Mycorrhizae on dipterocarps in rubber agroforests (RAF) in Sumatra

Made Hesti Lestari Tata

The research presented in this thesis was carried out within the framework of the Plant Ecology and Biodiversity groups and the Prince Bernhard Cair for International Nature Conservation, Institute of Environmental Biology, University of Utrecht, World Agroforestry Centre (ICRAF - South East Asia), *Centraalbureau voor Schimmelcultures* (CBS) - Fungal Biodiversity Centre, and Forest and Nature Conservation Research and Development Centre.

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Mycorrhizae op Dipterocarpaceae in rubber agroforest (RAF) in Sumatra
(met een samenvatting in het Nederlands)

Mikoriza pada dipterokarpa di wanatani karet di Sumatra
(dengan rangkuman dalam bahasa Indonesia)

Proefschrift

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geboren op 25 Mei 1970
te Denpasar, Indonesië

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“Goals are a like a map. They help us determine where we want to end up, and give us personal direction on which to focus our energy”
(Chatarine Pulsifer)

Dedicated to:
Anya, Ibu Sridanti, Bapak Alit Tata,
mbak Trisna, Diah, Ayuk and mas Joko

Contents

CHAPTER 1

General introduction

Introduction

This thesis explores the way rubber agroforests (RAF) in the lowland humid forest zone of Sumatra (Indonesia) can be enriched with dipterocarp timber trees, whether or not the fungi that the tree needs to make ectomycorrhiza (EcM) partnerships in its root system are still present depending on land use history, what the identities of these fungi are and to what degree inoculation in the nursery stage is necessary. This topic of research was selected as it represents a practical problem that is relevant for countering the negative effects of tropical deforestation and can benefit from recent advances in using molecular techniques for identification of fungi. The thesis is based on field surveys, a field experiment and laboratory assays and experiments.

To provide background to this research, this introduction will briefly review literature for a number of steps in the argument:

1. Tropical forests in Indonesia are rapidly changing in nature and forms of planting and management of tropical timber in smallholder agroforestry systems can at least partially fill the demand for sustainably produced wood.
2. Rubber agroforests or 'jungle rubber' have become an important refuge for forest biodiversity in lowland Sumatra. A large part of the tree flora of the natural forest still regenerates within these agroforests and is tolerated or selectively retained by farmers. Although current policies aimed at 'illegal logging' provide negative incentives to farmer management of tropical timbers in their gardens, farmers are potentially interested in enrichment planting with timber.
3. Dipterocarp trees are prominent in the lowland forest zone and have been sought after in logging operations. As is common for trees that occur in middle and late stages of the forest succession cycle they are fully dependent on the formation of EcM with suitable fungal partners.
4. Two classes of fungi (Ascomycetes and Basidiomycetes) can be involved in ECM formation. They depend on spore dispersal and active mycelial networks for their survival and propagation.
5. Previous research, mostly on the island of Borneo, has indicated that presence of the ectomycorrhizal partners required by dipterocarp trees depends on the history of land use, and the loss of such fungi after forest conversion is a clear indication of the loss of functionally important belowground biodiversity.
6. On the basis of this brief review, the more specific objectives of the research are described, followed by an outline of the chapters that describe the various surveys and experiments.

Lowland tropical forests, deforestation and reforestation

> Tropical forests in Indonesia are rapidly changing in nature and forms of planting and management of tropical timber in smallholder agroforestry systems can at least partially fill the demand for sustainably produced wood.

Indonesia, an archipelago of islands with a complex biogeographical history of plate tectonics and consequent association with both mainland Asia and Australian flora and fauna, may well be the hottest hot-spot of current concern over the loss of tropical rain forests (Whitmore, 1984). Indonesia has the world's third largest area of tropical forest. The country still has the largest remaining area of tropical rain forests in the Pacific and South-East Asia. An estimated 50% of the country's total land area still has forest cover (FAO, 2005), but the nature of the forest is rapidly changing, even if cover is maintained. Indonesia has become the global leader in carbon-dioxide emissions from land use change, due to the rapid loss of forest biomass and destruction of peat-lands (Archard *et al.*, 2002; de Fries *et al.*, 2002; Swallow *et al.*, 2007). Tropical rain forests are characterized by their high diversity and species richness. Indonesia's tropical rain forests are among the worlds richest in flora and fauna, with major differences between the forests in the west part of Indonesia, i.e. Sumatra, Borneo, Java and Bali, and all

the islands to the east with close Australian affinity (Whitmore, 1984). West of the so-called Wallace line the forests are characterized by the prominence of trees of the Dipterocarpaceae family (Ashton, 1982), but contain trees of many other families as well (Whitmore, 1984).

Forests have played an important role in economic development in Indonesia, especially after independence. The forest industry sectors have contributed more than 12.7% to the national foreign exchange balance in the period 1998 to 2003 (Fauzi, 2006). Unfortunately, the concept of selective logging and sustainable forest management with sufficiently long periods of recovery proved to be an interesting theory, but not achievable in practice. Large areas of forests were lost in the interaction of logging concessions, increased accessibility of formerly remote areas, spontaneous and state-sponsored migrants, overcapacity in the pulp and paper industry and profitable opportunities for a tree-crop plantation sector (Sunderlin and Resosudarmo, 1996; Anonymous, 2007; Chomitz, 2007).

The United Nations Framework Convention on Climate Change (UNFCCC) defined deforestation as directly human-induced conversion of forested land to non-forested land (Schoene *et al.*, 2007). The internationally accepted definition of forest has two components: one that specifies **canopy cover** and **tree height**, and one that refers to the institutional framework of forestry, as it includes "*areas normally forming part of the forest area which are temporarily un-stocked as a result of human intervention such as harvesting or natural causes but which are expected to revert to forest".* The second part gives priority to the institutional expectation that land will revert to forest cover. This can explain that official statistics of deforestation indicate much smaller areas than remote-sensing data on loss of forest cover.

The overall loss of forest cover in Indonesia was 1.7 million ha year^{-1} (Holmes, 2002). The annual deforestation rate in Central Sumatra as a percentage of 1990 forest cover was estimated as 3.2 to 5.9% (Archard *et al.*, 2002). In the Bungo district of Jambi province alone, forest cover decreased by 25% between 1993 and 2002 (Ekadinata and Vincent, 2005).

To counter the high rate of loss of forest cover, the government of Indonesia (GOI) has initiated tree-planting efforts during the last three decades (Nawir *et al.*, 2007); technically such efforts do not qualify as reforestation, as the land remained institutionally within the forest category. According to the International Tropical Timber Organization (ITTO) guideline, forest rehabilitation is a management strategy applied on degraded forest land that aims at restoring the capacity of a forest to produce products and services (Schoene *et al.*, 2007). In 2005, tree planting activities encompassed 163,282 ha in Indonesia (MOF, 2005); a significant part of such tree planting occurs in the context of monoculture tree plantations with short-rotation species (often not native in the area where they are planted) that can be used for the pulp and paper industry. Although forest cover may be recovered, the biological richness of the natural rain forest is lost with this approach to rehabilitation of the wood-based industry rather than of the natural forests. Part of the forest rehabilitation is, however, based on enrichment planting with native tree species.

In many of the forest areas, the local community has objected to the designation of the land as state forest, and the legal debate is complex (Fay and Michon, 2005; Contreras-Hermosilla and Fay, 2005), as the Forestry Law is in apparent conflict with preceding laws that clarify land ownership. Aware of these challenges, the Indonesian Ministry of Forestry (MOF) and its partners have implemented many projects related to forest rehabilitation in association with local communities, e.g., the Collaborative Forest Management Project (*Proyek Pengelolaan Hutan Bersama Masyarakat – PHMB*, in 2001), the Programme of Specific Allocated Funds – Reforestation Funds (*Dana Alokasi Khusus-Dana Reboisasi – DAK DR*, in 2001), Farm Forestry (*Hutan Rakyat*, in 1970), Community based Forest Management (1994-1999), and the Rehabilitation of Logged-over Area (1996-2003). These efforts, however, were insufficient to significantly counterbalance the ongoing loss of forest cover according to Nawir *et al.* (2007). These authors documented some constraints hindering reforestation initiatives in Indonesia. These constraints were mainly rooted in policies and in the approaches used in projects.

Substantial areas outside the official forest zone in Indonesia have a level of tree cover that meets the internationally agreed forest definition. Such lands are managed by local communities and/or individual farmers. The use of trees, either domesticated from the local flora or introduced from elsewhere for 'non-timber forest products' from such lands, is not problematic. The harvesting of timber, however, is facing increasing challenges due to the increased control of the timber trade to stop illegal logging and the absence of legal certificates of origin for much farm-grown timber. Forms of enrichment planting may be attractive, as far as the future benefit flows will be controlled by the farmer and provide a clear incentive. Revision of the policies regulating market access, however, may be needed to make farm-grown timber a reality (Roshetko *et al.*, 2008; van Noordwijk *et al.*, 2007, 2008a).

Rubber agroforests (RAF)

> Rubber agroforests or 'jungle rubber' have become an important refuge for forest biodiversity in lowland Sumatra. A large part of the tree flora of the natural forest still regenerates within these agroforests and is tolerated or selectively retained by farmers. Although current policies aimed at 'illegal logging' provide negative incentives to farmer management of tropical timbers in their gardens, farmers are potentially interested in enrichment planting with timber.

The introduction to Asia in the second half of the 19th century of *Hevea brasiliensis*, a tree from floodplain forests along the Amazon river and known for its latex as rubber tree, has led to a dramatic change in the lowland tropical rainforests of Sumatra and Borneo, especially along rivers with good accessibility (Joshi *et al.*, 2002; Tengwall, 1945). The subsequent emergence (a hundred years ago) of rubber agroforestry in relatively remote areas did not require any formal research, extension or policies. It was driven by active market agents (providing rubber seed free of charge as investment in their rubber processing plants) and booming prices, linked to the start of the automobile industry and the economic recovery from World War I. The effect of the transformation on forest resources was mixed: the rubber agroforests allowed for sustainable livelihoods on 3-5 ha per household (the area that can be tapped with household labour) and natural forests remained part of the landscape while the population density grew to some 50 persons per km^2, both by attracting migrants and by local youth staying in the area (van Noordwijk *et al.*, 1995; Joshi *et al.*, 2002). All accessible parts of the landscape, however, were transformed to rubber agroforests, and the part of the local flora and fauna that could live in this habitat became the part that survived.

Currently, rubber agroforest (RAF) is an important agro-ecosystem type in Sumatra (Laumonier, 1997). Rubber agroforests can range in intensity from secondary forests with some rubber (e.g. 5-10% of tree basal area) to vegetation dominated by rubber with a complement of native forest trees. So-called 'complex agroforest' systems are characterized by a substantial (but less than 50%) proportion of rubber trees in the total biomass and a large diversity of native forest trees and understory plants (Gouyon *et al.*, 1993). Such complex agroforests are the most forest-like forms of agroforestry (Long and Nair, 1999), and have gained recognition for their value in biodiversity conservation (Beukema *et al.*, 2007; Griffith, 2000; Schroth *et al.*, 2004; Rasnovi, 2006). Initial studies of plant diversity in these rubber agroforests focused on epiphytic and terrestrial pteridophytes, that have effective spore dispersal and may indicate habitat characteristics as such (Beukema and van Noordwijk, 2004; Beukema *et al.*, 2007). Subsequent studies of the tree flora of RAF and forests clarified the role these agroforests can play for tree species, including Red List and threatened species (Rasnovi, 2006).

Although there are anecdotes that rubber seeds were catapulted into existing secondary forests as a low-cost establishment method, most rubber has probably been planted after slash-and-burn land clearing and alongside upland rice and other food crops. Rather than the natural fallow succession via bush ('belukar') to secondary forest, the resulting vegetation became an agroforest, with rubber as the primary economic species, but many local tree species with use value were retained when the plot was partially opened to start tapping the rubber trees (Gouyon *et al.*, 1993; Penot, 2007). As long as land was available, efforts focused on establishment of a larger rubber area, and old rubber gardens or plots with few rubber

trees were left to grow to justify the tapping effort. When land became more limited, old rubber agroforests were again opened by slash-and-burn land clearing and the cycle repeated. An alternative technique for rejuvenation emerged as gap-planting ('sisipan'). The gaps in jungle rubber simulate natural forest regeneration and give young rubber trees a chance to grow (Joshi *et al.*, 2002). Management of the rubber trees in the rubber agroforests varies with household conditions and current rubber price, from very intensive to extensive. Farmers start to tap the latex of the rubber trees approximately 8-10 years after planting and continue to do so for approximately 30 years; however trees planted in the 1920's are still found and can still be tapped (Gouyon *et al.*, 1993; Joshi *et al.*, 2002).

Indonesia, Malaysia and Thailand have dominated the international markets for natural rubber, but production systems in Malaysia and Thailand have historically been more intensive than Indonesia, although Indonesia has the largest area of rubber agroforests (Joshi *et al.*, 2006). Rubber has played a significant role in Indonesia's economic development, and the economy of a province such as Jambi in the 20th century has largely been based on rubber production and processing until logging, oil palm plantations and pulpwood production for pulp and paper mills started to diversify the economy. Rubber latex production increased from 2.1 million tons in 2005 to 2.4 million tons in 2006. Latex earned substantial foreign exchange reserves (equivalent to USD 2.6 million in 2005; Export magazine, 2008).

The productivity of traditional rubber systems is relatively low, about 400 to 600 kg of dry rubber ha^{-1} year^{-1}, whereas the productivity of rubber rose in monoculture is usually 1000 to 1800 kg ha^{-1} year^{-1} (Joshi *et al.*, 2006). The returns to labour, however, are generally comparable between jungle rubber and more specialized plantations. Income from rubber tapping has been estimated to be up to USD 1,429 ha^{-1} year^{-1} by Rosyid *et al.* (2002), but has significantly increased with rising rubber prices on the global market since then. Despite the low latex productivity of rubber grown in RAF, farmers benefit from other resources of the RAF, such as food, fruits, fodder, fuel wood and timber (Gouyon *et al.*, 1993; Michon, 2005). Complex agroforest systems in RAF provide both tangible and intangible benefits. Tangible benefits include latex, fuel woods for local consumption, and other tree products for the market. Intangible benefits include soil conservation, protection of water quality, carbon sequestration and landscape beauty (Joshi *et al.*, 2003; Suyanto *et al.*, 2005).

Rubber wood has become an important commodity in Malaysia and Thailand after the development of techniques for treating the wood at the sawmill to prevent fungal-wood-decay infection. In Indonesia, the plantation sector has been able to benefit from the rubber wood market in its replanting programs, but until recently there were obstacles for smallholders to benefit from such opportunity. Access to the timber market was restricted by regulations and questions about the right to use and sell the products (Ketterings *et al.*, 1999; Tomich and Lewis, 2001; Long and Nair, 1999; van Noordwijk *et al.*, 2003). Recently rubber wood has been deregulated by the Ministry of Forestry (MoF) and the main constraints are now in the organization of access to sawmills with the capacity to treat the wood.

Natural forest trees that are part of the RAFs have traditionally been used locally, especially when the remaining natural forest that is within reach became depleted, or access to this resource restricted by regulations. Sale of timber from RAFs became more difficult when the control on markets for 'illegal logging' products tightened.

In contrast to the decreasing area of natural forests in Jambi, the area occupied by RAF remained constant during the last decade (Ekadinata and Vincent, 2005), although the last years conversion to more intensive rubber systems is prominent. RAF represents a substantial opportunity for smallholders to benefit from the expected future increase in prices for legal and sustainably produced tropical hardwoods. Enrichment planting of rubber agroforests with selected timber species may complement efforts to rehabilitate production forests under control of government-sanctioned concessions. Interviews with farmers confirmed that farmers are increasingly interested in planting timber trees inside their rubber gardens, but they may lack the practical skills involved (van Noordwijk *et al.*, 2004; Tata *et al.*, 2008a). The adoption of some technical guidelines for agroforestry, combined with policy regulation of the agroforestry

timber trade would be necessary to maximize the potential of this ecologically friendly forest management system (Long and Nair, 1999; Tomich and Lewis, 2001; Schroth *et al.*, 2004). Among the tree species suitable for such enrichment planting the dipterocarps take a special place.

Dipterocarpaceae

Dipterocarp trees are prominent in the lowland forest zone and have been sought after in logging operations. As is common for trees that occur in middle and late stages of the forest succession cycle they are fully dependent on the formation of ectomycorrhiza with suitable fungal partners.

The Dipterocarpaceae are a family of flowering tree species with wing-like seeds [from Greek words: *di*= two; *pteron*= wing; *karpos* = fruit]. Dipterocarps dominate the canopy of tropical rain forests in Indonesia West of the Wallace line (Ashton, 1982). Although the family name refers to two wings, the Dipterocarpaceae also include genera with fruits lacking wings or having more than two wings. The wings, when present, are modified sepals. The Dipterocarpaceae of Indonesia includes several genera such as *Anisoptera, Cotylelobium, Dipterocarpus, Shorea, Parashorea, Upuna* and *Vatica*. *Dipterocarpus* fruits have indeed two wings, while other genera, such as *Anisoptera, Shorea, Parashorea,* and *Upuna* have five wings consisting of two large ones and three small. *Vatica* fruits generally lack wings or have highly reduced wings (Ashton, 1982). Although winged, dipterocarp fruits tend not to disperse more than 50 m from the mother tree (Osada *et al.*, 2001). Occasionally fruits can be wind-lifted or float in a stream or river and be transported over larger distances.

The natural distribution of Dipterocarpaceae encompasses a wide range of ecosystems; these species are native to lowlands and few species are present in mountainous areas (up to 1200 m above sea level, asl). Examples of higher elevation species include *Hopea beccariana, Shorea pinanga* and *Vatica micrantha* (Silk, 2006). In drier areas, species such as *Shorea teysmanniana* and *Shorea uliginosa* are found along riverbanks and in peat swamps (Mamose and Shimamura, 2002). In heath forests, *Shorea balangeran, Shorea scabrida, Vatica brunigii* and other species may be found (Nagy and Proctor, 2000; Silk, 2005).

Dipterocarp species tend to fruit in mass fruiting seasons with a periodicity ranging from 1 to 10 years depending on species (van Schaik *et al.*, 1993). For instance, *Hopea odorata* and *Anisoptera marginata* in the Arboretum of the Forest Research and Development Agency (FORDA) in Bogor, Indonesia, bloom annually (Subiakto, 2005, personal communication). However, *Dipterocarpus costulatus* and *D. kerrii* fruit only every 4-5 years (Krishnapillay and Tompsett, 1998). The ecological interpretation of this masting is usually avoidance of seed predation, although the physiological triggers that allow synchronized response across the landscape are still unclear; climatic signals may relate to the absence of clouds and abundance of sunlight in years with long dry seasons (Van Schaik *et al.*, 1993).

Dipterocarp seeds are ephemeral, germinating easily when conditions are warm and moist, and therefore in Indonesian conditions, they cannot be stored long (Tompsett, 1998). When planted, dipterocarp seedlings tend to be shade-tolerant, allowing seedlings to grow under a closed forest canopy for long periods, sometimes more than 10 years (Ashton, 1998). It has been reported that an increase in the amount of “photosynthetically active radiation” (PAR) accelerates the early growth of dipterocarp seedlings (Ashton and de Zoysa, 1989; Tennakoon *et al.*, 2005).

As is common in trees that occur in mid and late stages of forest succession, dipterocarps have a strong association with fungi by jointly forming ectomycorrhiza.

Ectomycorrhizae (EcM)

Two phyla of fungi, i.e. Ascomycota and Basidiomycota, can be involved in EcM formation; they depend on spore dispersal and active mycelial networks for their survival and propagation.

