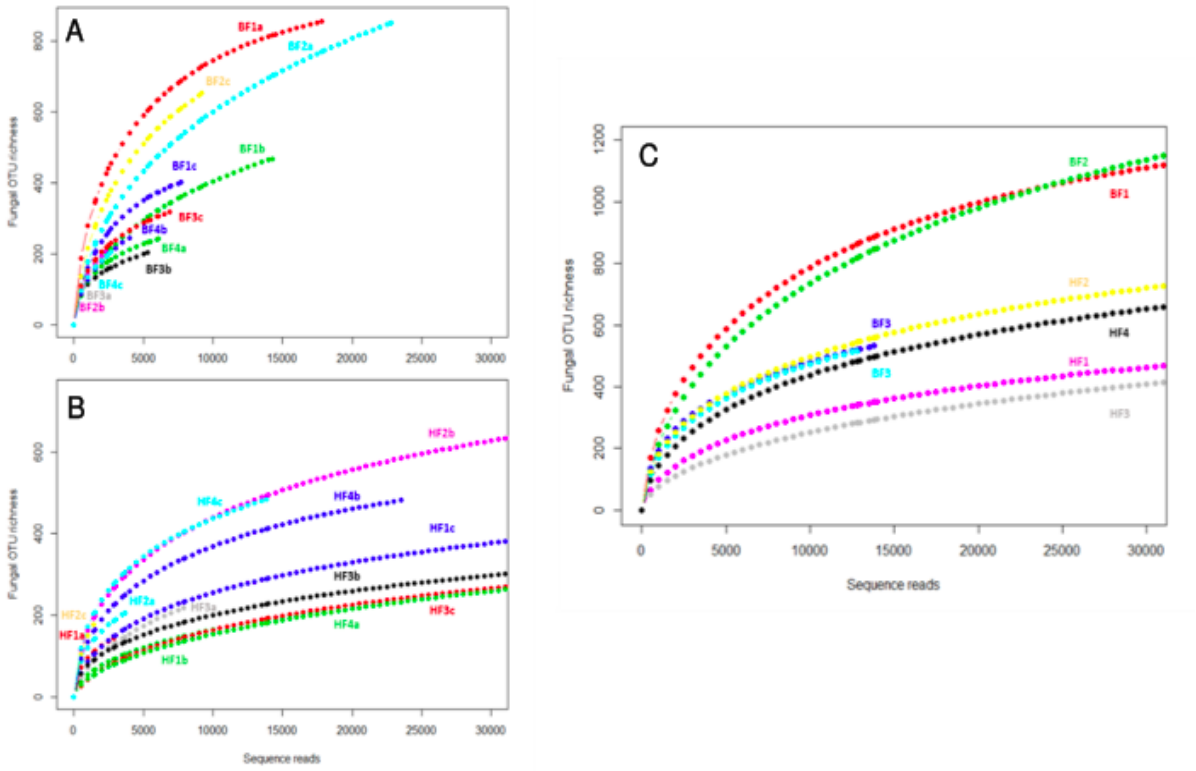

Figure S 3.3.1: Rarefaction Curves of Non-Rarified Sequences from Jungle Rubber Sites. A) Rarefaction curves of non-rarified sequences of subplot samples in Bukit 12 landscape **B)** Rarefaction curves of non-rarified sequences of subplot samples in Harapan landscape **C)** Rarefaction curves of non-rarified sequences of subplot samples plot by core plots in Bukit 12 and Harapan landscape.

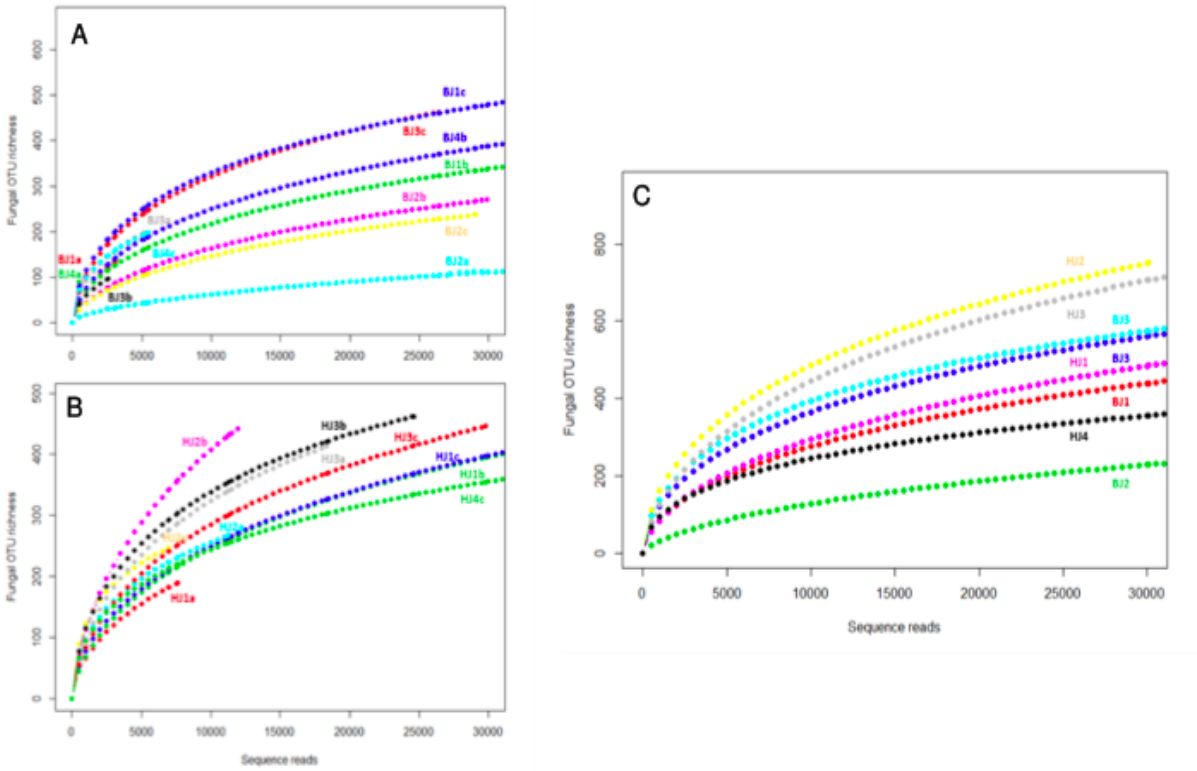

Figure S 3.3.2: Rarefaction Curves of Non-Rarified Sequences from Rubber Sites. A) Rarefaction curves of non-rarified sequences of subplot samples in Bukit 12 landscape **B)** Rarefaction curves of non-rarified sequences of subplot samples in Harapan landscape **C)** Rarefaction curves of non-rarified sequences of subplot samples plot by core plots in Bukit 12 and Harapan landscape.