Mycorrhiza (from the Greek words: μύκης *[mykes or mukos]* = fungus and ριζα *[rhiza]* = root) is the term given to the symbiosis formed between fungi and the roots of plants. The term was first

used by Frank (1885 *cited in* Trappe, 2004), who discovered the structure and hypothesized that it represented a mutual symbiotic relationship in which the fungus obtains carbohydrates (sugars) from the plant, and, in return, supplies the plant with minerals. Since then, as noted in reviews by Harley (1972) and Smith and Read (1997), several different forms of mycorrhizal associations between fungi and plants have been discovered. Early descriptions of mycorrhiza include the work of Janse (1897) in the Botanical Garden in Bogor, who provided detailed descriptions of what were later recognized to be arbuscular mycorrhiza (AM) on a large number of plant species. The most common forms are VAM, ectomycorrhiza, ericoid mycorrhiza, monotropoid mycorrhiza, and orchid mycorrhiza. Ectomycorrhiza is formed between woody plants (most conifers and many broad-leaved trees, many woody shrubs, and some herbaceous plants) and fungi that are mainly in the phyla Basidomycota and Ascomycota. Many reports have shown that members of the Dipterocarpaceae have ectomycorrhizal associations with fungi (Smits, 1994; Lee *et al.*, 1997; Wang and Qiu, 2006; Amaronpitak *et al.*, 2006; Tedersoo *et al.*, 2007).

Anatomically, EcM are characterized by three structures, viz. the mantle, the Hartig net and the external mycelium. Short-roots of ectomycorrhizal plants are always covered by a *mantle* (or "sheath") of hyphae, as a boundary between the roots and soil (Agerer, 1987-97). The mantle can serve as a locus of nutrient and carbon storage for the fungus (Harley, 1972). The *Hartig net*, first described by T. Hartig in 1840 (Trappe, 2004), is a network of hyphae growing between cortical cells of the root. This provides the main interface between the fungus and the host plant, where carbohydrates can be exchanged for minerals. In contrast to the situation in vesicular-arbuscular, ericoid and orchid mycorrhizal associations, the fungus does not penetrate the host cell walls. Lastly the *external mycelium* consists of hyphae extending from the fungal mantle out into the soil (Linderman, 1988).

Initiation of mycorrhizal symbioses on tree roots can be achieved in different ways (Kuyper *et al*., 2004), such as: (i) tree seedlings connecting to existing hyphal networks (a mechanism studied via field bioassays); (ii) tree seedlings stimulating germination of spores and establishing contact (studied in greenhouse bioassays); and (iii) tree seedlings picking up mycorrhizal partners in the nursery. These different pathways for mycorrhization can be applied in silvicultural management practices in the field. We need to know, however, which methods are feasible to work with under tropical conditions.

Identification of EcM fungi can be achieved based on study of sporocarp morphology (Brundrett *et al.*, 1996), morphological characterization of the EcM themselves (Agerer, 1987-1998; Ingleby *et al.*, 1990) and molecular analysis. The advanced technique of molecular analysis allows identification of mycobionts from mycelia and directly from EcM root tips (Gardes *et al.*, 1991; Gardes and Bruns, 1993; Bruns *et al.*, 1998; Martin, 2001).

Land use effects on availability of mycorrhiza inoculum

> Previous research, mostly on the island of Borneo, has indicated that presence of the ectomycorrhizal partners required by Dipterocarp trees depends on the history of land use, and the loss of such fungi after forest conversion is a clear indication of the loss of functionally important belowground biodiversity.

Efforts to rehabilitate dipterocarps after intensive logging in Kalimantan encountered difficulties in tree establishment. Lack of EcM partners in the soil and high sensitivity of fungi to disturbance of the soil were seen to be the main reason. Practical techniques were developed to ensure inoculation in the nursery stage (Santoso *et al.*, 2006; Badan Standarisasi Nasional, 2006; Tata *et al.*, 2008b). Parallel studies in Sumatra also explored feasible inoculation techniques. Critical studies, with appropriate controls, on the need for inoculation, however, were not carried out (Anonymous, 2004).

Earlier studies with endomycorrhiza or vascular arbuscular mycorrhiza (VAM), as it used to be named, had shown a low dependence on land use history. Severely degraded sites in South East Asia tend to be covered by *Imperata cylindrica*, a very resilient rhizomatous grass, that is subject to and tolerant of annual fire. The grass, however, has effective association with

VAM, and spore density and diversity tends to be higher in such grasslands than in forests or other vegetation. Murniati (2002) concluded from experiments in East Kalimantan that VAM inoculum potential is sufficiently high, therefore tree planting campaigns do not need to provide nursery-level inoculum for endomycorrhizal trees. A small positive effect on initial survival, however, was confirmed for nursery stage inoculation. Onguene (2000) found for Cameroon increased endomycorrhiza spore density and mycorrhizal colonization after forest converted to shifting cultivation, however forestry practices, such as logging, decreased spore number and mycorrhization. He suggested that mycorrhiza (ecto- and endomycorrhiza) inoculation in the nursery is required to improve forest sustainability in logged over areas.

While the belowground components of global biodiversity are only gradually recognized (Giller *et al.*, 2005, 2006; Swift *et al.*, 2004), the case of EcM fungi appeared to be a clear case with high functional significance. Functions of soil biota, such as decomposition and creation of soil porosity, may not require a high species diversity, due to the fact that the function may saturate as soon as a few species are present. The biological specificity of EcM formation, however, suggests that a high fungal diversity may need to be maintained to support a high tree diversity. If enrichment planting with multiple species is to be successful, the existing inoculum EcM potential of a soil is part of its 'natural capital'. Few quantitative studies of functionally important belowground biodiversity in the tropics have been completed, and a study of EcM diversity was welcomed as part of a global project on the functional significance and sustainable management of belowground biodiversity (Giller *et al.*, 2005; 2006).

Objectives of the study

The work described in this thesis has focused on the diversity of vegetation in native forests and RAF and the relation of this plant diversity with the diversity of EcM in the soil. We have also considered how EcM are affected by changes in land use and by enrichment planting practices.

The research was based on several interrelated objectives:

(1) Characterizing, identifying and comparing the diversity of vegetation in seven different land use types, with particular attention to
    a) the status of plants with known dependency on EcM,
    b) the successional status of the tree species and
    c) the applicability of the 'shadow species' concept.
(2) Determining the impact of land use change on the inoculum potential of EcM fungi
(3) Determining, for test species of ectomycorrhizal trees, the availability of fungal partners in soil as affected by prior land use
(4) Identification of constraints and other factors affecting growth of *Shorea* species tested in the context of rubber agroforests.

Outline of thesis

Since farmers' interest in planting timber trees in their rubber gardens is gradually increasing, this thesis focuses on the practical technical issue of whether sufficient EcM inoculum compatible with dipterocarp partners is present in non-forest soil converted to RAF, or whether special nursery inoculation is necessary or advantageous for dipterocarp outplanting. Other factors related to the success of dipterocarp afforestation are not dealt with in this thesis, including policy and regulation, market trends, farmer access to planting materials, options for using oil palms or faster growing forest trees. A diagram of the thesis outline is shown in Fig. 1.

The research presented is a synthesis of several years of work involving enrichment planting of RAF with dipterocarp species and the relation of these species with their mycobionts, i.e. EcM fungi. This work aims to provide an ecologically based understanding of the persistence of EcM inoculum in the soil after changes in land use type. In addition, this work aims to establish the identity of the available EcM inoculum based on DNA analysis.

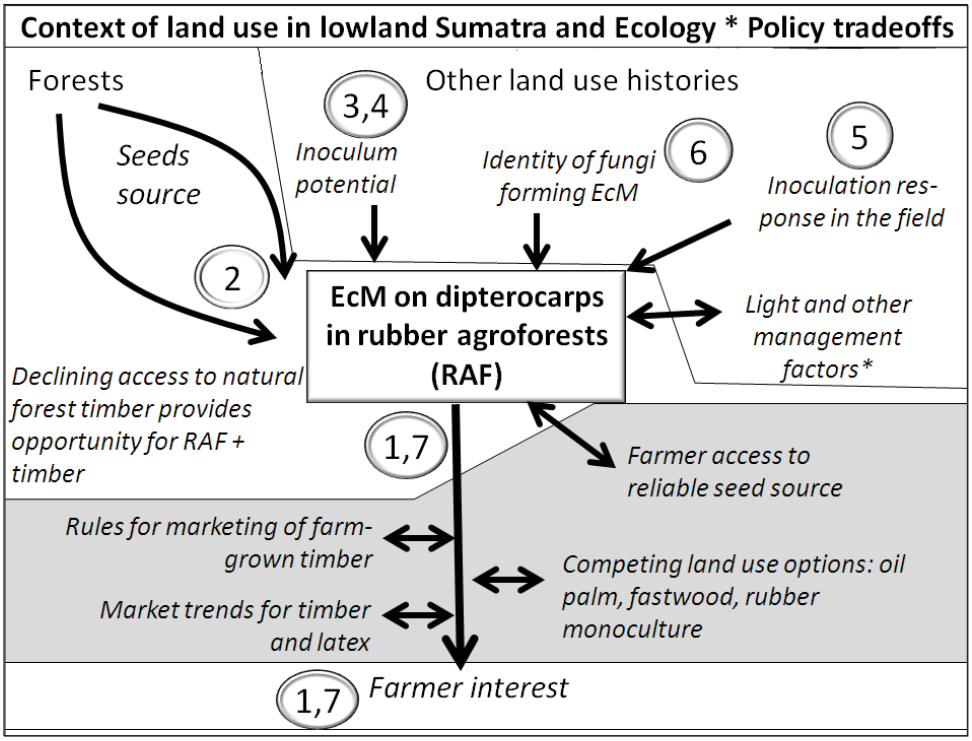

Fig. 1 Outline of the thesis: context of land use in lowland Sumatra in relation with conomic and policy tradeoffs. Number(s) in a circle shows number of related chapter. Four factors in the grey area and factor with asterisk are not discussed in this thesis.

Chapter 1 provides an introduction about tropical forests, deforestation in Indonesia, Indonesian initiatives towards forest rehabilitation, RAF, Dipterocarpaceae and EcM.

Chapter 2 describes vegetation diversity in seven land use types, and compares between forests and RAF. Related aspects of obligate EcM symbiosis, successional status of tree species and 'shadow species' in forests and RAF are discussed.

Chapter 3 focuses on land use effects on EcM inoculum available to dipterocarp trees planted on acid forest-derived soils. Two species of *Shorea* were used as bait in the nursery study. This chapter probes the effects of different soils on growth and EcM formation in *S. lamellata* and *S. selanica*.

Chapter 4 studies the effect of soil heating and drying in nursery trials on EcM formation and plant growth in five dipterocarp species. Soils were preheated and dried prior to being planted with five dipterocarp cuttings. This procedure was to answer a question raised in chapter 3.

Chapter 5 deals with enrichment planting of dipterocarp species in RAF. This chapter addressed the question: "are the dipterocarp species used less dependent on existing inoculum potential in the soil for their EcM formation than might be assumed?"

Chapter 6 analyses the diversity of EcM community obtained in chapters 3, 4 and 5, studied by directly amplifying and sequencing the fungal ITS rDNA locus from EcM root tips and by culture isolation from similar EcM root tips.

Chapter 7 synthesizes and summarizes the results and makes recommendations on the application of this knowledge in silvicultural practices aimed at sustainable management of forest resources in Indonesia.

CHAPTER 2

Trees and regeneration strategies in rubber agroforests and other forest-derived vegetation in Jambi (Sumatra, Indonesia)

with M. van Noordwijk and M.J.A. Werger

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Abstract

The rubber agroforests (RAF) of Indonesia provide a dynamic interface between natural processes of forest regeneration and human management targeting the harvesting of latex with minimum investment of time and financial resources. The composition and species richness of higher plants across an intensification gradient from forest to monocultures of tree crops have been investigated in six land use types (viz. secondary forest, RAF, rubber monoculture, oil palm plantation, cassava field and *Imperata* grassland) in Bungo, Jambi Province, Indonesia. We emphasize comparison of four different strata (understory, seedling, sapling and tree) of vegetation between forest and RAF, with specific interest in plant dependence on ectomycorrhiza fungi. Species richness and species accumulation curves for seedling and sapling stages were similar between forest and RAF, but in the tree stratum (trees > 10 cm dbh) selective thinning by farmers was evident in a reduction of species diversity and an increase in the proportion of trees with edible parts. Very few trees dependent on ectomycorrhiza fungi were encountered in the RAF. However, the relative distribution of early and late successional species as evident from the wood density distribution showed no difference between RAF and forest.

Keywords: *diversity indices, species richness, structure, tropical secondary forest*

Introduction

Sumatra is the worlds' fifth largest island and part of the biogeographical 'Sundaland' domain that is widely recognized for its high biodiversity (Whitten *et al.*, 2000). It harbours a wide variety of natural and derived vegetation types (Laumonier, 1997), from forestland shrub land, wet lands, agriculture and grassland. Although Sumatra is not as rich in Dipterocarpaceae as the island of Borneo (Ashton, 1998), this tree family is still considered to be characteristic of the lowland forests and contributes 3.1% of total tree species diversity in Sumatra as against 6.3% in Borneo (Roos *et al.*, 2004). Dipterocarp trees are mostly shade tolerant and characteristic of middle to late successional stages. Most big trees in late successional stages of the lowland forests, such as Dipterocarpaceae and Fagaceae, have a close association with ectomycorrhiza (EcM) fungi (Smits, 1994). In contrast, associations with endomycorrhizal (also indicated as arbuscular mycorrhiza) fungi mainly dominate in early successional trees and agricultural plants (Janos, 1980; Wang and Qiu, 2006). Early successional stages tend to have low wood density, while late successional trees usually have high wood densities (Swaine and Whitmore, 1988). So, distribution of the wood density of the trees in a mixed-vegetation can be used as an indicator of successional status.

During the nineteen-nineties forest cover in Sumatra declined dramatically. The rate of deforestation or forest conversion in Sumatra was estimated to be about 61% within 12 years (FWI/GFW, 2001). In Bungo district in Jambi province alone, the conversion rate of forest areas was about 25% within 10 years, from 1993 to 2002 (Ekadinata and Vincent, 2004). Loss of forest biodiversity depends largely on the type of land cover to which the natural forest was converted (Gillison and Liswanti 2004, Tomich at al., 2002). Some forest-derived land cover types still maintain substantial sub-sets of the original forest vegetation and approach the structure of secondary forests (Murdiyarso *et al.*, 2002). From some derived land cover types the forest vegetation can still recover. From other, the loss of biodiversity is likely to be permanent on a relevant time scale of decades. As the late succession dipterocarp trees depend on EcM, their recovery potential likely depends on the belowground as well as aboveground impacts of forest conversion on species persistence. RAF is the main forest-derived land cover type of interest in this regard.

The introduction of *Hevea brasiliensis* ('para rubber') in Sumatra in the first decade of the 20th century caused a revolutionary change in the land use pattern, when the new cash crop was found to be compatible with local forest conditions. The upland rice – crop fallow systems that had been the mainstay of the local economy were replaced with RAFs, of various management intensities (Gouyon *et al.*, 1993, van Noordwijk *et al.*, 1998). Complex RAF is characterized by a substantial share of rubber trees in the total tree biomass, but also by a large diversity in species of native forest trees and understory plants (Laumonier, 1997; Beukema *et al.*, 2007). These RAF systems may well represent the best example of 'domesticated forests' (Michon, 2005; Schroth *et al.*, 2004) that maintain basic forest ecological processes of regeneration in a highly productive context, and that allows weekly income to be derived by tapping off rubber (Tomich *et al.*, 2002).

Earlier studies have clarified vegetation structure and species composition of RAF (Gouyon *et al.*, 1993; Gillison and Liswanti, 2003; Michon, 2005) and analysed the pteridophyte flora (Beukema and Van Noordwijk, 2004) of RAF in Jambi. Local ecological knowledge and farmer management styles for regeneration in cyclical or semi-permanent RAF were analysed by Joshi *et al.* (2003, 2005), Ketterings *et al.* (1999) and Wibawa *et al.* (2005). However, none of the existing data sets has compared species richness in the different stages of tree regeneration (seedlings, saplings and trees) in relation to farmer management decisions.

Our analysis of tree and understory data collected in the Jambi project on the options for sustainable management of belowground biodiversity quantified the effect of land use on the composition and species richness of higher plants, with particular attention to plants with known dependency on EcM, successional status of the tree species and applicability of

the 'shadow species' concept (Rennols and Laumonier, 2006). Comparison of the seedling, sapling and tree strata focused on evidence of successful regeneration of forest diversity in agriculturally managed landscape units.

Method

Study area

The study was conducted in Bungo district of Jambi province, which lies between 101°27' and 102°30'E, and between 1°08' and 1°55' S. The Bungo River after which the district is named starts in the piedmont (foothills, 150 – 500 m a.s.l.) where the mountain range of the Bukit Barisan rises above the lowlands (Fig. 1). The Bungo river joins the Batang Hari in the flat or mildly undulating lowland peneplain that forms most of Jambi province, with elevations ranging from 50 to 150 m (a.s.l.). Soils of the lowland peneplain are very acid, have low fertility status, leached soils (Ultisols) deposited under marine conditions in the past, with higher clay contents close to the river (Van Noordwijk *et al.*, 1998). The piedmont hills were built mainly by granite and andesitic lava. The soils range from shallow to very deep, very acid, moderate to fine texture, well to moderately-excessive drained and generally higher fertility. Soil types are Entisols, and Inceptisol (van Noordwijk *et al.*, 1998).

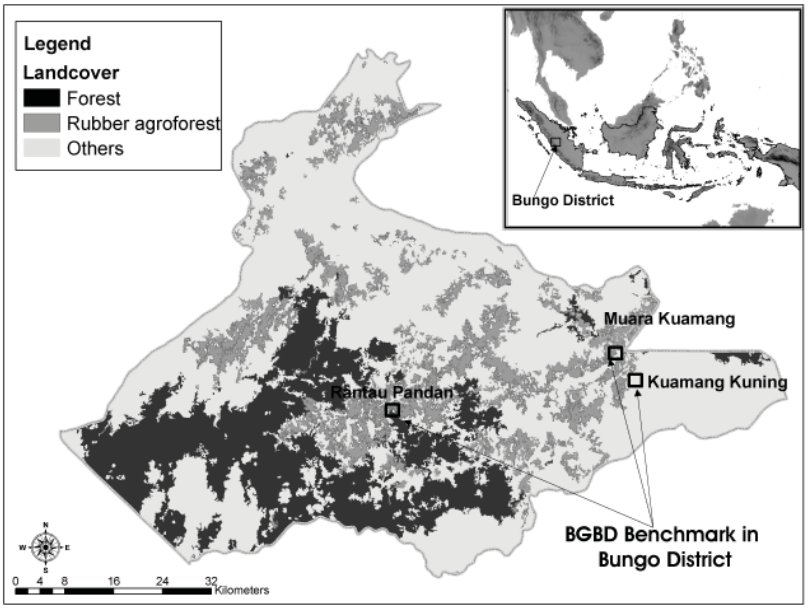

Fig. 1 Map of study area in Bungo district, Jambi province, Indonesia.

Bungo district has vegetation ranging from forest, agroforest, swamp forest along the river, tree crop plantations and agriculture (upland rice, maize, cassava and paddy rice). Some surveys were initiated in April 2005 for the Sustainable Management of Belowground Biodiversity (CSM-BGBG) project (Giller *et al.*, 2005), with a 'sampling window' in the foothills in Rantaupandan and two in the lowland peneplain in Muara Kuamang and Kuamang Kuning. Selection criteria for these approximately 25 km^2 windows were the opportunity to capture diversity through the presence of a range of land use types. Sampling within the windows was done in an equidistant grid of points, with additional points to obtain a minimum number of replicates of all major land use strata. To implement this scheme, land cover in Bungo district

was interpreted from satellite images of Landsat ETM taken in 2002. The benchmark area was divided during the field inventory into six classes describing land use type (LUT) as follow:

- Secondary forest: community managed forest used for extraction of timber for local use and non-timber forest products, forests recovering from selective logging and mature untapped RAF, usually with low density of rubber trees
- Rubber agroforest (RAF); complex rubber agroforest that is currently being tapped
- Rubber monoculture (RM); rubber monoculture with intensive management
- Oil palm plantation (OP)
- Cassava field (CS); when floristic inventories were conducted, all cassava had been harvested
- *Imperata cylindrica* grassland (IG)

Relatively scarce land cover classes (river, road, and village) were excluded from the sample design. The number of sample points per land cover class varied between 5 and 12.

The 'sampling window' in Rantau Pandan and Muara Kuamang contained secondary forest (FO) and RAF, each represented by 8 sample plots in each window. Rubber monoculture (RM), was sampled in 6 plots each in Rantau Pandan and Kuamang Kuning. The other land uses, viz. oil palm (OP), crop cassava (CS) and *Imperata* grass land (IG), were only represented in Kuamang Kuning, with 5, 9, and 12 sample plots, respectively.

Plot size

In total seventy circular plots of 200 m^2 (8 m in radius) were laid out. Each plot was divided into a subplot of 50 m^2 (4 m in radius) and a subplot of 25 m^2 (2.8 m in radius), nested within the larger plots. All strata of vegetation were recorded. The diameter at breast height (dbh; 1.3 m) of trees ≥ 10 cm within circular plots of 200 m^2 was measured. Saplings and woody climbers, with dbh less than 10 cm and height of more than 2 m, were recorded from the 50 m^2 subplots. Similar data were collected for seedlings (consisting of shrubs and woody plants less than 2 m high) and understory (consisting of lianas, herbs, terrestrial ferns and grasses) were recorded within the 25 m^2 subplots.

Herbarium specimens were collected from each individual tree, except very well known species, and deposited at the Herbarium of the World Agroforestry Centre (ICRAF-SEA). Herbarium specimens were identified at the Herbarium Bogoriense, Bogor, Indonesia. Among all trees sampled in the 6 land use types in Bungo district (544 herbarium specimens), 88.2% was identified to species level, 5.0% was identified with a *cf*. note, 6.4% was identified at genus level, and the rest (0.4%) remained unidentified.

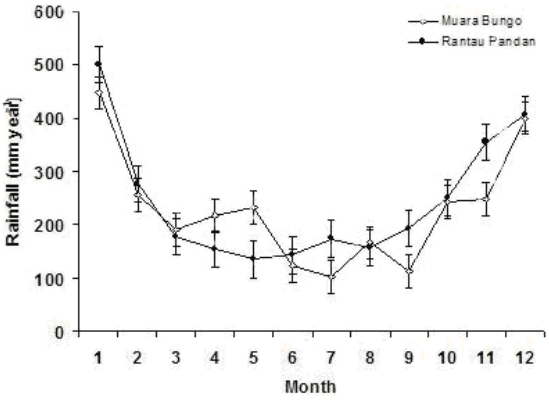

Fig. 2 Rainfall data for climate station in Muara Bungo and Rantau Pandan from 1998 to 2002 (data: ICRAF).

Climate

Generally the climate in Bungo district belongs to A type (Schmidt and Ferguson, 1951). Rainfall data were collected from the nearest climate station in the sub-districts of Muara Bungo and Rantau Pandan for the period 1998 to 2002 (Fig. 2). The mean annual rainfall and number of rainy days in Muara Bungo were 2,602 mm per annum and 126 days per annum, while in Rantau Pandan these were 2,888 mm per annum and 130 days per annum, respectively.

Data analysis: diversity indices and shadow species

Comparison of index diversities (Shanon Wiener and Simpson Index) was made between two land use types, e.g. forest and other land use types, using a *t*-test. Species richness, number of individual flora, number of family, density and basal area were compared between forest and other land use type using analysis of variance (*F*-test), and continued with Dunnet test when it was significant using Statistica 6.0 (StatSoft Inc., USA).

The relationship between species richness and sample size was compared between both land use types in curves of species accumulation, generated from randomly resampling the sample plot data in six reiterations, using R 2.1.1 software developed by Kindt and Coe (2005).

The data were analyzed using ecological standard methods. Abundance of ground cover species was calculated as percentage of a species relative to all species. For each LUT, species richness (the total number of species per land use type) and species diversity, was calculated as the Shanon-Wiener index (Ludwig and Reynolds, 1988):

$$H' = -\sum_{i=1}^{s} pi \ln pi \qquad \text{Eq.1}$$

where *pi* is the proportion of individuals found in the *i*-th species in each concentric plot or in the whole plot. This index considers the number of species (species richness) and the evenness of their abundance.

Floristic diversity of each LUT was also calculated as the Simpson's diversity index (Ludwig and Reynold, 1998):

$$D_s = 1 - \Sigma \left[n_i * (n_i-1) / N*(N-1) \right] \qquad \text{Eq.2}$$

where n_i is the number of individuals in the i^{th} species collected, and N is the total number of individual organisms in the plot sample. Comparison of diversity was made between LUT of forest and RAF using a *t* - test with each plot considered as an independent replicate.

The concept of 'shadow species', as recently introduced by Rennolls and Laumonier (2006) on the basis of a natural forest data set from Jambi was applied separately to the data for seedlings, saplings and trees of the forest and RAF plots. The number of 'shadow species' for species observed once, twice and multiple times was calculated using the relative frequency of observation and a procedure introduced by Rennolls and Laumonier (2006). Shadow species are species whose existence in the land use types can be inferred from the data, but that have not been actually observed. A single observance of the species is called a singleton.

Based on literature, we classified all species of woody plants according to their EM dependency, human use of their edible parts and wood density. Pioneers typically tend to have low wood densities, linked to rapid growth rates and medium-sized trees, while late successional species have high wood densities, grow slowly and reach to greater heights. To classify plants according to their wood density we used a database developed by World Agroforestry Centre (ICRAF-SEA) and available at www.icraf.org/SEA to obtain a midpoint estimate of the wood densities of tree growing in the forest and RAF, and calculated the cumulative frequency of the species according to wood density.

Plants are considered edible if they produce fruits, vegetables, nuts, gums or spices that are used by man. This information is given by Whitmore (1983), Whitmore and Tantra (1986)

and Keβler and Sidiyasa (1995), and was cross checked in the context of local ecological knowledge in Bungo district.

To classify species on their EcM dependency, we used the information from Smits (1994). Independence in two-way classification of data (e.g. LUT and properties of the trees) was tested using a $\chi 2$ – test, pooling the data for forest and RAF for the three sampling windows.

Results

Floristic characteristics of six land use types in Jambi

The stratum of seedlings and saplings shows considerable regeneration in forest and RAF. Species richness of saplings and trees in forest was higher than in RAF, but seedlings' species richness was higher in RAF than in forest. In the further discussion we will focus on a comparison of forest and RAF.

Average plot-level richness and species accumulation curves (Fig. 3) for forest and RAF overlapped for seedlings and saplings, but trees and understory differed significantly between the two LUT's when 10 or more plots were considered.

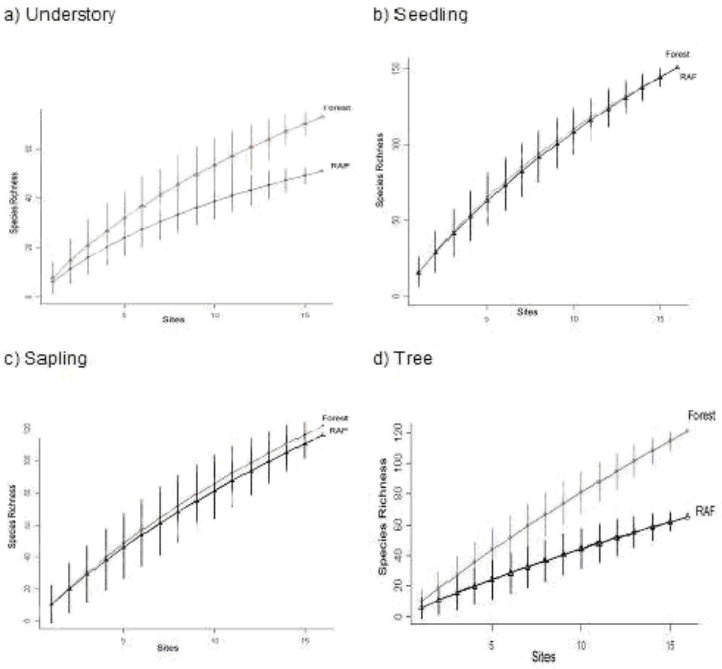

Fig. 3 Relationship between number of observed plant species and number of plots included in the analysis for four strata in forest and rubber agroforest (RAF); vertical lines show standard deviation of results obtained by re-sampling the data.

Diversity indices

The diversity indices of Shanon Wiener and Simpson showed that diversity of each stratum in forest is consistently higher than in RAF (Table 1).

Diversity of understory in the forest was significantly higher than in RAF (*t* test = 10.5; probability 0.01). Seedling diversity in the forest was also higher than in RAF (*t* test = 3.0; probability 0.01), as was the diversity of saplings in the forest and RAF (*t* test result = 2.5;

Table 1 Structural characteristics and floristic diversity of land use type of forest, rubber agroforest (RAF), rubber monoculture (RM), oil palm plantation (OP), Cassava field (CS) and *Imperata* grassland and (IG) in Bungo District (Jambi, Indonesia). The area of each land use type is 0.32 ha, 0.32 ha, 0.20 ha, 0.10 ha, 0.18 ha, and 0.24 ha, respectively. Number of plant families given the total number of families found in all plots of a LUT.

Stratum	Parameter	Land use types					
		SF	RAF	RM	OP	CS	IG
Tree	# Individu	12.4 (1.2)	12.7 (4.5) ns	12.3 (1.6) ns	0	1.0 *	0
	# species	9.6 (1)	6.0 (0.9) *	1.5 (0.2) **	0	1.0 *	0
	# family	8.0 (0.8)	5.3 (0.9) **	1.4 (0.2) ***	0	1.0 *	0
	Density (n ha^{-1})	621.9 (58.1)	634.4 (56.6) ns	61.6 (78.4) ns	0	50.0 *	0
	Total BA (m^2 ha^{-1})	5.6 (0.7)	4.9 (0.6) ns	2.5 (1.4) **	0	0.2 *	0
	BA rubber (m^2 ha^{-1})	0.9 (0.3)	2.6 (1.5) **	2.3 (0.4) *	0	0	0
	Shanon Index	4.5 (6.5E-05)	2.6 (1.8E-03)**	0.2 (8.6E-12) ***	-	0	-
	Samson's Index	0.98 (2.8E-05)	0.72 (1.3E-03)**	0.07 (9.6E-04) ***	-	0	-
Sapling	# Individu	18.2 (2.8)	18.0 (3.2) ns	5.3 (1.1) *	0	0	0
	# species	11.2 (1.4)	10.6 (1.6) ns	2.0 (0.8) **	0	0	0
	# family	8.8 (0.97)	8.0 (1) ns	1.8 (0.7) **	0	0	0
	Density (n ha^{-1})	3650.0 (561.7)	3600.0 (642.4) ns	1066.7 (229.0) *	0	0	0
	Shanon Index	4.3 (7.2E-04)	4.2 (9.6E-04) *	1.1 (2.1E-03) ***	-	-	-
	Samson's Index	0.99 (3.8E-06)	0.98 (1.3E-05) *	0.46 (0.01) ***	-	-	-
Seedling	# Individu	45.6 (4.2)	60.9 (7.4) ns	52.2 (4.6) ns	29.8 (15.6) ns	12.2 (2.1) ***	4.8 (2.0) ***
	# species	15.4 (1.3)	15.7 (5.5) ns	8.5 (1.0) ***	2.6 (0.5) ***	2.3 (0.5) ***	1.1 (0.3) ***
	# family	11.3 (1.1)	11.9 (0.9) ***	6.8 (0.9) ***	10.6 (0.9) ***	2.0 (0.4) ***	1.1 (0.3) ***
	Shanon Index	4.3 (8.2E-04)	4.0 (9.0E-04) **	2.6 (4.4E-03) ***	1.3 (0.02) ***	1.6 (0.02) ***	0.95 (0.004) ***
	Samson's Index	0.98 (3.6E-06)	0.97 (5.3E-06) **	0.83 (1.5E-04) ***	0.7 (5.6E-04) ***	0.8 (2.3E-04) ***	0.4 (0.006) ***
Under-	# Individu	38.4 (14.6)	84.1 (30.7) *	176.3 (37.7) *	174.2 (34.0) ns	12.2 (2.1) ns	281.6 (59.9) **
story	# species	6.1 (0.7)	7.8 (0.9) ns	7.6 (1.1) ns	14.2 (1.2) ***	2.3 (0.5) *	9.2 (1.4) ns
	# family	5.2 (0.5)	7.1 (0.7) ns	5.8 (0.8) ns	9.0 (0.7) *	2.0 (0.4) *	7.1 (1.1) ns
	Shanon Index	2.8 (3.0E-03)	2.1 (2.1E-03) **	2.7 (5.9E-03) ***	2.8 (0.1) ***	2.1 (0.01) ***	1.7 (0.008) ***
	Samson's Index	0.9 (1.2E-04)	0.7 (2.0E-04) **	0.89 (1.5E-05) *	0.9 (1.8E-05) ***	0.8 (1.4E-5) ***	0.7 (5.8E-05) ***

Note: Asterisk denote significant value RAF compare to forest; * at the $p < 0.05$, ** at the $p < 0.01$, *** at the $p < 0.001$, ns denote not significant based on Dunnet test.

probability 0.05). Furthermore, diversity of trees in the forest was higher than in RAF (*t* test = 7.2; probability 0.01).

Rubber monoculture is dominated by rubber, and has a lower diversity of trees, saplings and seedlings than forest or RAF. The floristic diversity of OP, CS and IG were lower than the diversity of RAF and forest. Neither saplings nor trees were present in OP and IG land use, except for a single tree present in CS at the time of this study.

Dominant family and species in the forest and RAF

Arecaceae was the most common family in the understory, and *Euphorbiaceae* was the most common in all other strata in the forest and RAF (Table 2). *Fagaceae*, associated with EM fungi, was one of the five most frequent plant families in the forest. Most of the Dipterocarpaceae species encountered were growing in forest plots.

Table 2 The five commonest families present at forest and RAF, in Bungo district, Jambi.

Landuse types	No.	Understory	Seedling	Sapling	Tree
Forest	1	Arecaceae (13.2)	Euphorbiaceae (12.3)	Euphorbiaceae (15.6)	Euphorbiaceae (14.8)
	2	Selaginellaceae (5.7)	Rubiaceae (7.5)	Myrtaceae (8.2)	Fagaceae (9.8)
	3	Annonaceae (3.8)	Annonaceae (6.2)	Rubiaceae (6.6)	Myrtaceae (8.2)
	4	Connaraceae (3.8)	Fabaceae (5.5)	Annonaceae (4.9)	Fabaceae (5.7)
	5	Dioscoreaceae (3.8)	Lauraceae (5.5)	Fabaceae (4.1)	Lauraceae (5.7)
RAF	1	Arecaceae (8.2)	Euphorbiaceae (11.1)	Euphorbiaceae (16.4)	Euphorbiaceae (13.6)
	2	Annonaceae (6.9)	Rubiaceae (9.2)	Annonaceae (11.2)	Burseraceae (7.6)
	3	Connaraceae (6.9)	Fabaceae (7.2)	Fabaceae (6.0)	Fabaceae (7.6)
	4	Dilleniaceae (5.5)	Annonaceae (5.9)	Lauraceae (6.0)	Moraceae 7.6)
	5	Vitaceae (4.1)	Lauraceae (5.2)	Rubiaceae (6.0)	Lauraceae (6.1)

The five commonest species were ranked by their importance value index (IVI) in forest and RAF. None of the five commonest species were dipterocarps (Table 3). In the understory, *Selaginella ornata* is a shared species among the top 5 of both forest and RAF. Although *Euphorbiaceae* are prominent within both land use types, the rubber tree that is dominant in RAF was found in low density in the forests – suggesting either that it spreads as 'invasive exotic' into forests or that part of the 'forest' represents failed attempts in the past to establish RAF. Other tree species dominant in RAF were *Artocarpus integer* (group of fruit trees), *Parkia sumatrana* (group of fodder trees) and *Parkia speciosa* (group of nuts).

Table 3 The five commonest species in forest and RAF, in Bungo district, Jambi.

Landuse types	No.	Understory	Seedling	Sapling	Tree
Forest	1	*Selaginella ornata*	*Spatholobus* sp.1	*Acronychia porteri*	*Alangium javanicum*
	2	*Phacelophrynium matinum*	*Syzygium splendens*	*Actinodaphne glabra*	*Alseodaphne* sp.
	3	*Arenga obstifolia*	*Gleichenia microphylla*	*Aglaia forbesii*	*Alstonia angustifolia*
	4	*Calamus ciliaris*	*Urophyllum ferrugineum*	*Aglaia lawii*	*Antidesma montanum*
	5	*Calamus javensis*	*Ixora brunonis*	*Ancistrocladus tectorius*	*Aporosa nervosa*
RAF	1	*Buettnera curtisii*	*Fordia splendidissima*	*Adina dumosa*	*Hevea brasiliensis*
	2	*Selaginella ornata*	*Hevea brasiliensis*	*Agelaea macrophylla*	*Artocarpus integer*
	3	*Selaginella intermedia*	*Symplocos cochinchinesis*	*Alseodaphne nigrescens*	*Macaranga trichocarpa*
	4	*Taenitis blechnoides*	*Clidemia hirta*	*Ancistrocladus tectorius*	*Parkia sumatrana*
	5	*Scleria purpuracens*	*Urophyllum corybosum*	*Antidesma cuspidatum*	*Parkia speciosa*

Shadow species' in the forest and RAF

With our limited sample size, many species were observed only once (singletons) within or across land use types. The Rennols-Laumonier equation for 'shadow species' estimated species richness of the forest + RAF data as close to (but not numerically identical) to that of the sum of the forest and RAF alone plus species observed in both RAF and forest (Table 4); species observed at least once in both RAF and forest were doubletons (or higher k-tons) and consequently represented a small number of shadow species. The estimated number of shadow species was 34.4 and 33.5% of the number of observed species, for forest and RAF, respectively, and 2.7% for species observed in both LUT's.

Table 4 Number of observed and shadow (estimated) species observed only in forest, only in RAF, in both forest and RAF, and in the combined data set.

Stratum		Number of species observed in			
		Union Forest+RAF	**Forest only**	**RAF only**	**Forest + RAF**
Trees	Observed	164	99	44	21
	Shadow	69	46	20	2
Saplings	Observed	200	84	76	40
	Shadow	66	32	32	1
Seedlings	Observed	244	91	93	60
	Shadow	61	29	30	1
Understories	Observed	97	24	45	28
	Shadow	21	8	13	0

Distribution of early and late successional species

To describe the distribution of the successional status of the species in each stratum in forest and RAF, we compared the cumulative frequency of wood density of the plant species observed (Fig. 4). The lowest wood density of species observed in the plots is 250 kg m^{-3} (*Trichospermum javanicum*) and the highest is 1100 kg m^{-3} (*Dialium patens*). The cumulative frequency of wood density had a similar pattern within all strata in RAF and forest.

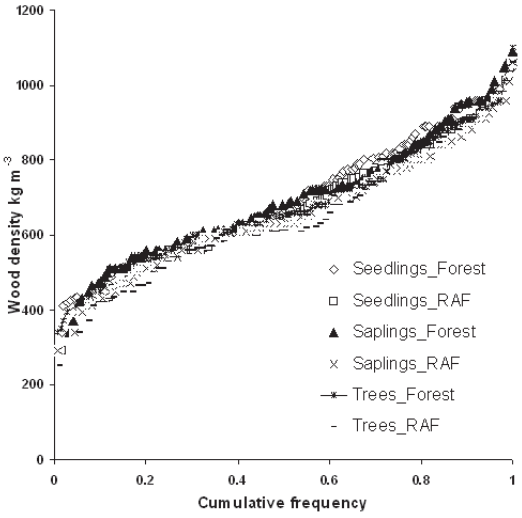

Fig. 4 Cumulative frequency of wood density of species found in forest and RAF in three strata, as indicator of successional status.