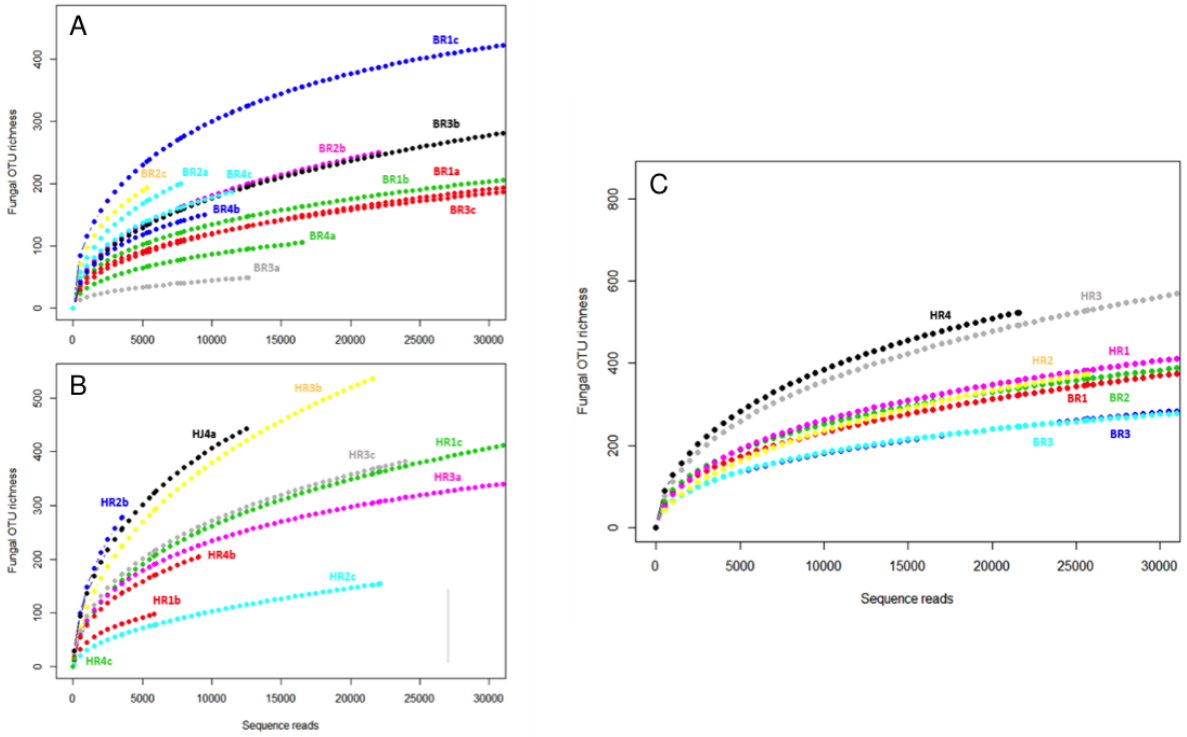

Figure S 3.3.3: Rarefaction Curves of Non-Rarified Sequences from Rubber Sites. A) Rarefaction curves of non-rarified sequences of subplot samples in Bukit 12 landscape**B)** Rarefaction curves of non-rarified sequences of subplot samples in Harapan landscape **C)** Rarefaction curves of non-rarified sequences of subplot samples plot by core plots in Bukit 12 and Harapan landscape.

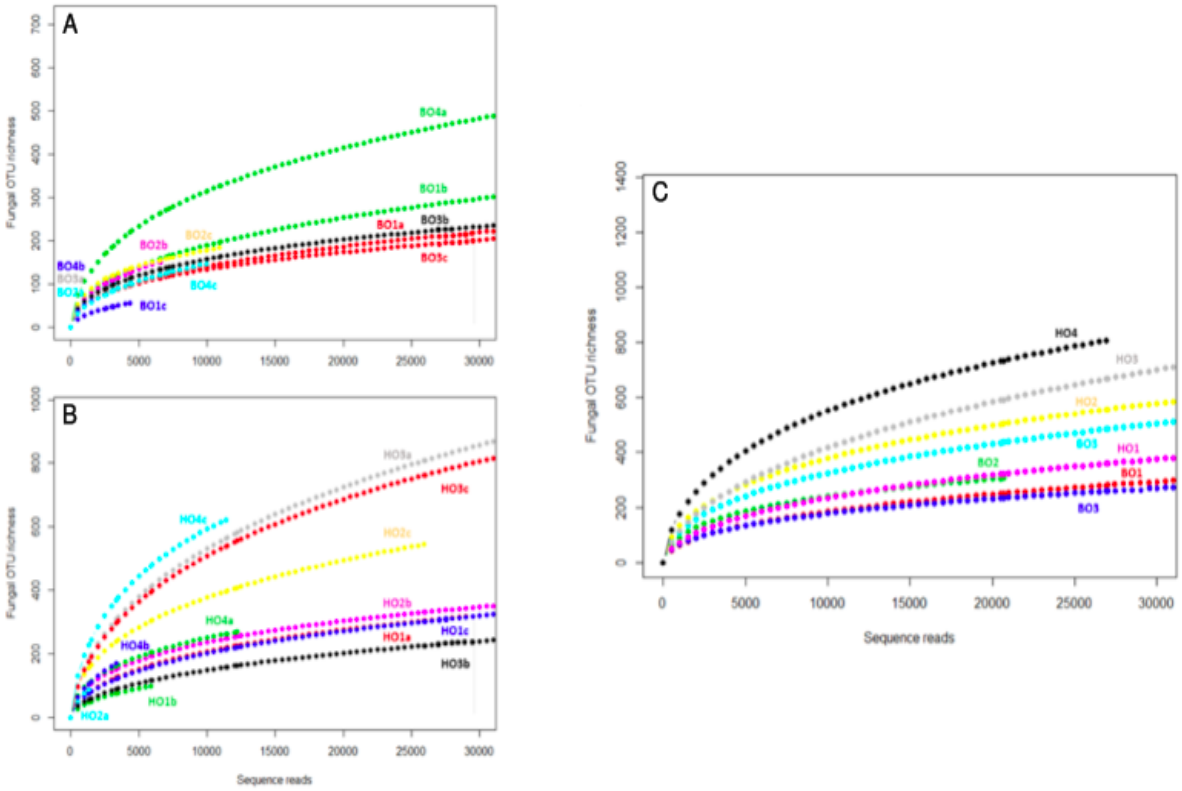

Figure S 3.3.4: Rarefaction Curves of Non-Rarified Sequences from Oil Palm Plantations. A) Rarefaction curves of non-rarified sequences of subplot samples in Bukit 12 landscape **B)** Rarefaction curves of non-rarified sequences of subplot samples in Harapan landscape **C)** Rarefaction curves of non-rarified sequences of subplot samples plot by core plots in Bukit 12 and Harapan landscape.