Tree dependency on EcM and trees with edible parts

Three families with EM dependency were found in Bungo area, e.g. Dipterocarpaceae, Fagaceae and Gnetaceae. The relative abundance in terms of species numbers is shown in Table 5**.**

Species dependent on EM in the three strata (seedlings, saplings and trees) were more abundant in forest than in RAF. Occurrence of seedlings and trees was significantly different, based on a χ^2 – test (χ^2 was 12.1 and 19.8; with probabilities of 0.01 and 0.001, respectively), while the occurrence of EM dependence in saplings was on the margin of statistical significance significantly different (χ^2 = 5.4; probability 0.05).

The relative abundance of trees with edible parts among seedlings and saplings in forest seemed higher than in RAF, but this difference was not statistically significant (χ^2 were 2.4 and 3.3, for seedlings and saplings, respectively). However, trees with edible parts are far more abundant in RAF than in forests (χ^2 = 51.5; probability 0.001) (Table 5**)**. A list of edible species is given in the Appendix.

Table 5 Relative abundance of species with EM dependency and trees with edible parts in forest and RAF, Bungo district, Jambi.

Relative abundance (%)	Landuse types	Stratum		
		Seedlings	Saplings	Trees
EM dependency	Forest	2.8*	5.3	10.6**
	RAF	0.6	1.7	0.5
Tree species with edible parts	Forest	14.3	18.3	28.8**
	RAF	11.7	12.9	64

Note: * value in the same column indicates significant difference at p = 0.01; ** at p = 0.001.

Discussion

The high floristic diversity of the lowland tropical forests of Sumatra means that the sample size in a study of this size is insufficient to account for the species richness and diversity (Richards, 1996, Plotkin *et al.*, 2000, Kindt *et al.*, 2006) or presence of rare species with high priority for conservation planning programs (Lawton, 1993, Rennols and Laumonier, 2006).

Rennolls and Laumonier (2006) reported a total of 499 observed species and an estimated number of 'shadow species' of 175 trees in a 3-ha area in Batang Ule, Jambi. Their ration of shadow to observed tree species (0.35) was only slightly lower than the ratios we found (0.47 and 0.46 for forest and RAF, respectively), despite the lower absolute numbers. The larger data set of Rasnovi (2006) that included the sapling stratum only for RAF and forests in the Bungo and neighbouring districts of Jambi includes a total species count of 930, in 108 sample plots. If we can assume that the taxonomic skill involved in the different surveys is comparable (and all refer to the Bogor Herbarium as source of knowledge in this regard), it seems likely that the total number of species encountered keeps increasing with sample effort. The 'shadow species' estimate of Rennols and Laumonier (2006) provides a substantial underestimate of what can be expected for increased sample effort and is not a reliable indicator.

Closer analysis of the forest – RAF comparison showed only slight differences in understory vegetation, seedlings and saplings, indicating high plant regeneration potential of the RAF. As most of the RAF occurs outside of a direct forest neighbourhood, access is probably highest for plants with seed dispersal by wind (anemochory) or animals (zoochory). Rasnovi (2006) reported that about 71% of the seedlings observed only in RAF belong to long-range zoochorous species. Expressed as fraction of the species pool, she found that

far-zoochory was the dispersal mode of 27.9 and 31.3% of species observed in forest and RAF, respectively, while autochory (large seeds with limited dispersal range) was represented by 35.1 and 23.1% of species. These differences in ecological signature should be taken into account, despite the overall numerical similarity of RAF and forest regeneration patterns.

The tree composition of RAF as agroecosystem managed by farmer differed significantly from that of the forest. Tree diversity and species richness in RAF were lower than in forest. In the RAF, non rubber trees, such as food and cash crops grow spontaneously. After the seedling and sapling stage (where forests and RAF are similar), the farmers selectively remove trees that don't have economic or use value, before the time for tapping rubber (about 6-8 year after planting rubber). Farmers maintain (and occasionally transplant) species of non timber products, such as latex, resin, fruits, rattan, for instance, since they can easily harvested the products (Michon 2005). Rasnovi (2006) found that the intensity of management within RAF had a negative correlation with species richness and similarity of composition with forest.

Relative to the total vegetation, plants with edible parts were more abundant in RAF than in forest. Although several plants with edible parts have moderate to high wood density, the cumulative frequency distribution of wood density indicates a slight shift towards early successional plants in RAF (Fig. 4). So far, farmers in Bungo have not been interested to plant and maintain timber trees in RAF, as other sources of timber were accessible to them. This, however, may be changing now, as indicated by farmer interest in enrichment planting with timber. The frequency of trees dependent on EcM was less in RAF than in forest. Tree dependency on EcM is common in late successional species that produce good timber, except for the family of *Gnetaceae*, which is well known as tree with edible part (fruits and leaves), i.e. *Gnetum gnemon*. We encountered liana *Gnetum* sp., *G. cuspidatum* and *G. latifolium* in the forest plots that were grouped as understory stage.

Most Dipterocarpaceae have large seeds and short-range dispersal, which may hinder spontaneous regeneration in RAF far away from forest. Therefore dipterocarp regeneration in RAF may require enrichment planting, if farmers become interested in and receive economic incentives for more diverse and forest-like species composition of RAFs. Evidence so far indicates that the RAFs represent an ecological 'tipping point' – they still allow for ecological restoration of lowland forest diversity if management intensity is reduced, but they are already depleted in species of late successional signature.

Acknowledgements

We thank Ratna Akiefnawati, Syahril and ICRAF – Muara Bungo team for assistance during data collection. Saida Rasnovi shared her insights in the ecology of RAFs. Identification of herbarium specimens was confirmed by J.J. Afriastini and her colleagues at Herbarium Bogoriense, Bogor. H.L.T. is grateful for the Netherlands organization for international cooperation in higher education (NUFFIC) – Netherlands Fellowship Programme (NFP) scholarship, World Agroforestry Centre (ICRAF-SEA) and Conservation Sustainable Management – Belowground Biodiversity (CSM-BGBD) Indonesia for financial supports.

Appendix List of plants with edible parts in Bungo district, Jambi

Family	Species	Part can be eaten
Anacardiaceae	*Mangifera foetida*	Fruits
	Mangifera indica	Fruits
Bombacaceae	*Durio zibethinus*	Fruits
Burseraceae	*Canarium littorale*	Seeds
	Canarium patentinervium	Seeds
Clusiaceae	*Garcinia atroviridis*	Fruits
	Garcinia gaudichaudii	Fruits
	Garcinia macrophylla	Fruits
	Garcinia maingayi	Fruits
	Garcinia parvifolia	Fruits, spices
Euphorbiaceae	*Baccaurea cf. bracteata*	Fruits
	Baccaurea kunstleri	Fruits
	Baccaurea lanceolata	Fruits
	Baccaurea motleyana	Fruits
	Baccaurea pyriformis	Fruits
	Hevea brasiliensis	Young seed, vegetables
	Sauropus androgynus	Leaves, vegetables
Fabaceae	*Archidendron bubalinum*	Fruits (pods)
	Archidendron ellipticum	Fruits (pods)
	Archidendron fagifolium	Fruits (pods)
	Archidendron jiringa	Fruits (pods)
	Archidendron microcarpum	Fruits (pods)
	Dalbergia latifolia	Fruits (pods)
	Dialium indum	Fruits (pods)
	Dialium patens	Fruits (pods)
	Parkia speciosa	Fruits (pods)
	Parkia sumatrana	Fruits (pods)
Fagaceae	*Castanopsis argentea*	Seeds
	Castanopsis javanica	Seeds
	Castanopsis lucida	Seeds
	Castanopsis malaccensis	Seeds
	Lithocarpus conocarpus	Seeds
Flacourtiacea	*Pangium edule*	Seeds, spices
Lauraceae	*Cinnamomum cuspidatum*	Bark, spices
	Cinnamomum iners	Bark, spices
Meliaceae	*Sandoricum koetjape*	Fruits
Moraceae	*Artocarpus anisophyllus*	Fruits (pods)
	Artocarpus cf. kemando	Fruits (pods)
	Artocarpus elasticus	Fruits (pods)
	Artocarpus integer	Fruits
	Artocarpus kemando	Fruits
Myrtaceae	*Eugenia cf. clavimyrtus*	Fruits
	Eugenia zippeliana	Fruits
	Syzygium antisepticum	Leaves, vegetables
	Syzygium jambos	Fruit, leaves, vegetables
Olacaceae	*Scorodocarpus borneensis*	Leaves, vegetables
Sapindaceae	*Dimocarpus longan*	Fruits
	Lansium domesticum	Fruits
	Nephelium eriopetalum	Fruits
	Nephelium lappaceum	Fruits
	Nephelium rubescens	Fruits
	Nephelium uncinatum	Fruits

CHAPTER 3

Land use effects on ectomycorrhiza inoculum potential for dipterocarp tree establishment on acid forest-derived soils fromlowland Sumatra

with M. van Noordwijk, M.J.A. Werger and G.S. de Hoog

Abstract

Dipterocarp trees are supposedly obligatory ectomycorrhizal and regrowth of natural forests after land use change may depend on presence of inoculum potential. Soil samples collected from seven different land use types in Jambi (Sumatra, Indonesia) were transferred to a nursery. Using two dipterocarp trees species (*Shorea lamellata* and *S. selanica)* as bait, we investigated the effect of land use type on the ectomycorrhiza (EcM) inoculum potential and soil properties. The major part (72.6%) of variation in soil properties could be described by two principle components, one texture related and one pH related. Phosphorus availability was only weakly related to both principle components. The soils had differential effects on growth of shoot and stem diameter of the tree seedlings and on EcM formation. Variation in EcM formation (18 – 41% of roots) had no consistent relation to tree growth in the nursery stage. The best growth of both *Shorea* species was obtained on soils derived from annual cropping, which had increased P availability, reduced Al-saturation of the exchange complex, but supported a relatively low percentage of EcM colonization. Variation in effective inoculum potential due to land use history is probably not a major constraint for establishment of dipterocarps across the landscape.

Keywords*: Agroforestry, Dipterocarpaceae, Ectomychorriza (EcM), tropical forests*

Introduction

Dipterocarp trees are a commercially important component of the natural forests of lowland Sumatra, but occur only in low frequencies in the 'rubber agroforests' that have replaced this forest in the more accessible parts of the landscape (originally along the rivers, later along the roads). With the rapid loss of remaining natural forests from the landscape (Murdiyarso *et al.*, 2002), through conversion to oil palm, rubber or fastwood plantations, the species-rich rubber agroforests have rapidly gained in importance as reservoir of useful trees (Joshi *et al.*, 2003). While for many local fruit trees natural dispersal and establishment is still adequate, dipterocarps probably require specific enrichment planting if farmers want to benefit from the current trend towards increased value for quality timbers. Dipterocarps, however, are known to be obligate EcM trees, and the presence of inoculum potential in the soil is a pre-requisite for success in enrichment planting.

According to previous research, mostly on the island of Borneo, the fungi that can form ectomycorrhiza (EcM) are sensitive to land use change (Smits 1994; Yasman 1995; Turjaman *et al.*, 2007). Absence of EcM host trees in non-forests and abandoned agriculture land may make soils deficient in native EcM propagules and lacking in antagonists against pathogenic microbes (Wilberforce *et al.*, 2003). Thus, we started the experiments reported here with the expectation to find strong effects of land use history on inoculum EcM potential of the soil, with samples derived from a range of land use histories including permanent forest, long-term rubber agroforest, open field crops, *Imperata* grassland and oil palm plantation. An *ex situ* test of inoculum potential was set up as part of a broad characterization of the impacts of land use change on belowground biodiversity in tropical landscapes (Giller *et al.*, 2006).

Using species of dipterocarp trees as tests of the inoculum potential requires a separation of the effects of EcM from other properties of the soil (either 'inherent' or modified by recent land use) that may also influence tree performance, either directly or in interaction with the mycorrhiza formation (Fig. 1).

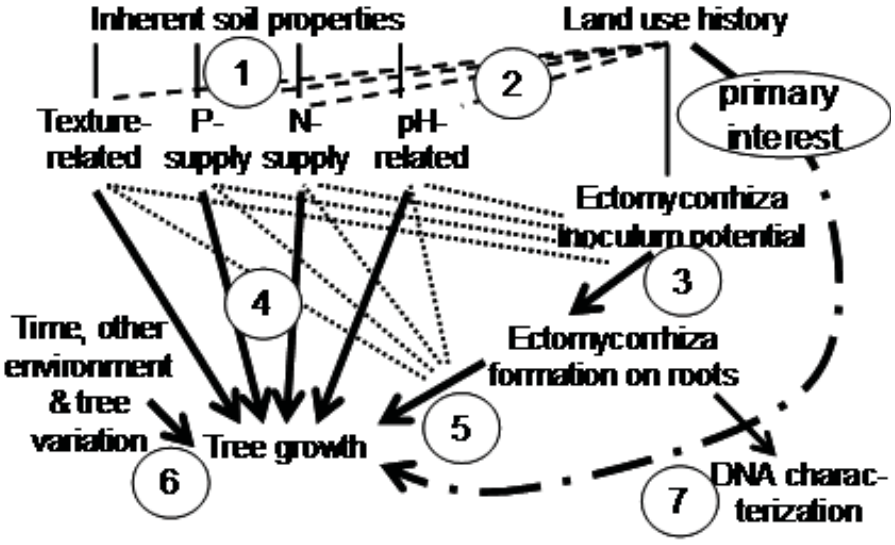

Fig. 1 Schematic presentation of the relationships involved in the ex situ test of inoculum potential of soils for EcM formation on dipterocarp trees, and the 6 steps required in the analysis of the data: 1.Grouping of soil variables; 2. Land use history vs soil; 3. Mycorrhization vs land use; 4. Soil properties vs tree growth; 5. Tree growth vs mycorrhization; 6. Combined model; 7. Further characterization of the ectomycorrhizal fungi with molecular techniques. Solid lines show direct relation; dashed lines show indirect relations; dotted lines show indirect effect; dash-dotted line shows hypothesis of indirect effect.

The test of land use history on inoculum potential requires the test of three interlinked hypotheses:

1. Variation in inherent soil properties and soil fertility related to landscape position and land use history have a modifying effect on tree growth,
2. Land use history determines the EcM inoculum potential of a soil, irrespective of soil texture and fertility,
3. EcM development is essential (obligatory) for growth of the tree seedlings and dominates over differences in chemical soil fertility.

Method

Origin of soil

Bungo district (western part of Jambi, Indonesia) is geographically located at 101°27' E, 1°08' S. Three sampling windows were established for the Sustainable Management of Belowground Biodiversity (CSM-BGBD) project: e.g. Rantau Pandan (foothills area) and Muara Kuamang and Kuamang Kuning (lowland peneplain) (Fig. 2). The sampling windows in Rantau Pandan and Muara Kuamang contain secondary forest (SF) and rubber agroforest (RAF), each represented by 8 sample plots in each window. Rubber monoculture (RM) was sampled in 6 plots each in Rantau Pandan and Kuamang Kuning. The other land uses, e.g. oil palm (OP), cassava garden (CS), annual crops (AC) and *Imperata* grassland (IG) were only represented in Kuamang Kuning, with 5, 9, 5 and 12 sample plots, respectively. Soil cores were dug up to 15 cm depth in each plot and 4 point samplings at each plot were taken to make a soil composite. Soils were kept in the plastic container and transferred to the nursery of the Forest and Nature Conservation Research and Development Centre (FNCRDC) in Bogor, Indonesia. The soils were collected in September 2004.

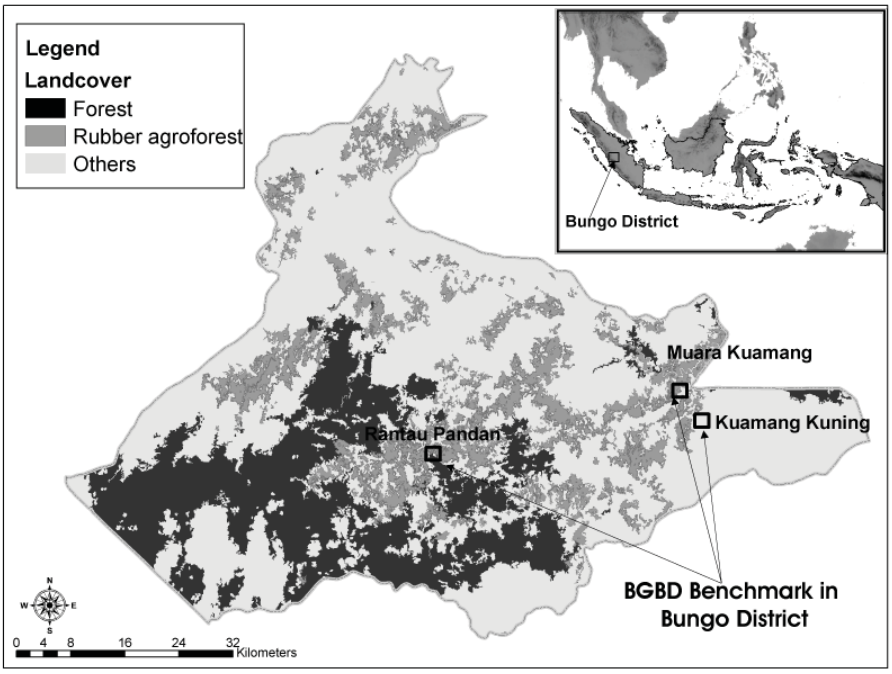

Fig. 2 Study site of soil sampling collection in Bungo district, Jambi Province, Indonesia.

Soil samples of half (randomly selected) of the plots (e.g. 8 plots of each SF and RAF, 5 plots of RM, 3 plots of each OP and AC, 5 plots of CS and 6 plots of IG) were analyzed in the Centre for Soil and Agroclimate Research (CSAR) in Bogor, Indonesia with standard soil physical and chemical laboratory methods. The soil samples were analyzed for texture (sand, silt, clay), pH (in a 1:25 soil:solution extract with water or 1 N KCl), P_{total} (P_2O_5 using ammonium molybdate method), available P_Bray1, $C_{organic}$ (Walkley and Black), N_{total} (Kjeldhal), exchangeable K, Ca, Mg, Na (exchanged with 1N NH_4-acetate solution, pH 7) and exchangeable Al and H (exchanged with a 1 N KCl solution). The effective cation exchange capacity (ECEC) was obtained by summation of these cation values. Initial soil conditions were assessed in May 2005. The reference organic C (C_{ref}) content for forest soils was calculated using a regression equation derived from a large data set for Sumatra (van Noordwijk *et al.* 1997):

$$C_{ref} = (\text{SampleDepth\_cm}/7.5)^{-0.42} * \text{EXP}(1.333 + 0.00994 * \text{clay\%} + 0.00699 * \text{silt\%} - 0.156 * \text{pH(KCl)} + 0.000427 * \text{elevation\_masl}) \quad \text{Eq. 1}$$

Preparation of pot trials, growth and harvesting condition

Soils from each plot were sieved thoroughly to disaggregate soil from gravel and roots or wood debris. Soil was filled into plastic pots of ca. 500 cm^3 each. As a control for EcM effects, a composite soil mixture from all sites was sterilised in an autoclave at 121 ^{o} C, at 1 bar, for 1 hour. Sterilised soil was filled into plastic pots and placed on a separate bench in the screen-house to reduce cross contamination.

Seeds of *Shorea lamellata* and *S. selanica* were collected from the arboretum of the FNCRDC, Bogor, Indonesia, in October 2004. Each seed was planted directly into a plastic pot. Most seeds germinated within two weeks. Non-germinated seeds were removed from pots and a new and vigorous seed was put into the pot.

The seedlings were kept in a plastic house for ten months and watered regularly once to twice a day using water from a well. Splashing of water from one treatment block to another was avoided. Growth of height and stem diameter at 1 cm above ground level was monthly measured.

Ten months after planting, all seedlings were harvested. Biomass was calculated as a function of shoot height and stem diameter. Dry weight (including leaves, stems and roots) of the seedlings were recorded after drying the seedlings at 70 ^{o}C for 48 hours. Prior to drying, the roots were washed under tap water to clean them from soil. We randomly selected root tips from about 20% of the harvested seedlings and these were spread into Petri dishes. The number of root tips and roots with EcM were counted under a dissecting microscope and expressed as a fraction of the whole sample. Confirmation of EcM colonization was obtained by examining a cross section of the root tips using a compound microscope and recording the presence of a mantle and a Hartig net (Brundrett *et al.*, 1995).

Data analysis

To study the relative effect of land use types on EcM inoculum potential (MIP) and soils, we carried out five steps in the data analysis (compare Fig. 1), viz.

(1) Grouping of soil variables; principal component analysis (PCA) was performed to determine the dominant axes of soil variation.

(2) Land use types versus soil properties, using the principle component groupings of autocorrelated variables.

(3) Soil properties versus tree growth; linear regressions were analyzed based on dominant soil factor versus tree growth (height, diameter and biomass).

(4) Mycorrhization versus land use; a linear regression was performed on data of mycorrhization of two species of *Shorea* at each land use type.

(5) Tree growth versus mycorrhization; linear regression based on growth versus mycorrhization was examined.

Basic statistical analyses for growth parameters (height and diameter) and other parameters were conducted using GenStat 9.1 edition for windows (VSN International Ltd., U.K.). Data were checked for homogeneity of variance and normality by analysis of the residual. Reliable growth data available from two months after planting (MAP) and from 7 to 10 MAP were used for statistical analysis.

Results

Grouping of soil variables

The PCA showed that two axes accounted for 72.6% of variation in the dataset that included all measured (and a number of derived) soil variables. Analysis of the Eigen values of the soil parameters revealed a group mostly associated with 'factor 1', a group with 'factor 2', a mixed group and two 'independent' variables. Factor 1 was mostly texture based, factor 2 mostly pH and exchangeable cation related, with the soil organic C and N parameters related to both factors. The 1-dimensional texture factor 'sand minus clay%' was strongly related to factor 1 (Y1 = 0.18 'Sand_minus_Clay' factor – 0.94; R2 = 0.99) and the exchangeable concentration of Ca + Mg had the strongest association with factor 2 (Y2 = 0.69 'Ca+Mg' – 0.05; R2 = 0.80). Eigen values and means for the land use representatives are shown in Table 1, grouping the soil variables according to their relative association with factors 1 and 2. A correlation matrix of soil properties (Table 2) provides further detail on the grouping. The Bray-I measure of available P was not statistically related to any of the other soil variables; the exchangeable Na content was also independent, but probably not important for this study.

Land use history versus soil properties

Generally soils were very acid in all land use types, and the lowest pH value was obtained in the forest (pH(KCl) and pH(H_2O) both 3.7). Soil properties varied among different land use types and a null-hypothesis of homogeneity was rejected for all variables except for exchangeable K content and C/N ratio of soil organic matter (Table 1). The forest sites in the survey had significantly higher clay contents than the other land uses, reflecting the landscape positions where forests still survive; the RAF had the highest sand contents. The higher C_{org} content of the forest soils was fully accounted for by the higher clay content, according to the reference value C_{ref} (Eq. 1).

Forests and RAF had very high exchangeable Al^{3+} contents (around 80%), while that in the other land uses (around 50%) was still considered too high for most food crops. The relationship between pH and Al-saturation across land uses was not very tight, except in rubber monoculture (RM) (Fig 3a). Available P content was lower in the forests, RAF and RM than in the other land uses, but a wide range of combinations of texture and available P (Fig 3b) was part of the EcM assessment.

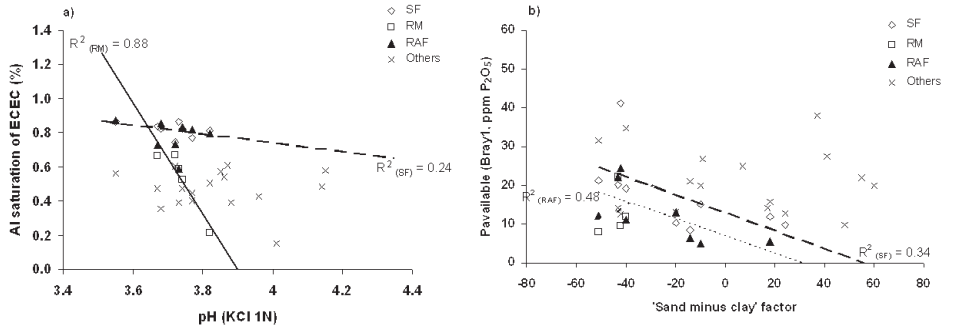

Fig. 3 a) Relationship between pH and Al saturation in the top soil of all land use types; b) Relationship between the 'sand minus clay' factor and Pavailable at all land use types; SF: secondary forest; RAF: rubber agroforests; RM: rubber monoculture; Others: oil palm plantation, cassava field, annual crops and *Imperata* grassland.

Table 1 Soil properties (grand means and standard errors of the mean [S.E.M.]) in 7 land use types and their Eigen value on the two principle components of overall variance; the parameters are grouped into a texture-related group, mostly associated with Factor 1, and a pH related group with Factor 2; a small intermediate group (soil organic matter) relates to both; SF: secondary forest; RAF: rubber agroforests; RM: rubber monoculture; OP: oil palm plantation; IG: *Imperata* grassland; CS: cassava field; AC: annual crops.

	Land use type										eigen-	eigen-
	SF	RAF	RM	OP	CS	AC	IG	Mean	s.e.m.	vr	value 1	value 2
Texture related												
'Sand-Clay' Factor	-25.3	35.1	19.8	3.3	24.4	27.7	3	10.8	4.6	7.9 ***	0.072	0.010
Sand (%)	27.3	61.8	54.4	45	56.6	55.3	45	48.4	2.7	7.4 ***	0.009	0.001
P2O5_H	31.2	14.5	16.1	22.4	17.7	17.2	19	20.2	1.5	4.1 **	0.011	0.001
Fe total (%)	3.4	1.8	2.3	2.5	2.2	0.52	2.1	2.2	0.17	5.4 ***	0.010	0.002
K2O_KOH	9.8	5	4.6	6.4	5.4	5	6.6	6.4	0.47	4.3 **	0.009	0.000
Clay (%)	52.5	26.6	34.6	41.7	32.2	27.7	42	37.6	2	7.7 ***	0.009	0.002
Silt (%)	20.3	11.6	11	13.3	11.2	17	13	14.1	1	2.7 *	0.008	0.002
K (cmol+kg-1)	0.11	0.08	0.06	0.1	0.08	0.07	0.1	0.09	0.01	1.9 ns	0.005	0.001
pH & texture related												
C/N	13.8	14.7	14.8	14.3	14.9	15.2	14.4	14.5	0.16	1.4 ns	0.001	0.000
N_{tot} (%)	0.24	0.14	0.14	0.13	0.11	0.22	0.13	0.16	0.01	11.6 ***	0.007	0.002
$C_{organic}$ (%)	3.2	2	2.1	1.8	1.6	3.4	1.9	2.3	0.13	10.4 ***	0.005	0.002
ECEC ($cmol^+kg^{-1}$)	6.6	4.3	4	3.7	3.6	4.4	4.1	4.6	0.25	5.6 ***	0.006	0.006
C_{ref} (%)	4.2	3	3.2	3.5	3.1	3.1	3.5	3.4	0.1	9.3 ***	0.005	0.001
Exchangeable H^+	0.67	0.5	0.34	0.25	0.3	0.3	0.35	0.43	0.03	10.4 ***	0.007	0.014
Exchangeable Al^{3+}	4.7	3	1.8	1.7	1.4	1.5	1.6	2.5	0.26	10.1 ***	0.010	0.023
C_{org}/C_{ref}	0.77	0.66	0.65	0.53	0.52	1.1	0.54	0.67	0.03	9.8 ***	0.001	0.000
pH related												
pH (KCl)	3.7	3.8	4	4	4.1	4	4	3.9	0.03	8.1 ***	0.001	0.002
pH (H_2O)	3.7	4.1	4.3	4.4	4.4	4.1	4.4	4.2	0.06	6.1 ***	0.001	0.003
Base Saturation (%)	18.2	22.2	46.7	47.3	52.6	59.5	53.3	38.4	3.2	12.4 ***	0.004	0.025
ECEC/clay	0.13	0.16	0.13	0.09	0.11	0.16	0.1	0.13	0.01	3.1 ***	0.003	0.007
Mg ($cmol^+kg^{-1}$)	0.34	0.19	0.42	0.34	0.44	0.59	0.48	0.37	0.03	2.6 *	0.003	0.023
Ca ($cmol^+kg^{-1}$)	0.75	0.55	1.39	1.27	1.4	1.9	1.6	1.1	0.09	11.8 ***	0.000	0.023
Ca+Mg ($cmol^+kg^{-1}$)	1.1	0.74	1.8	1.6	1.8	2.5	2	1.51	0.12	8.5 ***	0.001	0.023
Independent												
P-Brayl, P_2O_5 ppm	18.5	12.5	12.3	26.9	19.2	29.1	15.8	17.6	1.5	2.8 *	0.004	0.011
Na ($cmol^+kg^{-1}$)	0.01	0.02	0.01	0.02	0	0.07	0.03	0.02	0	3.0 *	0.001	0.033

Asterisks denote probability of a 'no-LU difference' null hypothesis. *, **, *** indicate statistical significant differences at *p* value 0.5, 0.1, 0.01, respectively, ns: not significant.

Soil properties versus tree growth

The ANOVA (Table 3) showed that land use type (U) and *Shorea* species used (S) affected growth of height and stem diameter, biomass and EcM colonization. The interaction between time (T) and land use (U) was significant at all response variables. Tree growth was reflected in significant effects of repeated time of measurement on tree height and biomass. The three-way interaction of T * U * S was not statistically significant.

The height and diameter growth of *S. lamellata* and *S. selanica* for the various land use types are shown in Fig. 4. Most differences occurred between planting and 7 MAP, with approximately parallel growth curves in the period 7 – 10 MAP. Diameter growth of the two *Shorea* species planted in RAF soils was better than those planted in soil from other land use types. However, height growth of the two *Shorea* seedlings was better in AC soils than in soil from other land use types.

Table 2 Correlation matrix of soil properties measured across 7 land use types in Jambi, Sumatra.

	Sand-Clay' Factor	Sand (%)	P_2O_5_H	Fe total (%)	K_2O_KOH	Clay (%)	Silt (%)	K ($cmol^+kg^{-1}$)	C/N	N (%)	$C_{organic}$ (%)	ECEC ($cmol^+kg^{-1}$)	C-ref	Exchangeable H^+	Exchangeable Al^{3+}	C/Cref	pH (KCl)	pH (H_2O)	Base Saturation (%)	ECEC/clay	Mg ($cmol^+kg^{-1}$)	Ca ($cmol^+kg^{-1}$)	*Ca+Mg ($cmol^+kg^{-1}$)*	Bray1_P2O5	Na
Sand-Clay' Factor	1																								
Sand (%)	0.99	1																							
P_2O_5_H	-0.8	-0.83	1																						
Fe total (%)	-0.77	-0.76	0.73	1																					
K_2O_KOH	-0.71	-0.72	0.82	0.68	1																				
Clay (%)	-0.98	-0.94	0.73	0.76	0.67	1																			
Silt (%)	-0.64	-0.75	0.72	0.49	0.55	0.49	1																		
K ($cmol^+kg^{-1}$)	-0.47	-0.46	0.48	0.35	0.79	0.47	0.27	1																	
C/N	0.45	0.49	-0.53	-0.57	-0.44	-0.39	-0.52	-0.05	1																
N (%)	-0.63	-0.67	0.64	0.39	0.66	0.56	0.66	0.45	-0.32	1															
$C_{organic}$ (%)	-0.53	-0.56	0.51	0.22	0.55	0.47	0.55	0.45	-0.06	0.96	1														
ECEC ($cmol^+kg^{-1}$)	-0.66	-0.68	0.59	0.52	0.72	0.61	0.56	0.61	-0.14	0.81	0.8	1													
C-ref	-0.97	-0.98	0.82	0.75	0.72	0.93	0.74	0.48	-0.48	0.69	0.59	0.74	1												
Exchangeable H^+	-0.5	-0.52	0.45	0.41	0.55	0.47	0.42	0.47	-0.09	0.62	0.62	0.87	0.63	1											
Exchangeable Al^{3+}	-0.52	-0.54	0.48	0.48	0.58	0.47	0.5	0.47	-0.2	0.66	0.63	0.91	0.65	0.93	1										
C/Cref	-0.07	-0.11	0.09	-0.23	0.18	0.01	0.26	0.23	0.27	0.73	0.86	0.51	0.12	0.37	0.35	1									
pH (KCl)	0.32	0.34	-0.23	-0.27	-0.31	-0.28	-0.35	-0.33	0.12	-0.49	-0.49	-0.71	-0.48	-0.84	-0.27	-0.88	1								
pH (H_2O)	0.34	0.38	-0.34	-0.25	-0.42	-0.29	-0.41	-0.31	0.15	-0.6	-0.59	-0.76	-0.49	-0.76	-0.41	-0.87	0.84	1							
Base Saturation (%)	0.2	0.23	-0.19	-0.29	-0.2	-0.16	-0.28	-0.11	0.22	-0.29	-0.25	-0.52	-0.37	-0.75	-0.06	-0.82	0.89	0.77	1						
ECEC/clay	0.37	0.31	-0.19	-0.31	0	-0.44	0.07	0.1	0.25	0.26	0.35	0.4	-0.24	0.41	0.57	0.45	-0.41	-0.52	-0.36	1					
Mg ($cmol^+kg^{-1}$)	-0.24	-0.24	0.35	0.19	0.37	0.25	0.13	0.22	-0.06	0.27	0.25	0.09	0.11	-0.26	0.19	-0.3	0.52	0.33	0.72	-0.17	1				
Ca ($cmol^+kg^{-1}$)	-0.05	-0.03	-0.05	-0.17	-0.03	0.08	-0.08	0.03	0.24	0	0.06	-0.2	-0.12	-0.53	0.17	-0.59	0.69	0.55	0.91	-0.26	0.79	1			
Ca+Mg ($cmol^+kg^{-1}$)	-0.11	-0.09	0.07	-0.08	0.09	0.13	-0.02	0.09	0.17	0.08	0.12	-0.13	-0.06	-0.48	0.19	-0.53	0.67	0.51	0.9	-0.24	0.89	0.98	1		
Pav-Bray1, P_2O_5 ppm	-0.27	-0.32	0.44	0.01	0.22	0.2	0.46	0.17	-0.08	0.27	0.27	0	0.26	-0.13	0.22	-0.14	0.23	0.1	0.27	-0.25	0.3	0.3	0.31	1	
Na ($cmol^+kg^{-1}$)	0	-0.04	-0.01	-0.27	-0.16	-0.05	0.2	-0.09	0.04	0.24	0.28	-0.06	-0.03	-0.23	0.37	-0.26	0.17	0.23	0.38	0.04	0.37	0.48	0.47	0.37	1

Table 3 Variance ratio (F) of the data on growth of height and stem diameter for four observation periods for the factor land use types (U), tree species (S) and time observation (T).

Response Variable	Time (T)	Land Use (U)	Tree species (S)	UxS	TxU	TxS	TxUxS
Height (cm)	756.7 ***	4.1 ***	70.3 ***	1.5	2.9 ***	8.8 ***	0.33
Diameter (cm)	1,821.6 ***	4.6 ***	162.9 ***	0.48	5.2 ***	18.4 ***	0.76
Biomass (cm^3)	1,077.8 ***	5.12 ***	158.6 ***	0.54	5.1 ***	64.2 ***	0.4
EcM colonization (%)		7.1 ***	4.3 *	0.3			
df of F	(3,420)	(7,140)	(1,140)	(7,140)	(21,420)	(3,420)	(21,420)

Asterisk denotes significant value: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

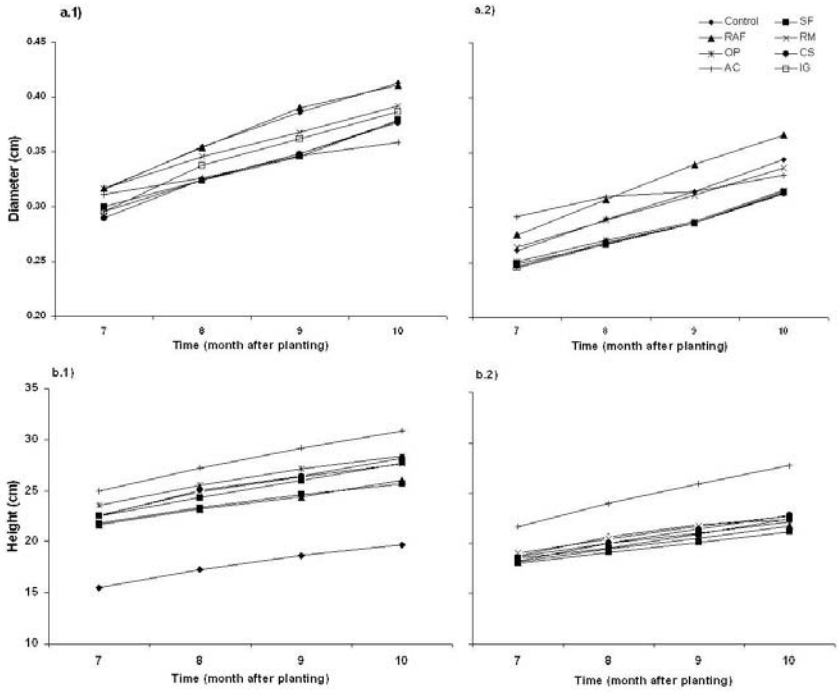

Fig. 4 Effects of land use type on a) stem diameter and b) shoot height of 1) *S. lamellata* and 2) *S. selanica*, planted on soil from secondary forest (SF), rubber agroforest (RAF), rubber monoculture (RM), oil palm plantation (OP), cassava field (CS), annual crops (AC) and *Imperata* grassland (IG) at time series of observation.

The relationship between 'sand minus clay' factor and Ca+Mg as representatives of 'factor 1' and 'factor 2' in soil variation, with biomass and growth of height and diameter were analyzed using multiple linear regressions analysis. Both factors did not show significant relations with growth and biomass of the two *Shorea* species. The variance ratio $_{(8,29)}$ for regression analysis between Ca+Mg and biomass and growth of height and diameter were 0.97, 1.6, and 1.4, respectively, while the F-values $_{(8,29)}$ for regression analysis between 'sand minus clay' factor and biomass and growth of height and diameter were 0.41, 1.6, and 0.37,

respectively. No strong relationships were found between the two main groups of soil properties factors with growth and biomass of *S. lamellata* and *S. selanica*.

Relationship between available P (Bray1) with growth of height, diameter and biomass of *S. lamellata* and *S. selanica* were analysed using multiple linear analysis. Available P showed a significant relationship with diameter {F-values $_{(8.29)}$ = 2.7, at the *p* value < 0.05}, but no significant relation with height and biomass. F-values $_{(8,29)}$ for regression analysis between available P and biomass and height were 1.2 and 0.72, respectively.

The relationship between biomass increment, e.g. the difference between biomass at 7 and 10 MAP, on *S. lamellata* and *S. selanica* with soil parameters across all land use types is shown in Fig 5.