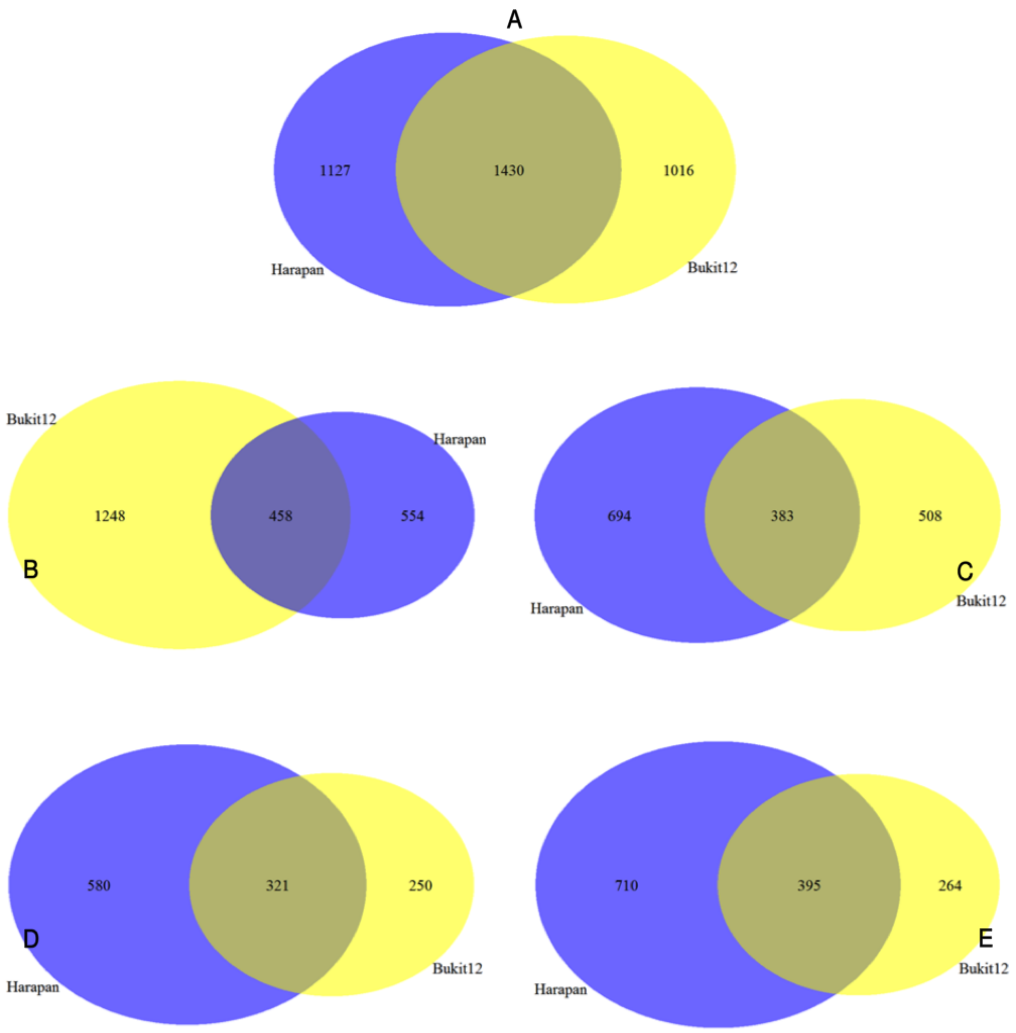

Figure S 3.3.5: Venn Diagram of Shared and Non-Shared Fungal OTUs. Each colored circles represents a landscape or land use system. Numbers in the circles and in overlaps between and among different circles indicate the number of fungal OTUs shared and non-shared between and among land use systems. **A)** Comparison of landscapes **B)** Comparison of forest sites of the two landscapes **C)** Comparison of jungle rubber sites of the two landscapes **D)** Comparison of rubber sites of the two landscapes **E)** Comparison of oil palm sites of the two landscapes.

Table S 3.1: Fungal Orders Found Across Land Use Systems.

Taxonomy of fungal orders found across the land use systems
k_Fungi; p_Ascomycota; c_Archaeorhizomycetes; o_Archaeorhizomycetales
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Botryosphaeriales
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Capnodiales
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Dothideales
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Hysteriales
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Jahnulales
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Myriangiales
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Patellariales
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Trypetheliales
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Tubeufiales
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Venturiales
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Chaetothyriales
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Eurotiales
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Mycocaliciales
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Onygenales
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Verrucarialis
k_Fungi; p_Ascomycota; c_Geoglossomycetes; o_Geoglossales
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Agyriales
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Lecanorales
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Ostropales
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Peltigerales
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Pertusariales
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Teloschistales
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Umbilicariales
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Erysiphales
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Helotiales
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Leotiales
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Rhytismatales
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Thelebolales
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Lichinales
k_Fungi; p_Ascomycota; c_Orbiliomycetes; o_Orbiliales
k_Fungi; p_Ascomycota; c_Pezizomycetes; o_Pezizales
k_Fungi; p_Ascomycota; c_Saccharomycetes; o_Saccharomycetales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Boliniales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Chaetosphaeriales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Coniochaetales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Diaporthales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Glomerellales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Lulworthiales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Magnaporthales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Microascales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Ophiostomatales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Sordariales

Table S 3.1 Continued

k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Trichosphaeriales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Xylariales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Atheliales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Auriculariales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Boletales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Cantharellales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Geastrales
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k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Phallales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Polyporales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Russulales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Sebacinales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Thelephorales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Trechisporales
k_Fungi; p_Basidiomycota; c_Atractiellomycetes; o_Atractiellales
k_Fungi; p_Basidiomycota; c_Cystobasidiomycetes; o_Cystobasidiales
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k_Fungi; p_Basidiomycota; c_Incertae sedis; o_Malasseziales
k_Fungi; p_Basidiomycota; c_Microbotryomycetes; o_Sporidiobolales
k_Fungi; p_Basidiomycota; c_Pucciniomycetes; o_Pachnocybales
k_Fungi; p_Basidiomycota; c_Pucciniomycetes; o_Septobasidiales
k_Fungi; p_Basidiomycota; c_Tremellomycetes; o_Cystofilobasidiales
k_Fungi; p_Basidiomycota; c_Tremellomycetes; o_Filobasidiales
k_Fungi; p_Basidiomycota; c_Tremellomycetes; o_Tremellales
k_Fungi; p_Basidiomycota; c_Tremellomycetes; o_Trichosporonales
k_Fungi; p_Basidiomycota; c_Ustilaginomycetes; o_Ustilaginales
k_Fungi; p_Basidiomycota; c_Wallemiomycetes; o_Geminibasidiales
k_Fungi; p_Chytridiomycota; c_Chytridiomycetes; o_Chytridiales
k_Fungi; p_Chytridiomycota; c_Chytridiomycetes; o_Rhizophydiales
k_Fungi; p_Glomeromycota; c_Glomeromycetes; o_Archaeosporales
k_Fungi; p_Glomeromycota; c_Glomeromycetes; o_Diversisporales
k_Fungi; p_Glomeromycota; c_Glomeromycetes; o_Glomerales
k_Fungi; p_Zygomycota; c_Incertae sedis; o_Mortierellales
k_Fungi; p_Zygomycota; c_Incertae sedis; o_Mucorales
k_Fungi; p_Ascomycota; c_Achaeorhizomycetes; o_unidentified
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Incertae sedis
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_unidentified
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_unidentified
k_Fungi; p_Ascomycota; c_Incertae sedis; o_Incertae sedis
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_unidentified
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Incertae sedis
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_unidentified
k_Fungi; p_Ascomycota; c_Orbiliomycetes; o_unidentified

Table S 3.1 Continued

k_Fungi; p_Ascomycota; c_Pezizomycets; o_unidentified
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Incertae sedis
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_unidentified
k_Fungi; p_Ascomycota; c_unidentified; o_unidentified
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_unidentified
k_Fungi; p_Basidiomycota; c_Exobasidiomycetes; o_Incertae sedis
k_Fungi; p_Basidiomycota; c_Tremellomycetes; o_unidentified
k_Fungi; p_Basidiomycota; c_unidentified; o_unidentified
k_Fungi; p_Chytridiomycota; c_unidentified; o_unidentified
k_Fungi; p_Glomeromycota; c_Glomeromycetes; o_unidentified
k_Fungi; p_Glomeromycota; c_unidentified; o_unidentified
k_Fungi; p_Incertae sedis; c_Incertae sedis; o_Incertae sedis
k_Fungi; p_Rozellomycota; c_unidentified; o_unidentified
k_Fungi; p_unidentified; c_unidentified; o_unidentified

Table S 4.1: Fungal Phyla (p), Classes (c) and Orders (o) Found in Root Samples Analyzed by Pyrosequencing.