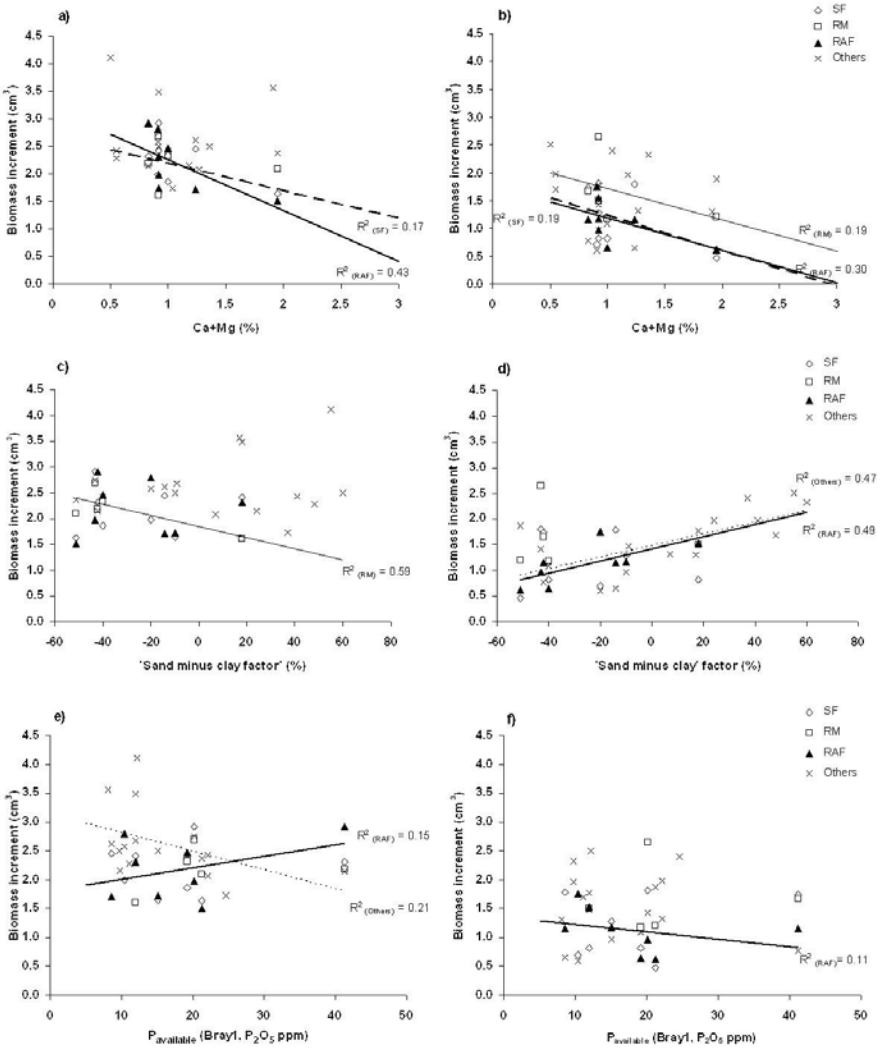

Fig. 5 Relationship between biomass increment, i.e. biomass difference between 7 and 10 months after planting (MAP), of two *Shorea* species with soil parameters planted in soil from secondary forest (SF), rubber monoculture (RM), RAF (rubber agroforests) and Others (consists of oil palm, cassava field, annual crops and *Imperata* grassland). a), c) and e) refer to *S. lamellata*; b), d) and e) to *S. selanica*; a) and b) relate biomass increment to Ca+Mg concentration on the soil (and associated soil variables in component 1); c) and d) to the 'sand minus clay' factor; e) and f) to available P.

Mycorrhization versus land use and growth

The highest percentage of EcM colonization was observed in the seedlings planted in RAF soil, while both *Shorea* seedlings planted in soil from AC had the lowest EcM colonization. The seedlings planted in sterilized soil (control) and IG soils were colonized by EcM fungi (Fig. 6), and, contrary to our expectations, seedlings planted in IG soil had less EcM colonization.

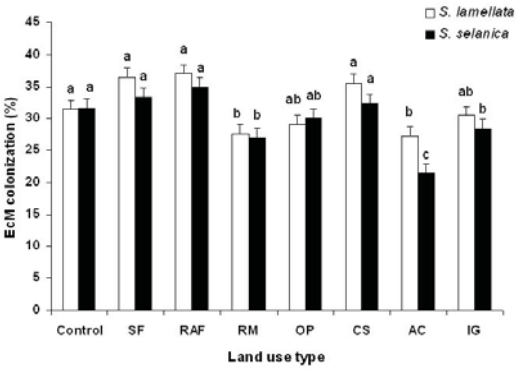

Fig. 6 Effects of land use type on EcM colonization of *S. lamellata* and *S. selanica*. Different letters indicate significant differences according to LSD test at p≤0.05. SF: secondary forest, RAF: rubber agroforests, RM: rubber monoculture, OP: oil palm, CS: cassava field, AC: annual crops, and IG: *Imperata* grassland..

Regression analysis showed a significant relationship between the 'factor 2' indicator Ca+Mg and EcM colonization {F-value $_{(2,35)}$ = 3.9, at the *p* value < 0.05}, but no significant relationship with the 'factor 1' indicator 'sand minus clay' factor, as the F-value $_{(2,35)}$ was 2.1. Relationship between mycorrhization and land use type are shown in Fig 7. On *S. lamellata*, EcM colonization was strongly correlated with the 'sand minus clay' factor obtained for RAF (Fig. 7a). EcM colonization was also strongly correlated with available P (Bray1) obtained for RAF and RM (Fig. 7c). On *S. selanica*, EcM colonization was strongly correlated with available P (Bray1) obtained for RAF (Fig. 7d).

Multiple regression analysis showed no significant relationship between mycorrhization with tree growth and biomass. The variance ratios on mycorrhization on *S. lamellata* and *S. selanica* with stem diameter ($F_{8,29}$) were 0.63 and 0.27, respectively, and with height ($F_{8,29}$) 0.88 and 1.03, respectively. Variance ratios of mycorrhization on *S. lamellata* and *S. selanica* with biomass ($F_{8,29}$) were 0.50 and 0.45, respectively. Relationships between EcM colonization with biomass at 10 MAP on *S. lamellata* and *S. selanica* is shown in Fig. 8.

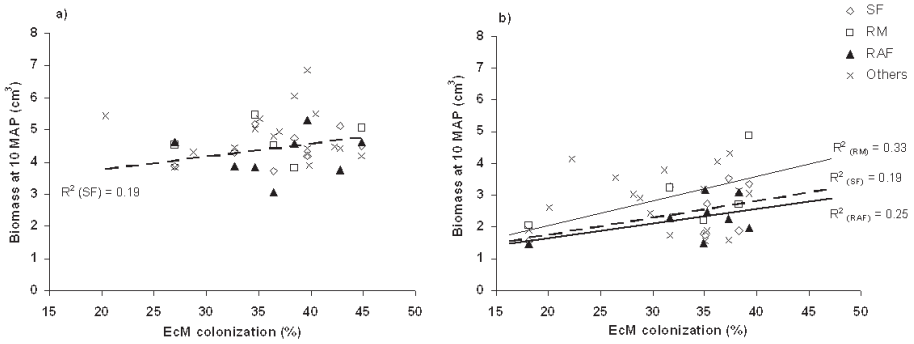

Fig. 8 Relationship between EcM colonization with biomass at 10 map on a) *S. lamellata*; b) *S. selanica*, planted on soil from secondary forest (SF), rubber monoculture (RM), rubber agroforestry (RAF), and Others (consists of oil palm, cassava field, annual crops and *Imperata* grassland)

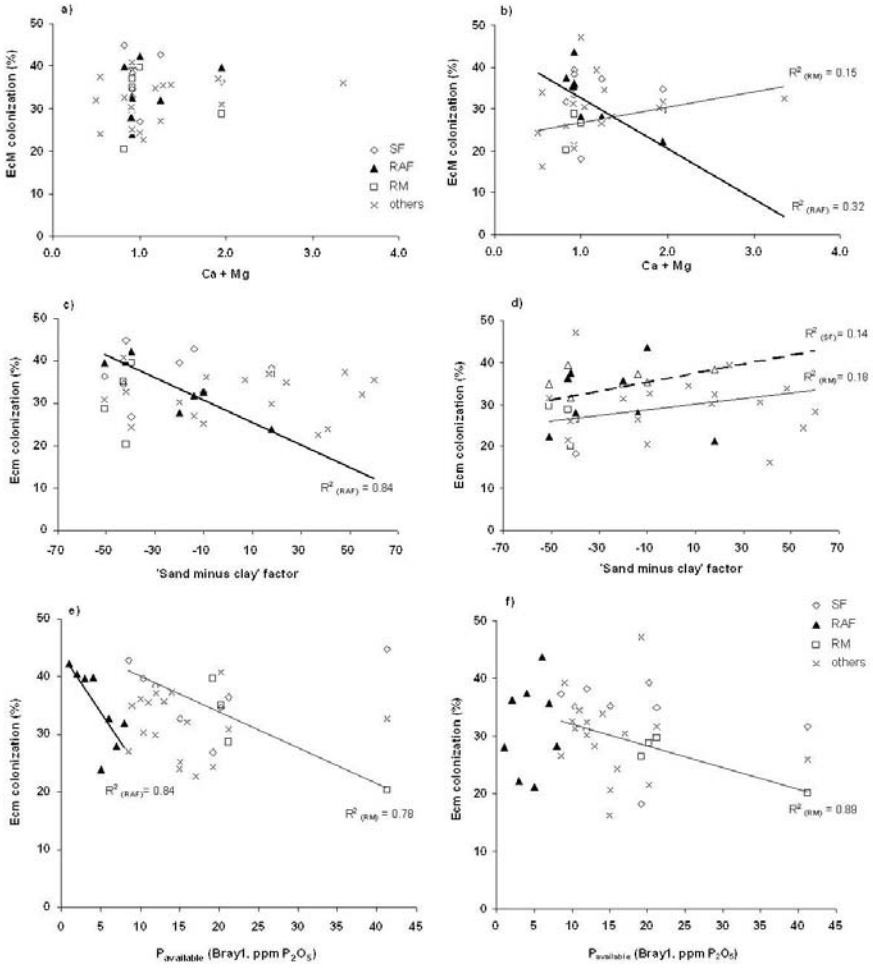

Fig. 7 Relationship between EcM colonization of two *Shorea* species with soil parameters planted in soil from secondary forest (SF), rubber monoculture (RM), RAF (rubber agroforests) and Others (consists of oil palm, cassava field, annual crops and *Imperata* grassland). a), c) and e) refer to *S. lamellata*; b), d) and e) to *S. selanica*; a) and b) relate EcM colonization to Ca+Mg concentration on the soil (and associated soil variables in component 1); c) and d) to the 'sand minus clay' factor; e) and f) to available. P

Discussion

Inherent soil variability in the replicated survey proved to be substantial, which established the relevance of the results across the full spectrum of soil types that occur in the area. The data groupings suggest two main groups, one with texture and total nutrient stock data, and one with soil pH and associated exchangeable cation data. Soil C and N_{tot} are strongly linked to clay content. Variation in indicators of available P was independent of these two groups. Land use types in the landscape are associated with different values for soil properties, as positions close to the river, with loamy textures, have traditionally been selected for rubber agroforest development, while forests were left on the poorest sandy soils and/or on ridges with high clay content. The difference in pH and exchangeable cation content may be more causally linked to land use differences, as the use of fire enhances cation content of the soil and increases its pH (Ketterings, 1999; Ketterings *et al.*, 2000; 2002).

The two *Shorea* species planted in the AC soil with relatively high P availability showed rapid growth in shoot height, despite relatively low EcM colonization values. Stem diameter of *S. lamellata* (but not *S. selanica)* responded similarly to the AC soil. This may be an effect of high available P contents in the soils. The higher available P contents of the oil palm and annual crop fields probably reflect P fertilization, but the lowest values (P-Bray1 of 12 ppm P_2O_5) obtained in the RAF and rubber monoculture are still sufficient for rubber tree growth (unpublished results ICRAF Muara Bungo). Other nursery studies showed that application of N and P fertilization reduced EcM colonization (Baum *et al.*, 2002; Liu *et al.*, 2004 and 2008).

The percentage of EcM colonization on *S. lamellata* and *S. selanica* in the IG soil, supposedly the most severely biologically and chemically degraded soil, was about 28-30%. The EcM colonization of the two species in sterilized soil (control) exceeded 30%, although it was placed on a separate bench to diminish effects of contamination from other treatments. However, we could not eliminate possible dispersal of air borne spores, which might be present in the surroundings of the nursery of the forestry research station of FNCRDC, where many dipterocarp trees grow. We expected that EcM fungi reinfected the seedlings planted in sterilized soil, as the sterilization technique used would normally be sufficient. Further tests of the soil sterilization treatment, however, are desirable. In other treatments, EcM inoculum may also persist in the soils in a dormant phase. Morphotype observations of the EcM in all treatments showed a dominance of monopodial regular pinnate mycorrhiza with white mantles. From a follow-up study on molecular identification of EcM based on sequencing DNA from root tips (which will be reported separately), we found that EcM roots were colonized by at least 3 different genera, including *Tomentella, Laccaria* and an unidentified genus of *Sclerodermataceae*. The three EcM fungi are considered to be early stage fungi (Deacon *et al.*, 1983; Sims *et al.*, 2004; Obase *et al.*, 2007), with effective spore dispersal. Thelephoroid fungi are commonly found to colonize seedlings in nurseries (Castellano and Molina, 1989; Menkis *et al.*, 2005; Teste *et al.*, 2006) .

The concept of 'inoculum potential' tested here of disturbed soils relies on effectiveness of spore survival and dispersal, rather than on connections to the wood-wide web through shared mycorrhizal networks. In nature, EcM fungi and hosts become interconnected through common mycorrhizal networks, exchanging nutrients and carbon (Helgason *et al.*, 1998; Sen, 2000; Peter, 2006). In pot trials or soil trenching experiments, EcM propagules have less ability to spread vegetatively via mycelia from one container to another; therefore spore propagules dominate the results (Casstellano and Molina, 1989; Simard *et al.*, 1997). EcM spores from sporocarps disperse and germinate easily when they find a suitable host tree and form ectomycorrhiza effectively (Casstellano and Molina, 1989). The small differences between EcM spores from sporocarps disperse and germinate easily when they find a suitable host tree and form ectomycorrhiza effectively (Casstellano and Molina, 1989). The small differences between land use types in the current experiment may indicate that survival of EcM spores and recolonization potential is not sensitive to the history of presence of Dipterocarp trees at the site.

We found soils with all land use histories to support EcM formation and dipterocarp tree growth. The overall effect of land use history on the inoculum potential was much smaller than expected. We expected that most non-forest land uses would have lost much of the EcM inoculum potential in the many years without vegetation and absence of lively interaction of EcM with the trees. While *Imperata* grassland soil is known to be rich in endomycorrhizal spores (Murniati, 2002), we expected poor EcM formation. But our alternative explanation for the presence of EcM inoculum in non-forest soils of coincidentally transported propagules from forests to other land use types may have a large impact. In addition to airborne dispersal, fungivores and sporivores may act as dispersal agents, such as mites (Oribatida), beetles (Coleoptera), flies (Diptera) and springtails (Collembola) (Lilleskov and Bruns, 2005). Small mammals like chipmunks and marmots (Cazares and Trappe, 1994) and big mammals, like wild pigs (*Sus scrofa*), which are common in the surroundings of the study area in Jambi, may also play a role. An indication may be the finding of fungal fragments in fecal materials of *Sus barbatus* (Setyowati *et al.*, 2005; Meijaard *et al.*, 2005).

This raises three follow-up questions:

A. Are at least some of the fungi that support EcM formation resistant to land use change and able to survive in the soil for several decades after forest conversion?

B. Have fungi that effectively form EcM been able to colonize the soil used for the *ex situ* test in sufficient spore densities to support tree growth?

C. Are dipterocarp tree species used less dependent on EcM formation than assumed?

To obtain more insights into question A, a follow up experiment was designed to test the effects of drying and heating on soil inoculum potential of a forest soil from the sampling area. Results will be described elsewhere, as chapter 4 ("Response of a soil heating and drying treatment on growth and EcM formation on five species of Dipterocarpaceae in a nursery trial"). A more direct answer to question B can be obtained by analysis of the DNA extracted from the EcM's for identification of the fungi. Results will be described in chapter 6. Question C can be answered in part by our data, and is further reported, based on field planitng experiments in rubber gardens of different histories: chapter 5 ("Limited response to nursery-stage mycorrhiza inoculation of *Shorea* seedlings planted in rubber agroforests in Jambi, Indonesia"). Indication so far suggest that enrichment planting with dipterocarp trees is not seriously constrained by lack of EcM inoculum.

Acknowledgements

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CHAPTER 4

Ectomycorrhiza formation and growth of five Dipterocarpaceae in response to soil heating and drying in a nursery trial

with M. van Noordwijk, M.J.A. Werger and G.S. de Hoog

Abstract

As an earlier nursery trial with soils derived from forest and other land uses showed remarkably small differences in ectomycorrhiza (EcM) formation of dipterocarp seedlings, we now tested the effects of soil drying and heating up to 150 ^{0}C for 3 hours, simulating fire impacts, on the inoculum potential of forest soils from a lowland tropical rainforest site on 5 different Dipterocarp species. Effects of these treatments on tree performance were small, for all five species tested. Mycorrhiza formation was not hampered by soil treatments in diversity or total infection rate, including the supposed sterilization of soil. Heating the soil increased P availability which may have reduced mycorrhiza dependence. Based on molecular identification, we identified at least three genera of EcM fungi as *Tomentella, Laccaria*, and *Inocybe.* The rapid colonization of the heated soil in the experiment and/or survival of inoculum potential may imply ample availability of ectomycorrhiza partners for dipterocarps in field conditions, regardless of land use history of the landscape.

Keywords*: Heat and drying disturbance,* Hopea odorata*, Propagule resistance,* Shorea selanica*,* Shorea lamellata*,* Shorea leprosula, Vatica sumatrana

Introduction

Fire, as wild fire or prescribed burning, is an important form of disturbance in Sumatra. Large estate crop plantations and small scale rubber producers in Sumatra commonly practice slash-and-burn to open the land to establish new plantations or new fields (Suyanto *et al.*, 2004). Fire is the most prevalent tool to open new fields as it is an efficient way to increase accessibility to the land (Ketterings *et al.*, 1999b; Suyanto *et al.*, 2004). In natural forests, high intensity of fire kills most of the trees (van Nieuwstadt *et al.*, 2001; Cleary *et al.*, 2006); but slash-and-burn practices starting with slashing the understorey and cutting valuable timber, followed by burning (Gouyon *et al.*, 1993; van Noordwijk *et al.*, 2008b), gives farmers the benefit from wood use (Ketterings *et al.*, 1999b; Suyanto *et al.*, 2004).

Besides smoke and air pollution from burning, fire also affects soil texture (Ketterings *et al.*, 2000), soil pH and soil nutrient supply (Iriansyah and Waliadi, 1999; Ketterings, 1999; Ketterings, *et al.*, 2002). Fire is also important for ectomycorrhizal (EcM) fungi which associate with late successional trees in forests and mature rubber agroforestry (RAF) ecosystems, such as Dipterocarpaceae, Fagaceae and Myrtaceae (Wang and Qiu, 2006). Damage and disturbance of host trees and soil properties because of fire, may affect mycorrhizal inoculum potential in the soils.

In a test of the mycorrhizal inoculum potential of forest-derived soils from lowland Sumatra, we found surprisingly little direct evidence of impacts of land use history on the ability of dipterocarps to develop ectomycorrhiza (EcM) in a simple pot trial (chapter 3). This raised three follow-up questions:

1. Are at least some of the fungi that support EcM formation resistant to land use change and able to survive in the soil for several decades after forest conversion?
2. Have fungi that effectively form EcM been able to colonize the soil used for the *ex situ* test in sufficient spore densities to support tree growth?
3. Are the dipterocarp tree species used less dependent on EcM formation than assumed?