Taxonomy
k_Fungi; p_Ascomycota; c_Archaeorhizomycetes; o_Archaeorhizomycetales
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Botryosphaeriales
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Capnodiales
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Dothideales
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Hysteriales
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Jahnulales
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Myriangiales
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Patellariales
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Trypetheliales
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Tubeufiales
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Venturiales
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Chaetothyriales
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Eurotiales
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Mycocaliciales
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Onygenales
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Verrucarialis
k_Fungi; p_Ascomycota; c_Geoglossomycetes; o_Geoglossales
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Agyriales
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Lecanorales
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Ostropales
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Peltigerales
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Pertusariales
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Teloschistales
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Umbilicariales
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Erysiphales
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Helotiales
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Leotiales
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Rhytismatales
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Thelebolales
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Lichinales
k_Fungi; p_Ascomycota; c_Orbiliomycetes; o_Orbiliales
k_Fungi; p_Ascomycota; c_Pezizomycetes; o_Pezizales
k_Fungi; p_Ascomycota; c_Saccharomycetes; o_Saccharomycetales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Boliniales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Chaetosphaeriales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Coniochaetales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Diaporthales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Glomerellales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Lulworthiales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Magnaporthales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Microascales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Ophiostomatales

Table S 4.1 Continued

k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Sordariales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Trichosphaeriales
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Xylariales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Atheliales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Auriculariales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Boletales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Cantharellales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Geastrales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Gomphales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Hymenochaetales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Hysterangiales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Phallales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Polyporales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Russulales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Sebacinales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Thelephorales
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Trechisporales
k_Fungi; p_Basidiomycota; c_Atractiellomycetes; o_Atractiellales
k_Fungi; p_Basidiomycota; c_Cystobasidiomycetes; o_Cystobasidiales
k_Fungi; p_Basidiomycota; c_Cystobasidiomycetes; o_Erythrobasidiales
k_Fungi; p_Basidiomycota; c_Incertae sedis; o_Malasseziales
k_Fungi; p_Basidiomycota; c_Microbotryomycetes; o_Sporidiobolales
k_Fungi; p_Basidiomycota; c_Pucciniomycetes; o_Pachnocybales
k_Fungi; p_Basidiomycota; c_Pucciniomycetes; o_Septobasidiales
k_Fungi; p_Basidiomycota; c_Tremellomycetes; o_Cystofilobasidiales
k_Fungi; p_Basidiomycota; c_Tremellomycetes; o_Filobasidiales
k_Fungi; p_Basidiomycota; c_Tremellomycetes; o_Tremellales
k_Fungi; p_Basidiomycota; c_Tremellomycetes; o_Trichosporonales
k_Fungi; p_Basidiomycota; c_Ustilaginomycetes; o_Ustilaginales
k_Fungi; p_Basidiomycota; c_Wallemiomycetes; o_Geminibasidiales
k_Fungi; p_Chytridiomycota; c_Chytridiomycetes; o_Chytridiales
k_Fungi; p_Chytridiomycota; c_Chytridiomycetes; o_Rhizophydiales
k_Fungi; p_Glomeromycota; c_Glomeromycetes; o_Archaeosporales
k_Fungi; p_Glomeromycota; c_Glomeromycetes; o_Diversisporales
k_Fungi; p_Glomeromycota; c_Glomeromycetes; o_Glomerales
k_Fungi; p_Zygomycota; c_Incertae sedis; o_Mortierellales
k_Fungi; p_Zygomycota; c_Incertae sedis; o_Mucorales
k_Fungi; p_Ascomycota; c_Achaeorhizomycetes; o_unidentified
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Incertae sedis
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_unidentified
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_unidentified
k_Fungi; p_Ascomycota; c_Incertae sedis; o_Incertae sedis
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_unidentified
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Incertae sedis
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_unidentified

Table S 4.1 Continued

k_Fungi; p_Ascomycota; c_Orbiliomycetes; o_unidentified
k_Fungi; p_Ascomycota; c_Pezizomycets; o_unidentified
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Incertae sedis
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_unidentified
k_Fungi; p_Ascomycota; c_unidentified; o_unidentified
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_unidentified
k_Fungi; p_Basidiomycota; c_Exobasidiomycetes; o_Incertae sedis
k_Fungi; p_Basidiomycota; c_Tremellomycetes; o_unidentified
k_Fungi; p_Basidiomycota; c_unidentified; o_unidentified
k_Fungi; p_Chytridiomycota; c_unidentified; o_unidentified
k_Fungi; p_Glomeromycota; c_Glomeromycetes; o_unidentified
k_Fungi; p_Glomeromycota; c_unidentified; o_unidentified
k_Fungi; p_Incertae sedis; c_Incertae sedis; o_Incertae sedis
k_Fungi; p_Rozellomycota; c_unidentified; o_unidentified
k_Fungi; p_unidentified; c_unidentified; o_unidentified

Table S 4.2: Fungal Phyla (p), Classes (c), Orders (o), Families (f) and Genera (g) Found in Root Samples Analyzed by Illumina Sequencing.

Taxonomy
k_Fungi; p_Ascomycota; c_Archaeorhizomycetes; o_Archaeorhizomycetales; f_Archaeorhizomycetaceae; g_Archaeorhizomyces
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Botryosphaeriales; f_Botryosphaeriaceae; g_Lasiodiplodia
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Botryosphaeriales; f_Botryosphaeriaceae; g_Microdiplodia
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Botryosphaeriales; f_Botryosphaeriaceae; g_Sphaeropsis
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Capnodiales; f_Capnodiaceae; g_Capnodium
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Capnodiales; f_Davidiellaceae; g_Cladosporium
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Capnodiales; f_Davidiellaceae; g_Davidiella
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Capnodiales; f_Incertae sedis; g_Capnobotryella
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Capnodiales; f_Incertae sedis; g_Cystocoleus
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Capnodiales; f_Incertae sedis; g_Meristemomyces
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Capnodiales; f_Mycosphaerellaceae; g_Mycosphaerella
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Capnodiales; f_Mycosphaerellaceae; g_Pseudocercospora
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Capnodiales; f_Mycosphaerellaceae; g_Ramichloridium
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Capnodiales; f_Mycosphaerellaceae; g_Ramularia
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Capnodiales; f_Mycosphaerellaceae; g_Uwebraunia
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Capnodiales; f_Teratosphaeriaceae; g_Catenulostroma
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Capnodiales; f_Teratosphaeriaceae; g_Devriesia
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Capnodiales; f_Teratosphaeriaceae; g_Readeriella
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Capnodiales; f_Teratosphaeriaceae; g_Teratosphaeria
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Dothideales; f_Dothioraceae; g_Aureobadidium
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Dothideales; f_Dothioraceae; g_Kabatiella
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Dothideales; f_Dothioraceae; g_Selenophoma
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Incertae sedis; f_Eremomycetaceae; g_Arthrographis
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Incertae sedis; f_Incertae sedis; g_Leptospora
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Incertae sedis; f_Incertae sedis; g_Zymoseptoria
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Jahnulales; f_Aliquandostipitaceae; g_Xylomyces
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Myriangiales; f_Incertae sedis; g_Endosporium
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Corynesporascaceae; g_Corynespora
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Cucurbitariaceae; g_Curreya
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Cucurbitariaceae; g_Pyrencohaetopsis
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Didymosphaeriaceae; g_Roussoella
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Incertae seids; g_Didymella
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Incertae seids; g_Letendraea
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Incertae seids; g_Periconia
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Incertae seids; g_Phoma
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Incertae seids; g_Pyrenochaeta
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Leptosphaeriaceae; g_Leptosphaeria
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Leptosphaeriaceae; g_Lophiostoma
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Massarinaceae; g_Helminthosporium
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Montagnulaceae; g_Alloconiothyrium
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Montagnulaceae; g_Montagnula
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Montagnulaceae; g_Paraconiothyrium
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Montagnulaceae; g_Paraphaeosphaeria
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Phaeosphaeriaceae; g_Ampelomyces

Table S 4.2 Continued

k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Phaeosphaeriaceae; g_Phaeosphaeria
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Pleosporaceae; g_Alternaria
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Pleosporaceae; g_Curvularia
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Pleosporaceae; g_Edenia
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Phaeosphaeriaceae; g_Epicoccum
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Sporomiaceae; g_Preussia
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Sporomiaceae; g_Westerdykella
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Pleosporales; f_Tetraplosphaeriaceae; g_Tetraplosphaeria
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Trypetheliales; f_Trypetheliaceae; g_Polymeridium
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Tubeufiales; f_Tubeufiaceae; g_Tubeufia
k_Fungi; p_Ascomycota; c_Dothideomycetes; o_Venturiales; f_Venturiaceae; g_Fusicladium
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Chaetothyriales; f_Chaetothyriaceae; g_Cyphellophora
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Chaetothyriales; f_Herpotrichiellaceae; g_Capronia
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Chaetothyriales; f_Herpotrichiellaceae; g_Cladophialophora
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Chaetothyriales; f_Herpotrichiellaceae; g_Exophiala
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Chaetothyriales; f_Herpotrichiellaceae; g_Phaeococcomyces
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Chaetothyriales; f_Herpotrichiellaceae; g_Phaeomoniella
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Chaetothyriales; f_Herpotrichiellaceae; g_Phialophora
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Chaetothyriales; f_Herpotrichiellaceae; g_Rhinocladiella
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Chaetothyriales; f_Incertae sedis; g_Coniosporium
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Eurotiales; f_Elaphomycetaceae; g_Elaphomyces
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Eurotiales; f_Trichocomaceae; g_Aspergillus
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Eurotiales; f_Trichocomaceae; g_Byssochlamys
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Eurotiales; f_Trichocomaceae; g_Paecilomyces
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Eurotiales; f_Trichocomaceae; g_Penicillium
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Eurotiales; f_Trichocomaceae; g_Phialosimplex
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Eurotiales; f_Trichocomaceae; g_Sagenomella
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Onygenales; f_Onygenaceae; g_Amauroascus
k_Fungi; p_Ascomycota; c_Eurotiomycetes; o_Verrucariales; f_Verrucariaceae; g_Hydropunctaria
k_Fungi; p_Ascomycota; c_Incertae sedis; o_Incertae sedis; f_Incertae sedis; g_Calcarisporiella
k_Fungi; p_Ascomycota; c_Incertae sedis; o_Incertae sedis; f_Incertae sedis; g_Cordana
k_Fungi; p_Ascomycota; c_Incertae sedis; o_Incertae sedis; f_Incertae sedis; g_Crinitospora
k_Fungi; p_Ascomycota; c_Incertae sedis; o_Incertae sedis; f_Incertae sedis; g_Dictyocatenulata
k_Fungi; p_Ascomycota; c_Incertae sedis; o_Incertae sedis; f_Incertae sedis; g_Dokmaia
k_Fungi; p_Ascomycota; c_Incertae sedis; o_Incertae sedis; f_Incertae sedis; g_Hansfordia
k_Fungi; p_Ascomycota; c_Incertae sedis; o_Incertae sedis; f_Incertae sedis; g_Knufia
k_Fungi; p_Ascomycota; c_Incertae sedis; o_Incertae sedis; f_Incertae sedis; g_Minimidochium
k_Fungi; p_Ascomycota; c_Incertae sedis; o_Incertae sedis; f_Incertae sedis; g_Ochroconis
k_Fungi; p_Ascomycota; c_Incertae sedis; o_Incertae sedis; f_Incertae sedis; g_Phaeoisaria
k_Fungi; p_Ascomycota; c_Incertae sedis; o_Incertae sedis; f_Incertae sedis; g_Pseudorobillarda
k_Fungi; p_Ascomycota; c_Incertae sedis; o_Incertae sedis; f_Incertae sedis; g_Retroconis
k_Fungi; p_Ascomycota; c_Incertae sedis; o_Incertae sedis; f_Incertae sedis; g_Scolecobadidium
k_Fungi; p_Ascomycota; c_Incertae sedis; o_Incertae sedis; f_Incertae sedis; g_Subulispora
k_Fungi; p_Ascomycota; c_Incertae sedis; o_Incertae sedis; f_Incertae sedis; g_Veronaea
k_Fungi; p_Ascomycota; c_Incertae sedis; o_Incertae sedis; f_Incertae sedis; g_Verruconis
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Agyriales; f_Agyriaceae; g_Trapeliopsis