To obtain more insights into question 1, a follow up experiment was designed to test the effects of drying and heating on EcM inoculum potential of a forest soil from the sampling area. Five dipterocarp tree species were used to test generalization across a range of dipterocarps that can be used for enrichment planting of the results obtained with two *Shorea* species in the first trial. Results will be discussed here. Question 2 was approached by molecular indentification based on polymerase chain reaction (PCR) of DNA extracted from the ectomycorrhizal root tips and sequencing.

Ketterings (1999) explored the soil chemical, physical and biological consequences of heating forest soils for different durations and temperatures, simulating the impact of land clearing fires on forest soils. Within a soil a strong temperature gradient can be expected during a fire, with only the top few cm of soil exposed to temperatures above 100 ^{0}C in all but the most intense fires. Usually sites are partially burned, but stumps often are piled up for a second burn experience of very hot fires, which may influence even soil texture by the formation of strong ('brick') aggregates in the sand fraction on the basis of clay particles.

We used a similar oven heating experiment as Ketterings (1999), in combination with intense drying of the soil, to study the impacts on survival of resistant propagules of EcM fungi and the growth of dipterocarp cuttings in a nursery.

Material and methods

Origin of soils

Soil samples were collected from two sites of mature rubber agroforests in the Muara Kuamang and Rantau Pandan sub-districts, Bungo districts, Jambi province, in February 2006. Characteristics of both sites were described in chapter 1 on "Tree and regeneration strategies

in rubber agroforests and other forest-derived vegetation in Jambi (Sumatra, Indonesia)." Top soils were sampled to 15 cm depth, stored in plastic bags and transferred to the nursery of the Forest and Nature Conservation Research and Development Centre (FNCRDC), Bogor, Indonesia.

Heating and drying

Soils from each site were sieved through a 0.5 cm sieve to disaggregate soil from gravel and roots or wood debris. The heat treatments of the soil were performed in an oven, in the laboratory of Soil Chemistry at the FNCRDC, a few weeks before cuttings of five dipterocarp species were transplanted. Soils were put on an aluminium tray of 5 cm thick, to ensure even heating of the soil samples. Soils were for three hours brought into an oven with the temperature set at 35 ^{o}C, 50 ^{o}C, 80 ^{o}C, 120 ^{o}C or 150 ^{o}C. A thermocouple was positioned in the middle of the soil and recorded temperature every 5 minutes. Control soil was unheated.

Following the heating treatment, sub-samples of the soils were treated in a drying machine (non-branded machine made locally in Bogor, Indonesia) at the nursery of Komatsu – Forest Research and Development Agency (FORDA), Bogor, Indonesia. The drying treatment was performed for 30 minutes, except for the controls (unheated soil), which were dried up to 1 hour. First, the sub-sample of 150 ^{o}C was dried, followed by soil samples of lower temperatures. After treatment all soils were dry. The drying machine worked on air movement in a tube and the heat was produced by the engine. The temperature in this stage was not measured. Treated soils were filled into polypropylene containers with a volume of 500 cm^3 and were placed in a glass-house. Pots that were filled with control treatments, e.g. unheated and undried soils, were placed separately from the other treatments.

Sub-samples of the soils from each treatment were randomly collected for further analysis. In total, 24 soil samples were analyzed in the laboratory of Soil Chemistry, CSAR, in Bogor, Indonesia. The soil samples were analyzed for pH (in a 1:25 soil: solution extract with water or 1 N KCl) and P-Brayl. Control soils from the two sites were analysed for texture (sand, silt, clay), pH (in a 1:25 soil: solution extract with water or 1 N KCl), P-Brayl, C-org (Walkley and Black), N-total (Kjeldahl), exchangeable K, Ca, Mg, Na (exchanged with 1N NH_4-acetate solution pH 7) and exchangeable Al and H (exchanged with a 1 N KCl solution). The effective cation exchange capacity (ECEC) was obtained by summation of these cations.

Plant materials

Five species of Dipterocarpaceae were used in this experiment, namely *Shorea leprosula, Shorea selanica, Shorea platyclados, Hopea odorata* and *Vatica sumatrana*. Cutting materials were used due to lack of seed availability. Shoot cuttings were produced by the nursery of Komatsu – Forestry Research and Development Agency (FORDA) project, Bogor, Indonesia, using KOFFCO techniques (Subiakto *et al.*, 2005). Cuttings were prepared and maintained in a glass-house for about 3 months. Root systems of the cuttings were randomly observed for free EcM colonization prior to transplanting to the experiment. Cuttings were planted into polypropylene containers containing treated soils or the controls in early March 2007. A total of 2,400 cuttings were planted. Some cuttings had produced new shoots at that time. Each treatment combination was placed on a separate bench, to avoid contamination as a result of soil splashing from regular watering.

Tree growth and mycorrhiza data

Height and stem diameter growth of the new shoots from the cuttings were recorded every month for 9 months. Due to un-uniformity of the growth of new shoots and mortality, only complete growth data were used for analysis, e.g. a 6 months period of observation for stem diameter and 9 months period of observation for height of new shoots.

After 9 months, all plants were harvested, making a total of 2,057 cuttings. The number of leaves was counted. Total biomass (including leaves, stems and roots) of cuttings was

recorded after drying the plant samples at 70 °C for 48 hours.

Prior to drying, the roots were washed in tap water to separate them from soil. We randomly selected root tips from about 20% of the harvested seedlings and these were spread into Petri dishes. The number of root tips and roots with EcM were counted under a dissecting microscope and expressed as a fraction of the whole sample. Confirmation of EcM colonization was obtained by examining a cross section of the root tips using a compound microscope and recording the presence of a mantle and a Hartig net (Brundrett *et al.*, 1996). The morphotype of the EcM was recorded according to Agerer (1987-1998).

Data analysis

The experiment was set up as a randomized factorial block design (Gomez and Gomez 1984; Steel and Torrie, 1960). In total there were 120 treatment combinations of temperature, drying, plant species and soil origin.

Basic statistical analyses were conducted using GenStat 9th Edition for windows (VSN International Ltd., U.K.). Data were checked for homogeneity of variance and normality by analysis of the residual. Data on growth in shoot height and stem diameter, number of leaves and biomass were $\log_{10}$ transformed. To test the effect of heating and drying on growth, we used general linear model with repeated measurement to RGRH and RGRD data. Due to mortality, we used 'unbalanced analysis of variance', using three factors, viz. temperature, drying and species; soil origin was used as a block to analyze data on biomass (dry weight of stems, leaves and roots) and EcM colonization. Fisher LSD tests were used for *posteriori* comparison of means (*posthoc*). Bench position was used as covariate.

Absolute growth rates (AGR) of height and stem diameter were calculated as the increment between two consecutive measurements six month apart. The relative growth rate of stem diameters (RGRD) was calculated as:

$$RGRD = \log_e D_2 - \log_e D_1 \qquad \text{(Eq. 1)}$$

where D_1 and D_2 are stem diameters measured at times 1 and 2, which were 1 month apart.

The relative height growth rate (RGRH) was calculated as:

$$RGRH = \log_e H_2 - \log_e H_1 \qquad \text{(Eq. 2)}$$

where $H_1$and H_2 are height at times 1 and 2, again 1 month apart.

Soil data were analysed using two-way analyses of variance (ANOVA), to test the effect of heating and drying on soil pH (H_2O), pH (KCl) and available P.

Results

Heat condition and soil properties

The final soil temperature did not reach the temperature that had been set in the oven (Fig. 1.). For example, soil temperature in the 150 °C treatment reached a highest value of 129 °C after 3 hours in the oven, before cooling down outside the oven.

Heating and drying treatments did have only a small positive effect on the pH and P availability in the soil. Heating affected pH measured in water, but not pH measured in 1 N KCl (Fig. 2).

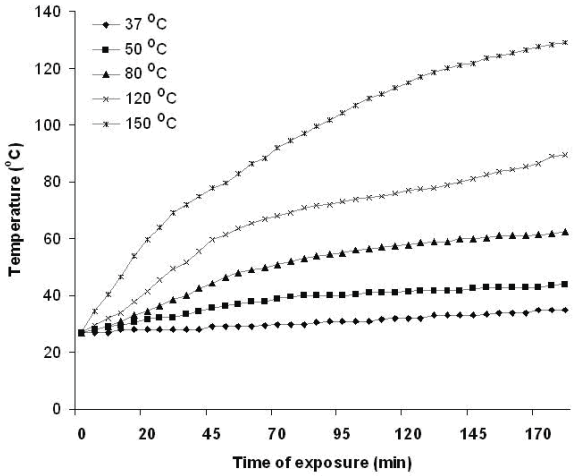

Fig. 1 Temperature profiles of soils during the heat treatments at different temperatures set to the oven.

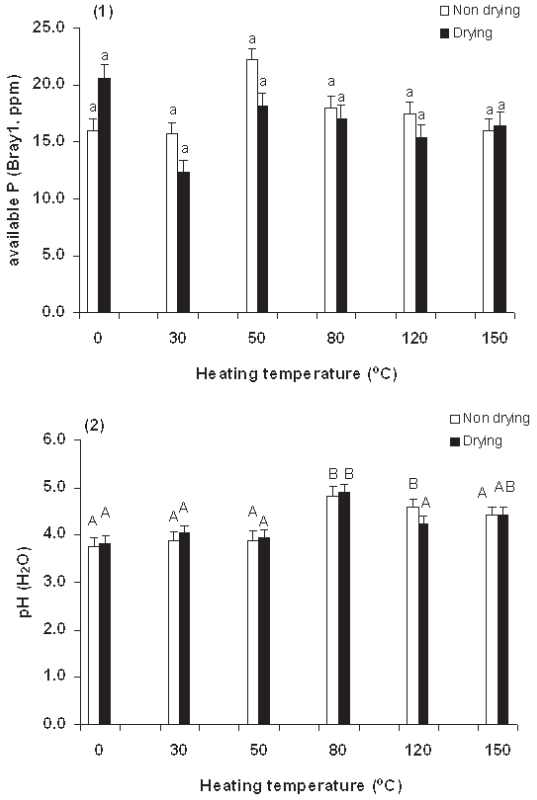

Fig. 2 Effect of heating and drying treatments on (1) available P (Bray1); (2) pH (H_2O). Different lower case letters indicate significant differences for available P; different capital letters indicate significant differences for pH (H_2O), according to LSD test at p≤0.05.

Growth of dipterocarp cuttings

Results of variance analysis (ANOVA) of heat and drying treatments, plant species and soil origin are shown in Table 1. Heat treatment significantly affected all response variables, and drying affected only RGRD and EcM colonization. RGRH and RGRD were significantly affected by interaction of all treatments and time of measurement. Position on the bench was included in the analysis of variance as a covariate, but did not significantly affect any variable measured.

The responses of plant species in terms of RGRH and RGRD varied among heat treatments (Fig. 3 and Fig. 4). *S. selanica, H. odorata* and *V. sumatrana* had higher RGRH and RGRD in the soils treated at 80 ^{o}C compared to other temperatures. In general, all five species planted in the controls had a low RGRD, and *V. sumatrana* had the highest RGRD in the 150 ^{o}C treatment.

Table 1 Probability of variance ratio (p value) of the data on relative growth rates of shoot height and stem diameter (RGRH and RGRD) for 8 and 5 observation periods, respectively for the factor of temperature (H), drying (D), plant species (S) and soil origin (O) and their interactions and time of observation (T).

	Variable response						
Source of variation	**RGRH** (cm month^{-1})	**RGRD** (mm month^{-1})	**Number of leaves**	**DW leaves (g)**	**DW stems (g)**	**DW roots (g)**	**EcM colonization (%)**
Temperature (H)	***	***	***	***	***	***	***
Drying (D)	ns	*	ns	ns	ns	ns	**
Plant Species (S)	ns	***	***	***	***	***	***
Soil origin (O)			ns	ns	ns	ns	**
Time (T)	***	***					
HxD	***	***	ns	***	***	***	***
HxS	***	***	*	*	***	*	**
DxS	***	ns	**	ns	ns	ns	**
HxDXS	***	*	*	ns	ns	*	**
TxH	***	***					
TxD	***	**					
TxS	***	***					
TxHxS	**	***					
TxDxS	ns	**					
TxHxDxS	*	**					

Asterisks denote significant value: *** p<0.001, ** p<0.01, * p<0.05, ns: not significant

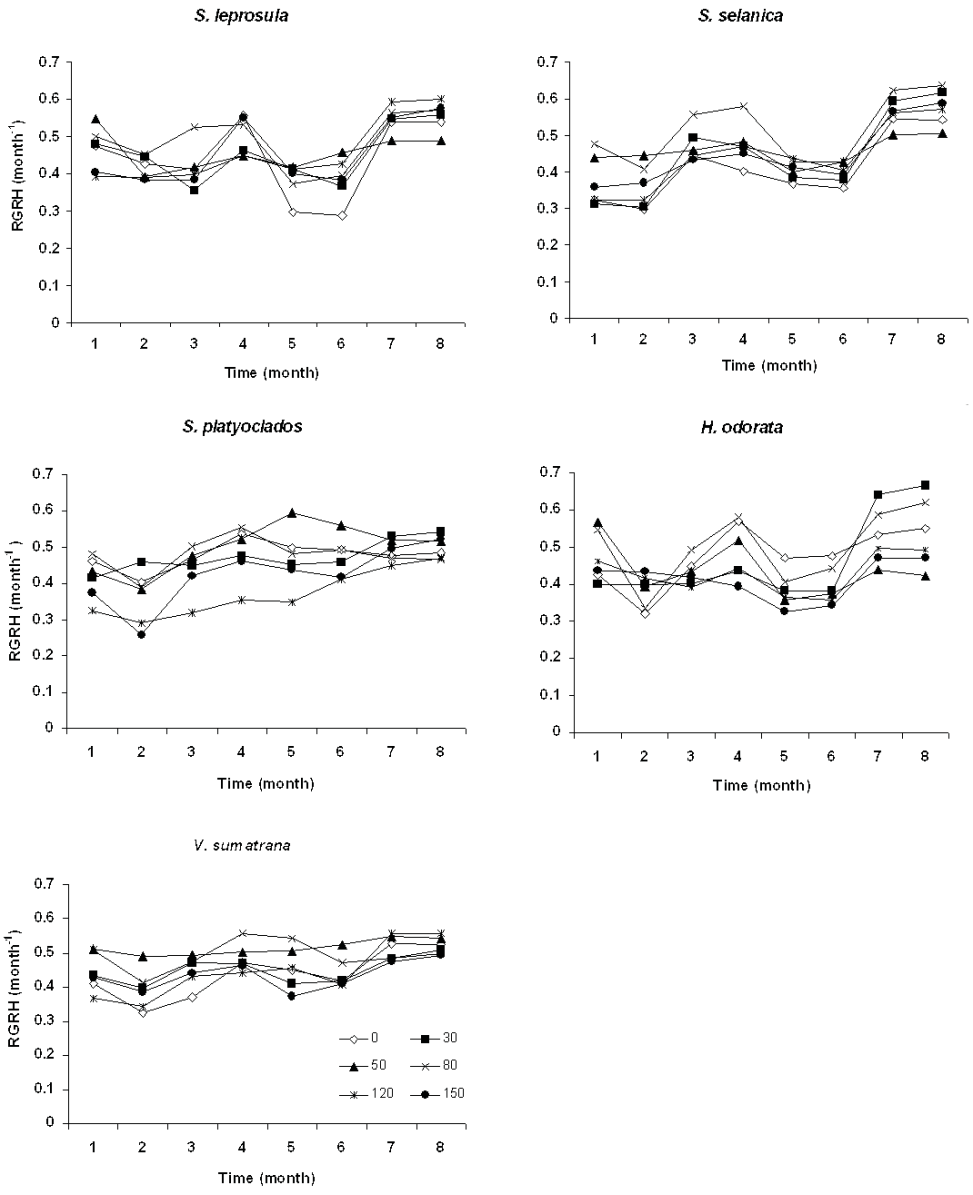

Fig. 3 Relative growth rate of height (RGRH) of *S. leprosula, S. selanica*, *S. platyclados, H. odorata* and *V. sumatrana* at six levels of heat treatment in the nursery for 9 months.

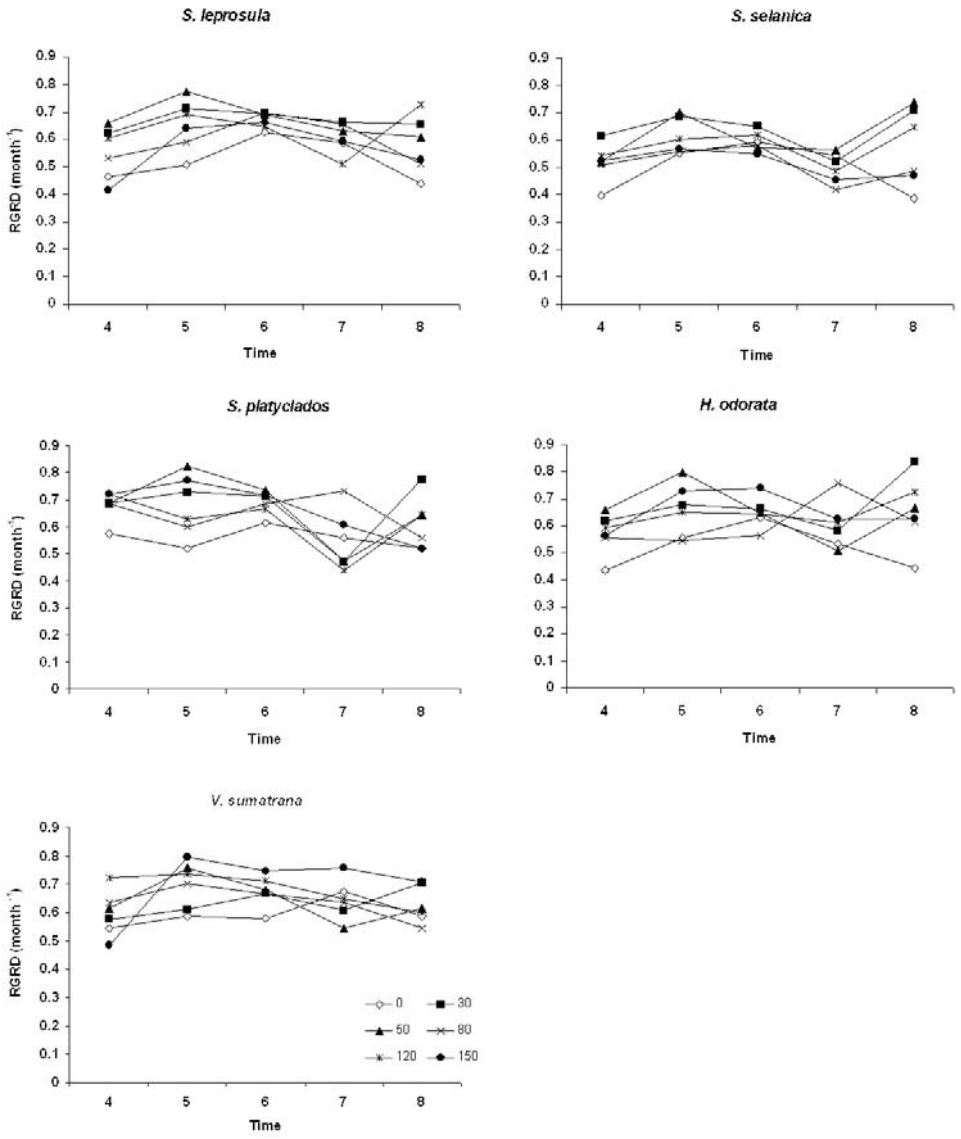

Fig. 4 Relative growth rate of diameter (RGRD) of *S. leprosula, S. selanica, S. platyclados, H. odorata* and *V. sumatrana* at six levels of heat treatment in nursery for 9 months.

Biomass and number of leaf

Heat treatment affected the DW of leaves, stems, and roots and the number of leaves, but the drying treatment did not. Interaction of 2 factors, e.g. heat (H) and drying (D), affected biomass, but not the number of leaves. In general, all five species planted in the control treatment had higher DW of stems, leaves and roots, whereas, all species planted in soil preheated at 150 ^{o}C had higher leaf numbers (Table 2).