Table S 4.2 Continued

k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Agyriales; f_Trapeliaceae; g_Placopsis
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Agyriales; f_Trapeliaceae; g_Sarea
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Lecanorales; f_Incerta sedis; g_Lecania
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Lecanorales; f_Incerta sedis; g_Leprocaulon
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Lecanorales; f_Incerta sedis; g_Flavoparmelia
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Lecanorales; f_Incerta sedis; g_Hypotrachyna
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Lecanorales; f_Ramalianaceae; g_Badidina
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Lecanorales; f_Sphaerophoraceae; g_Leifidium
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Lecanorales; f_Stereocaulaceae; g_Stereocaulon
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Ostropales; f_Stictidaceae; g_Cryptodiscus
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Ostropales; f_Thelotremataceae; g_Ocellularia
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Peltigerales; f_Collemataceae; g_Leptogium
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Peltigerales; f_Lobariaceae; g_Sticta
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Pertusariales; f_Megasporaceae; g_Aspicilia
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Teloschistales; f_Caliciaceae; g_Calicim
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Teloschistales; f_Teloschistaceae; g_Caloplaca
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Teloschistales; f_Teloschistaceae; g_Sirenophila
k_Fungi; p_Ascomycota; c_Lecanoromycetes; o_Umbilicariales; f_Umbilicariaceae; g_Umbilicaria
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Helotiales; f_Dermateaceae; g_Cryptosporiopsis
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Helotiales; f_Dermateaceae; g_Dermea
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Helotiales; f_Helotiaceae; g_Hymenoscyphus
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Helotiales; f_Helotiaceae; g_Idriella
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Helotiales; f_Hyaloscyphaceae; g_Incrucipulum
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Helotiales; f_Hyaloscyphaceae; g_Lachnum
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Helotiales; f_Hyaloscyphaceae; g_Unguicularia
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Helotiales; f_Incertae sedis; g_Scytalidium
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Helotiales; f_Incertae sedis; g_Tetracladium
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Helotiales; f_Incertae sedis; g_Trichosporiella
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Helotiales; f_Incertae sedis; g_Xylogone
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Helotiales; f_Sclerotiniaceae; g_Botrytis
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Helotiales; f_Sclerotiniaceae; g_Mycopappus
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Helotiales; f_Vibrisseaceae; g_Phialocephala
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Incertae sedis; f_Incertae sedis; g_Collophora
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Incertae sedis; f_Incertae sedis; g_Leohumicola
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Incertae sedis; f_Incertae sedis; g_Meliniomyces
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Leotiales; f_Leotiaceae; g_Leoia
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Rhytismatales; f_Rhytismataceae; g_Davisomycella
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Lichinales; f_Lichinaceae; g_Lichinella
k_Fungi; p_Ascomycota; c_Leotiomycetes; o_Lichinales; f_Peltulaceae; g_Peltula
k_Fungi; p_Ascomycota; c_Pezizomycetes; o_Pezizales; f_Chorioactidaceae; g_Neournula
k_Fungi; p_Ascomycota; c_Pezizomycetes; o_Pezizales; f_Pyronemataceae; g_Genea
k_Fungi; p_Ascomycota; c_Pezizomycetes; o_Pezizales; f_Pyronemataceae; g_Humaria
k_Fungi; p_Ascomycota; c_Pezizomycetes; o_Pezizales; f_Sarcoscyphaceae; g_Pithya
k_Fungi; p_Ascomycota; c_Pezizomycetes; o_Pezizales; f_Tuberaceae; g_Tuber
k_Fungi; p_Ascomycota; c_Saccharomycetes; o_Saccharomycetales; f_Debaryomycetaceae; g_Meyerozyma
k_Fungi; p_Ascomycota; c_Saccharomycetes; o_Saccharomycetales; f_Dipodascaceae; g_Geotrichum

Table S 4.2 Continued

k_Fungi; p_Ascomycota; c_Saccharomycetes; o_Saccharomycetales; f_Incertae sedis; g_Candida
k_Fungi; p_Ascomycota; c_Saccharomycetes; o_Saccharomycetales; f_Incertae sedis; g_Debaryomyces
k_Fungi; p_Ascomycota; c_Saccharomycetes; o_Saccharomycetales; f_Incertae sedis; g_Nadsonia
k_Fungi; p_Ascomycota; c_Saccharomycetes; o_Saccharomycetales; f_Lipomycetaceae; g_Lipomyces
k_Fungi; p_Ascomycota; c_Saccharomycetes; o_Saccharomycetales; f_Pichiaceae; g_Saturnispora
k_Fungi; p_Ascomycota; c_Saccharomycetes; o_Saccharomycetales; f_Trichomonascaceae; g_Blastobotrys
k_Fungi; p_Ascomycota; c_Saccharomycetes; o_Saccharomycetales; f_Trichomonascaceae; g_Spencermartinsiella
k_Fungi; p_Ascomycota; c_Saccharomycetes; o_Saccharomycetales; f_Trichomonascaceae; g_Sugiyamaella
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Boliniales; f_Boliniaceae; g_Camarops
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Chaetosphaeriales; f_Chaetosphaeriaceae; g_Australiasca
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Chaetosphaeriales; f_Chaetosphaeriaceae; g_Chaetosphaeria
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Chaetosphaeriales; f_Chaetosphaeriaceae; g_Chloridium
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Chaetosphaeriales; f_Chaetosphaeriaceae; g_Codinaeopsis
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Chaetosphaeriales; f_Chaetosphaeriaceae; g_Dictyochaeta
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Chaetosphaeriales; f_Chaetosphaeriaceae; g_Kylindria
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Chaetosphaeriales; f_Chaetosphaeriaceae; g_Thozetella
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Coniochaetales; f_Coniochaetaceae; g_Coniochaeta
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Coniochaetales; f_Coniochaetaceae; g_Lecythophora
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Coniochaetales; f_Incertae sedis; g_Wallrothiella
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Diaporthales; f_Cryphonectriaceae; g_Amphilogia
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Diaporthales; f_Cryphonectriaceae; g_Chrysoporthe
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Diaporthales; f_Diaporthaceae; g_Diaporthe
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Diaporthales; f_Diaporthaceae; g_Phomopsis
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Diaporthales; f_Gnomoniaceae; g_Greeneria
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Diaporthales; f_Incertae sedis; g_Harknessia
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Diaporthales; f_Sydowiellaceae; g_Sydowiella
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Diaporthales; f_Togniniaceae; g_Phaeoacremonium
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Glomerellales; f_Annulatascaceae; g_Conlarium
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Glomerellales; f_Apiosporaceae; g_Arthrinium
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Glomerellales; f_Glomerellaceae; g_Glomerella
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Bionectriaceae; g_Bionectria
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Bionectriaceae; g_Clonostachys
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Bionectriaceae; g_Stephanonectria
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Clavicipitaceae; g_Balansia
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Clavicipitaceae; g_Claviceps
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Clavicipitaceae; g_Metacordyceps
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Clavicipitaceae; g_Metacordyceps
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Clavicipitaceae; g_Metarhizium
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Cordycipitaceae; g_Beauveria
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Cordycipitaceae; g_Torrubiella
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Hypocreaceae; g_Gliocladium
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Hypocreaceae; g_Hypocrea
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Hypocreaceae; g_Hypomyces
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Hypocreaceae; g_Sepedonium
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Hypocreaceae; g_Trichoderma
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Incertae sedis; g_Acremonium

Table S 4.2 Continued

k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Incertae sedis; g_Calcarisporium
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Incertae sedis; g_Myrothecium
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Incertae sedis; g_Sarocladium
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Incertae sedis; g_Stachybotrys
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Incertae sedis; g_Stilbella
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Nectriaceae; g_Calonectria
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Nectriaceae; g_Chaetopsina
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Nectriaceae; g_Cosmospora
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Nectriaceae; g_Cylindrocladiella
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Nectriaceae; g_Flagellospora
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Nectriaceae; g_Fusarium
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Nectriaceae; g_Fusidium
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Nectriaceae; g_Gliocephalotrichum
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Nectriaceae; g_Haematonectria
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Nectriaceae; g_Mariannaea
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Nectriaceae; g_Nectria
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Nectriaceae; g_Pseudocosmospora
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Nectriaceae; g_Stylonectria
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Nectriaceae; g_Viridispora
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Nectriaceae; g_Volutella
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Nectriaceae; g_Xenocylindrocladium
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Ophiocordycipitaceae; g_Chaunopycnis
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Ophiocordycipitaceae; g_Ophiocordyceps
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Hypocreales; f_Ophiocordycipitaceae; g_Tolypocladium
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Incertae sedis; f_Incertae sedis; g_Custingophora
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Incertae sedis; f_Incertae sedis; g_Phialemoniopsis
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Incertae sedis; f_Plectosphaerellaceae; g_Gibellulopsis
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Lulworthiales; f_Lulworthiaceae; g_Lulwoana
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Magnaporthales; f_Magnaporthaceae; g_Gaeumannomyces
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Magnaporthales; f_Magnaporthaceae; g_Harpophora
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Magnaporthales; f_Magnaporthaceae; g_Magnaporthe
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Magnaporthales; f_Magnaporthaceae; g_Mycoleptodiscus
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Magnaporthales; f_Magnaporthaceae; g_Pseudophialophora
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Microascales; f_Microascaceae; g_Graphium
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Microascales; f_Microascaceae; g_Parascedosporium
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Microascales; f_Microascaceae; g_Pseudallescheria
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Ophiostomatales; f_Ophiostomataceae; g_Ophiostoma
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Ophiostomatales; f_Ophiostomataceae; g_Raffaelea
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Ophiostomatales; f_Ophiostomataceae; g_Sporothrix
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Sordariales; f_Cephalothecaceae; g_Cryptendoxyla
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Sordariales; f_Chaetomiaceae; g_Humicola
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Sordariales; f_Chaetomiaceae; g_Thielavia
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Sordariales; f_Lasiosphaeriaceae; g_Apodus
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Sordariales; f_Lasiosphaeriaceae; g_Fimetariella
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Sordariales; f_Lasiosphaeriaceae; g_Podospora
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Xylariales; f_Amphisphaeriaceae; g_Neopestalotiopsis

Table S 4.2 Continued

k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Xylariales; f_Amphisphaeriaceae; g_Pestalotiopsis
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Xylariales; f_Amphisphaeriaceae; g_Pseudopestalotiopsis
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Xylariales; f_Diatrypaceae; g_Peroneutypa
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Xylariales; f_Hyponectriaceae; g_Beltraniella
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Xylariales; f_Incertae sedis; g_Dendrophoma
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Xylariales; f_Incertae sedis; g_Microdochium
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Xylariales; f_Incertae sedis; g_Monographella
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Xylariales; f_Xylariaceae; g_Biscogniauxia
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Xylariales; f_Xylariaceae; g_Hypoxylon
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Xylariales; f_Xylariaceae; g_Nemania
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Xylariales; f_Xylariaceae; g_Obolarina
k_Fungi; p_Ascomycota; c_Sordariomycetes; o_Xylariales; f_Xylariaceae; g_Xylaria
k_Fungi; p_Ascomycota; c_Taphrinomycetes; o_Taphrinales; f_Taphrinaceae; g_Taphrina
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Agaricaceae; g_Cystolepiota
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Amanitaceae; g_Amanita
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Bolbitiaceae; g_Galerella
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Clavariaceae; g_Clavulinopsis
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Cortinariaceae; g_Cortinarius
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Entolomataceae; g_Clitopilus
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Entolomataceae; g_Entoloma
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Hygrophoraceae; g_Hygrophorus
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Inocybaceae; g_Crepidotus
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Inocybaceae; g_Inocybe
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Marasmiaceae; g_Clitocybula
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Marasmiaceae; g_Gerronema
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Marasmiaceae; g_Gymnopus
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Marasmiaceae; g_Hydropus
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Marasmiaceae; g_Marasmiellus
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Marasmiaceae; g_Neonothopanus
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Marasmiaceae; g_Rhodocollybia
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Marasmiaceae; g_Tetrapyrgos
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Mycenaceae; g_Mycena
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Mycenaceae; g_Panellus
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Physalacriaceae; g_Laccariopsis
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Porotheleaceae; g_Porotheleum
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Psathyrellaceae; g_Coprinellus
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Psathyrellaceae; g_Coprinopsis
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Psathyrellaceae; g_Psathyrella
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Strophariaceae; g_Gymnopilus
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Strophariaceae; g_Hypholoma
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Strophariaceae; g_Psilocybe
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Tricholomataceae; g_Delicatula
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Tricholomataceae; g_Pseudobaeospora
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Agaricales; f_Tricholomataceae; g_Tricholoma
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Auriculariales; f_Incertae sedis; g_Auricularia
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Auriculariales; f_Incertae sedis; g_Exidia

Table S 4.2 Continued

k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Boletales; f_Boletaceae; g_Boletus
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Boletales; f_Boletaceae; g_Octaviania
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Boletales; f_Boletaceae; g_Xerocomellus
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Boletales; f_Coniophoraceae; g_Coniophora
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Boletales; f_Sclerodermataceae; g_Scleroderma
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Cantharellales; f_Cantharellaceae; g_Craterullus
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Cantharellales; f_Ceratobasidiaceae; g_Ceratobasidium
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Cantharellales; f_Ceratobasidiaceae; g_Thanatephorus
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Cantharellales; f_Clavulinaceae; g_Clavulina
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Cantharellales; f_Hydnaceae; g_Hydnum
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Cantharellales; f_Tulasnellaceae; g_Epulorhiza
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Geastrales; f_Geastraceae; g_Geastrum
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Hymenochaetales; f_Hymenochaetaceae; g_Hymenochaete
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Hymenochaetales; f_Hymenochaetaceae; g_Phellinus
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Hymenochaetales; f_Schizoporaceae; g_Hyphodontia
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Hysterangiales; f_Mesophelliaceae; g_Mesophellia
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Phallales; f_Phallaceae; g_Phallus
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Polyporales; f_Fomitopsidaceae; g_Fomitopsis
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Polyporales; f_Ganodermataceae; g_Amauroderma
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Polyporales; f_Ganodermataceae; g_Ganoderma
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Polyporales; f_Incertae sedis; g_Phlebiella
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Polyporales; f_Meripilaceae; g_Rigidoporus
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Polyporales; f_Meruliaceae; g_Bjerkandera
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Polyporales; f_Meruliaceae; g_Hyphoderma
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Polyporales; f_Meruliaceae; g_Phlebia
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Polyporales; f_Meruliaceae; g_Scopuloides
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Polyporales; f_Meruliaceae; g_Steccherinum
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Polyporales; f_Phanerochaetaceae; g_Phanerochaete
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Polyporales; f_Phanerochaetaceae; g_Rhizochaete
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Polyporales; f_Polyporaceae; g_Coriolopsis
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Polyporales; f_Polyporaceae; g_Dichomitus
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Polyporales; f_Polyporaceae; g_Laccocephalum
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Polyporales; f_Polyporaceae; g_Perenniporia
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Polyporales; f_Polyporaceae; g_Trametes
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Polyporales; f_Xenasmataceae; g_Xenasmatella
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Russulales; f_Peniophoraceae; g_Entomocorticium
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Russulales; f_Russulaceae; g_Lactarius
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Russulales; f_Russulaceae; g_Lactifluus
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Russulales; f_Russulaceae; g_Macowanites
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Russulales; f_Russulaceae; g_Russula
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Sebacinales; f_Sebacinaceae; g_Sebacina
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Thelephorales; f_Thelephoraceae; g_Tomentella
k_Fungi; p_Basidiomycota; c_Agaricomycetes; o_Trechisporales; f_Hydnodontaceae; g_Trechispora
k_Fungi; p_Basidiomycota; c_Cystobasidiomycetes; o_Cystobasidiales; f_Cystobadidiaceae; g_Cystobasidium
k_Fungi; p_Basidiomycota; c_Cystobasidiomycetes; o_Cystobasidiales; f_Cystobadidiaceae; g_Occultifur
k_Fungi; p_Basidiomycota; c_Exobasidiomycetes; o_Exobasidiales; f_Exobasidiaceae; g_Exobasidium

Table S 4.2 Continued

k_Fungi; p_Basidiomycota; c_Exobasidiomycetes; o_Incertae sedis; f_Incertae sedis; g_Meira
k_Fungi; p_Basidiomycota; c_Exobasidiomycetes; o_Microstromatales; f_Microstromataceae; g_Sympodiomycopsis
k_Fungi; p_Basidiomycota; c_Incertae sedis; o_Malasseziales; f_Malasseziaceae; g_Malassezia
k_Fungi; p_Basidiomycota; c_Microbotryomycetes; o_Sporidiobolales; f_Incertae sedis; g_Rhodosporidium
k_Fungi; p_Basidiomycota; c_Microbotryomycetes; o_Sporidiobolales; f_Incertae sedis; g_Rhodotorula
k_Fungi; p_Basidiomycota; c_Microbotryomycetes; o_Sporidiobolales; f_Incertae sedis; g_Sporobolomyces
k_Fungi; p_Basidiomycota; c_Pucciniomycetes; o_Septobasidiales; f_Septobasidiaceae; g_Septobasidium
k_Fungi; p_Basidiomycota; c_Tremellomycetes; o_Cystofilobasidiales; f_Incertae sedis; g_Syzygospora
k_Fungi; p_Basidiomycota; c_Tremellomycetes; o_Filobasidiales; f_Filobasidiaceae; g_Filobasidium
k_Fungi; p_Basidiomycota; c_Tremellomycetes; o_Tremellales; f_Incertae sedis; g_Bullera
k_Fungi; p_Basidiomycota; c_Tremellomycetes; o_Tremellales; f_Incertae sedis; g_Cryptococcus
k_Fungi; p_Basidiomycota; c_Tremellomycetes; o_Tremellales; f_Incertae sedis; g_Fellomyces
k_Fungi; p_Basidiomycota; c_Tremellomycetes; o_Tremellales; f_Incertae sedis; g_Kockovaella
k_Fungi; p_Basidiomycota; c_Tremellomycetes; o_Tremellales; f_Incertae sedis; g_Mingxiaea
k_Fungi; p_Basidiomycota; c_Tremellomycetes; o_Tremellales; f_Incertae sedis; g_Sterigmatosporidium
k_Fungi; p_Basidiomycota; c_Tremellomycetes; o_Tremellales; f_Incertae sedis; g_Tremella
k_Fungi; p_Basidiomycota; c_Tremellomycetes; o_Trichosporonales; f_Trichosporonaceae; g_Trichosporon
k_Fungi; p_Basidiomycota; c_Ustilaginomycetes; o_Ustilaginales; f_Ustilaginaceae; g_Pseudozyma
k_Fungi; p_Basidiomycota; c_Wallemiomycetes; o_Geminibasidiales; f_Geminibasidiaceae; g_Geminibasidium
k_Fungi; p_Chytridiomycota; c_Chytridiomycetes; o_Chytridiales; f_Endochytriaceae; g_Endochytrium
k_Fungi; p_Chytridiomycota; c_Chytridiomycetes; o_Rhizophlyctidales; f_Rhizophlyctidaceae; g_Rhizophlyctis
k_Fungi; p_Chytridiomycota; c_Chytridiomycetes; o_Rhizophydiales; f_Rhizophydiaceae; g_Rhizophydium
k_Fungi; p_Chytridiomycota; c_Incertae sedis; o_Incertae sedis; f_Incertae sedis; g_Homolaphlyctis
k_Fungi; p_Glomeromycota; c_Glomeromycetes; o_Diversisporales; f_Acaulosporaceae; g_Acaulospora
k_Fungi; p_Glomeromycota; c_Glomeromycetes; o_Glomerales; f_Glomeraceae; g_Glomus
k_Fungi; p_Glomeromycota; c_Glomeromycetes; o_Glomerales; f_Glomeraceae; g_Rhizophagus
k_Fungi; p_Incertae sedis; c_Incertae sedis; o_Incertae sedis; f_Incertae sedis; g_Auratiopycnidiella
k_Fungi; p_Zygomycota; c_Incertae sedis; o_Kickxellales; f_Kickxellaceae; g_Ramicandelaber
k_Fungi; p_Zygomycota; c_Incertae sedis; o_Mortierellales; f_Mortierellaceae; g_Mortierella
k_Fungi; p_Zygomycota; c_Incertae sedis; o_Mucorales; f_Backusellaceae; g_Backusella
k_Fungi; p_Zygomycota; c_Incertae sedis; o_Mucorales; f_Cunninghamellaceae; g_Gongronella
k_Fungi; p_Zygomycota; c_Incertae sedis; o_Mucorales; f_Lichtheimiaceae; g_Rhizomucor
k_Fungi; p_Zygomycota; c_Incertae sedis; o_Mucorales; f_Mucoraceae; g_Hyphomucor
k_Fungi; p_Zygomycota; c_Incertae sedis; o_Mucorales; f_Mucoraceae; g_Mucor
k_Fungi; p_Zygomycota; c_Incertae sedis; o_Mucorales; f_Umbelopsidaceae; g_Umbelopsis

Declaration of the Authors Own Contributions

Chapter 2

Conceived and designed the experiments: Andrea Polle

Performed the experiments: Josephine Sahner, Sri Wilarso Budi, Henry Barus, Marike Meyer and Marife D. Corre

Analyzed the data: Josephine Sahner, Sri Wilarso Budi, Henry Barus, Nur Edy, Marike Meyer, Marife D. Corre and Andrea Polle

Contributed reagents/ materials/ analysis tools: Josephine Sahner, Sri Wilarso Budi, Henry Barus and Nur Edy

Wrote the paper: Josephine Sahner, Sri Wilarso Budi, Henry Barus, Nur Edy, Marife D. Corre and Andrea Polle

Chapter 3

Josephine Sahner and Nur Edy conducted the fieldwork. Dominik Schneider conducted the sequence processing.

Chapter 4

Dominik Schneider conducted the sequence processing.

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Eidesstattliche Erklärung

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