Table 2 Response of five Dipterocarp species on leaf number, dry weight (DW) of leaf, DW stem, DW root to heat and dry treatments. Values of each variable followed by same letter are not significantly different at $p<0.05$ according to LSD test. DW value is log10 transformed. D: drying treatment, ND: non-drying treatment, s.e.: standard error.

	Temp-	*S. leprosula*				*S. platyclados*				*S. selanica*				*H. odorata*				*V. sumatrana*			
Variable	erature (°C)	D	s.e.		ND	s.e.		D	s.e.		ND	s.e.		D	s.e.		ND	s.e.		D	s.e.		ND	s.e.	
DW roots (g)	0	0.29	0.06	b	0.36	0.06	bc	0.4	0.06	c	0.26	0.06	ab	0.35	0.06	c	0.41	0.06	a	0.29	0.07	b	0.38	0.06	bc	0.32	0.07	bc	0.26	0.06	ab
	30	0.18	0.06	ab	0.24	0.06	ab	0.18	0.06	ab	0.24	0.06	ab	0.19	0.06	ab	0.21	0.06	ab	0.2	0.09	ab	0.22	0.07	ab	0.25	0.06	ab	0.25	0.06	ab
	50	0.28	0.06	b	0.31	0.06	b	0.2	0.06	ab	0.31	0.06	b	0.26	0.06	ab	0.35	0.06	bc	0.17	0.08	ab	0.38	0.07	bc	0.21	0.06	ab	0.3	0.06	b
	80	0.36	0.06	bc	0.17	0.06	ab	0.27	0.06	ab	0.14	0.06	ab	0.38	0.06	bc	0.14	0.06	a	0.43	0.09	c	0.17	0.09	ab	0.38	0.06	bc	0.13	0.06	a
	120	0.26	0.06	ab	0.18	0.06	ab	0.15	0.06	ab	0.13	0.06	a	0.16	0.06	ab	0.18	0.06	ab	0.2	0.07	ab	0.16	0.08	a	0.1	0.06	a	0.16	0.06	ab
	150	0.22	0.06	ab	0.34	0.06	bc	0.13	0.06	a	0.17	0.06	ab	0.26	0.06	ab	0.23	0.06	ab	0.2	0.07	ab	0.24	0.08	ab	0.14	0.06	a	0.16	0.06	ab
DW_stem (g)	0	0.49	0.05	bc	0.5	0.05	bc	0.57	0.04	c	0.43	0.05	b	0.53	0.04	c	0.57	0.05	c	0.56	0.05	c	0.58	0.05	c	0.58	0.05	c	0.58	0.05	c
	30	0.23	0.04	a	0.33	0.05	ab	0.29	0.05	a	0.44	0.05	b	0.42	0.05	b	0.4	0.05	b	0.39	0.07	b	0.48	0.05	bc	0.5	0.07	c	0.5	0.05	c
	50	0.39	0.05	b	0.43	0.05	b	0.34	0.05	ab	0.44	0.04	b	0.5	0.05	c	0.57	0.04	c	0.42	0.06	bc	0.59	0.05	c	0.39	0.06	ab	0.57	0.05	c
	80	0.47	0.04	bc	0.27	0.05	a	0.4	0.04	b	0.31	0.05	a	0.43	0.04	b	0.25	0.05	a	0.43	0.07	bc	0.34	0.07	b	0.53	0.07	c	0.36	0.07	ab
	120	0.4	0.05	b	0.3	0.04	a	0.31	0.05	ab	0.28	0.05	a	0.43	0.05	b	0.41	0.05	b	0.4	0.06	b	0.39	0.06	b	0.39	0.06	ab	0.41	0.06	b
	150	0.39	0.04	b	0.42	0.05	b	0.22	0.05	a	0.28	0.05	a	0.47	0.05	bc	0.48	0.05	bc	0.42	0.05	bc	0.44	0.06	b	0.48	0.05	bc	0.4	0.06	b
DW_leaf (g)	0	0.52	0.06	bc	0.53	0.05	bc	0.58	0.05	c	0.42	0.05	b	0.63	0.05	d	0.68	0.05	de	0.46	0.05	b	0.48	0.05	bc	0.7	0.06	d	0.67	0.05	d
	30	0.27	0.07	a	0.35	0.06	a	0.25	0.05	a	0.45	0.05	b	0.44	0.05	b	0.48	0.05	bc	0.29	0.06	a	0.47	0.05	bc	0.48	0.05	bc	0.59	0.05	cd
	50	0.43	0.07	b	0.3	0.06	a	0.3	0.05	a	0.25	0.05	a	0.45	0.05	b	0.47	0.05	bc	0.34	0.05	ab	0.45	0.05	bc	0.51	0.05	bc	0.57	0.05	cd
	80	0.47	0.07	b	0.39	0.08	ab	0.44	0.05	b	0.3	0.05	a	0.43	0.05	b	0.39	0.05	ab	0.45	0.05	bc	0.34	0.05	ab	0.59	0.05	cd	0.56	0.05	cd
	120	0.39	0.06	ab	0.47	0.07	b	0.35	0.05	ab	0.28	0.05	a	0.45	0.05	b	0.46	0.05	b	0.4	0.05	b	0.44	0.05	b	0.41	0.05	b	0.52	0.05	bcd
	150	0.41	0.06	b	0.49	0.06	bc	0.27	0.05	a	0.33	0.05	ab	0.51	0.05	bc	0.49	0.05	bc	0.34	0.05	ab	0.43	0.05	b	0.45	0.05	b	0.47	0.05	bc
Leaf number	0	5.7	0.04	ab	4.6	0.04	a	8.9	0.03	de	8	0.04	cd	6.1	0.04	b	6.1	0.04	b	7.4	0.04	c	8.1	0.04	cd	6.7	0.04	b	6.9	0.04	bc
	30	5.9	0.03	b	6.6	0.04	b	10.9	0.04	ef	10.4	0.04	ef	6.6	0.04	b	6.5	0.04	b	7.9	0.05	c	9.8	0.04	e	6.8	0.04	bc	7.1	0.04	bc
	50	6.2	0.04	b	6.8	0.04	bc	11.2	0.04	f	10.8	0.04	ef	7.4	0.04	c	7.1	0.04	bc	8.2	0.05	cd	10.2	0.04	ef	7.2	0.04	c	7.2	0.04	c
	80	6.8	0.03	bc	7	0.04	bc	14.3	0.04	ghi	13	0.04	gh	7.7	0.04	c	7.5	0.04	c	8.9	0.05	de	10.5	0.06	ef	7.6	0.04	c	8.1	0.04	cd
	120	7.1	0.04	bc	7.6	0.04	c	15.1	0.04	i	13.2	0.04	gh	8.1	0.04	cd	8	0.04	cd	9.8	0.04	e	11.8	0.05	fg	7.8	0.04	c	8.6	0.04	d
	150	8.3	0.04	cd	7.9	0.04	c	16.4	0.04	j	14.9	0.04	hi	8.5	0.04	d	8.1	0.04	cd	10.6	0.04	ef	12.2	0.05	fg	9.2	0.04	de	9.3	0.04	de

EcM colonization

Heat and drying treatments affected EcM colonization; interaction among treatments also affected EcM formation (Table 1). Colonization of EcM in the control treatment was higher than in other soil heating treatments, but the effect was smaller than expected (Tables 3 and 4).

We found four morphotypes of EcM among all treatments and all dipterocarp species. Morphotype 1, i.e. monopodial to regular pinnate, mantle colour white to white brownish, is the commonest type in all treatments and dipterocarp plants. There was no significant effect of heat and dry treatments on the relative frequency of each morphotype (Fig. 5).

Table 3 Mean EcM colonization at five levels of heat treatment.

Temperature (°C)	EcM colonization (%)
0	58.3 (1.27) a
30	45.7 (1.34) c
50	47.9 (1.31) c
80	50.5 (1.36) b
120	41.2 (1.31) d
150	46.4 (1.30) c

Note: Value in brackets is standard error. Values followed by same letter are not significantly different at $p<0.05$ according to LSD test.

Table 4 Mean EcM colonization and standard errors (s.e.) on five species of Dipterocarpaceae planted in the soils treated with five levels of heat and drying treatments. Values in one column followed by same letter are not significantly different at $p<0.05$ according to LSD test.

Temperature (°C)	Drying treatment	*S. leprosula*		*S. platyclados*		*S. selanica*		*H. odorata*		*V. sumatrana*	
		Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
0	D	60.4	4.1 a	59.2	4.2 ab	73.9	3.9 a	60.7	3.9 a	63.6	3.8 a
	ND	51.8	3.8 ab	57.6	4.2 ab	52	4 bc	59.4	4.2 a	45.2	4 bc
30	D	44.5	3.8 c	43.4	5.6 c	49.2	3.9 bc	33.4	4.5 d	46	3.9 bc
	ND	47.3	3.9 bc	63.4	4.4 a	46.4	3.8 c	45.7	4.1 b	42.3	4.6 cd
50	D	49.9	3.8 ab	54	5.1 bc	46.4	4.3 c	35.9	4.3 c	38.4	3.9 de
	ND	52.7	3.9 ab	70.2	4.7 a	58.1	3.9 b	40.2	4 c	41.8	3.9 d
80	D	59.7	4.1 a	71.1	5.3 a	57.5	3.8 b	55.1	4 ab	54.1	3.7 ab
	ND	45	4.4 c	56	6 bc	44.4	4.5 cd	32	4 de	38.5	3.9 de
120	D	38.8	4.2 c	57.8	5.2 ab	42.7	4.1 cd	21	4 e	41.7	4 d
	ND	44.9	3.9 c	50.3	4.6 c	34	3.7 d	45.2	3.9 b	42.9	4 cd
150	D	48.7	4 bc	54.3	4.7 bc	51.8	4 bc	44.2	4 bc	49.1	3.9 b
	ND	48.7	3.9 bc	51.7	5.1 c	50.6	3.9 bc	39.9	4 c	29.7	4 e

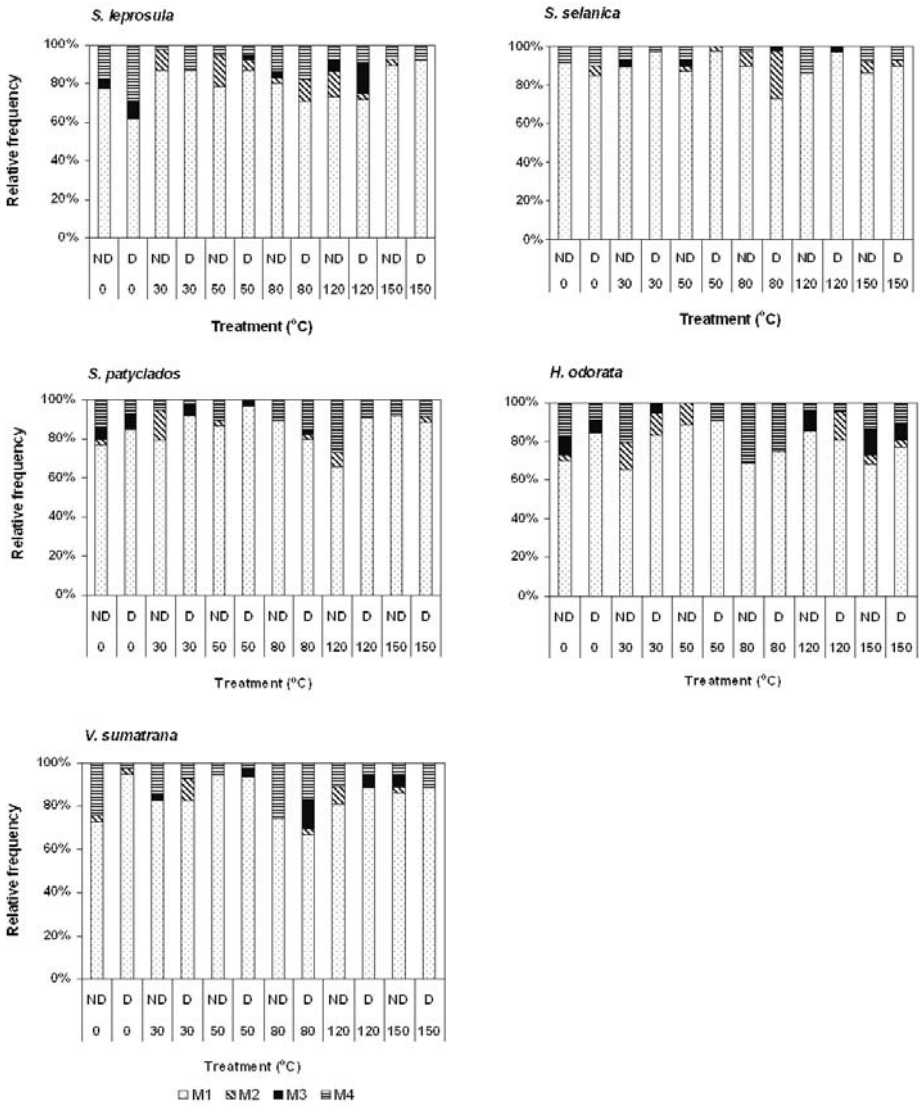

Fig. 5 Relative frequency of EcM morphotype on five dipterocarp species in heat and dry treated soils. Numbers indicate relative proportion of the EcM morphotype across all seedlings from the same species within one treatment. M1: monopodial to regular pinnate, mantle colour white brownish, external mycelia white; M2: irregular pinnate, mantle colour brown; M3: monopodial to regular pinnate, mantle colour greyish; M4: monopodial, mantle colour black.

Discussion

The heat and drying treatments in our study had little effect on available P in the soil. It has been reported that heat treatment tends to increase the content of available P in the soil and increases soil pH. During burning, litter and vegetation biomass are converted into ash which enriches the nutrient content and increases soil pH (Chandler *et al.*, 1983; Ketterings *et al.*, 2002). This may favour initial growth of dipterocarp seedlings after fire, before fungi colonize the roots.

The growth responses (RGRH and RGRD) of five dipterocarp species to heating and drying treatments differed over time. In general, *S. selanica, H. odorata* and *V. sumatrana* grew better in soil preheated at 80 ^{o}C.

Heating and drying treatments decreased EcM colonization of the dipterocarps. The control treatment showed a higher EcM colonization. This correlated with higher DW of leaves, stems and roots of the five dipterocarp species planted in the control treatment as compared to the treated soils. EcM has been reported to enhance nutrient uptake and increase of biomass of Dipterocarp trees (Turjaman *et al.*, 2006b) and this was also found in *Quercus ilex* (Fagaceae) (de Román *et al.*, 2005) and *Pinus radiata* (Liu *et al.*, 2008). Other studies, however, showed that fire increased EcM colonization on pine seedlings (Izzo *et al.*, 2006; Herr *et al.*, 1994). This result may have been due to the fact that the fire heated up to just 75 ^{o}C and this may have acted as a signal for temperature-resistant EcM spores, such as *Rhizopogon olivaceotinctus,* to germinate (Izzo *et al.*, 2006)

In general, the heating and drying treatments had little effect on the relative frequency of EcM morphotypes present in the five dipterocarp species. Morphotype M1 was encountered on all Dipterocarp species. It seems that M1 is a fungus with a high resistance to heat and dry treatment.

Ectomycorrhiza identification based on direct sequencing by Blast analysis, yielded at least three genera of EcM fungi colonizing the roots of the dipterocarps planted in the soil treated with heat up to 150 ^{o}C, viz. *Tomentella, Laccaria* and *Inocybe*. Four endophyte fungi were also encountered in the Dipterocarp cuttings, e.g. *Pestalotiopsis* sp.1, *Fusarium* sp. 6, *Muscodor* sp. and *Cosmospora vilior.* Detailed information about fungal identification is given in chapter 6. The commonest genus is *Tomentella*. Only one taxon, i.e. *Tomentella* sp. 1, is identical with EcM fungi identified *in-situ*. This suggests that abundent EcM fungi of the natural soil, have been killed by the heating and drying treatments, and the dipterocarp cuttings in this nursery study have been recolonized by other EcM and endophytic fungi.

Understanding the ecological effects of fire in Sumatra, where it has been used extensively for land clearing, is important, particularly the relationship between fire and the occurrence of EcM fungi. Fungi act as pioneer colonizers of forests and other land use types derived from forest following disturbance by fire. Particularly these so-called Phoenicoid fungi (Carpenter and Traper, 1985) which establish after disturbance by fire, play an important role in the nutrient cycling and immobilization in fire-disturbed ecosystems and contribute to accelerated plant growth and survival (Herr *et al.*, 1994).

Acknowledgements

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CHAPTER 5

Limited response to nursery-stage ectomycorrhiza inoculation of *Shorea* seedlings planted in rubber agroforests in Jambi, Indonesia

With M. van Noordwijk, M.J.A. Werger and R.C. Summerbell

(under revision)

Abstract

We transplanted *Shorea selanica* and *S. lamellata* seedlings that either had or had not received ectomycorrhiza (EcM) *Scleroderma columnare* inoculum in nursery into rubber gardens of different age and plot history. The objective was to assess whether or not absence of fungal inoculants restricted seedling survival, growth, nutrient uptake and EcM formation in the first 2 years after out-planting in Jambi. Inoculation with EcM fungi in nursery had only limited positive effects on growth in height and diameter or N and P uptake, but it enhanced survival in the period 6 – 24 months after outplanting in all plots. Presence of up to 5 morphotypes of EcM confirmed the availability of inoculum also in second generation rubber agroforests. With or without nursery stage inoculation, *S. selanica* and *S. lamellata* can be used for enrichment planting or reforestation in Sumatra as the species respond well to high light intensities.

Keywords*: Agroforestry, Dipterocarpaceae, Enrichment planting, Rubber agroforest,* Scleroderma columnare, *Sumatra*

Introduction

The natural vegetation in the lowland rain forest of Western Indonesia consists of mixed dipterocarp rain forest (Whitten *et al.*, 2000; Laumonier, 1997). The Dipterocarpaceae is a family of 17 genera and approximately 500 species worldwide. It is well known for valuable timber trees traditionally used in residential construction, as well as for essential oils, balsam and resins (Soerianegara and Lemmens, 1994). Under product names such as meranti, dipterocarps form the basis of Indonesia's timber and plywood industry. In domesticated forests, resin- (*dammar-*) producing dipterocarps are of local importance in Southwest Sumatra (Torquebiau, 1985; Michon, 2005).

In natural forests, dipterocarp trees form tall, cylindrical boles and characteristically dominate the canopy. The presence of wings on the seeds does not allow the large (11 to 59 mm diameter; Krishnapillay & Tompsett, 1998) seeds to disperse far; dispersal for the most part is not more than 50 m from the mother tree (Osada *et al.*, 2001). Seeds germinate directly, but this normally only occurs within the soil invested by roots of the mother tree (Tompsett, 1998); the irregular seed production and difficulties of seed storage (absence of dormancy) are constraints to silvicultural use of the species. Dipterocarps typically are shade tolerant and able to establish themselves under limited light availability.

The trees have an EcM association with fungi from either Basidiomycetes or Ascomycetes (Smits, 1992; Smits, 1994, Lee *et al.*, 1997; Wang and Qiu, 2006). Among the Basidiomycetes, Russulaceae tend to dominate and can be studied by collecting their fruiting bodies (Smits, 1994; Lee *et al.*, 1997; Ingleby *et al.*, 1998). The Ascomycetes have only become identifiable though molecular taxonomic techniques (Kovacs and Jakucs, 2006; Tedersoo *et al.*, 2006) and remain less well known. Some dipterocarp species potentially have dual associations, in that they may form both ecto- and endomycorrhiza (Tawaraya *et al.*, 2002).

Studies on the relationship between Dipterocarpaceae and EcM fungi in East Kalimantan showed that association with EcM fungi is necessary for early growth of dipterocarp seedlings, and that, in the nursery, inoculation may be necessary (Smits, 1994; Yasman, 1995; Priadjati, 2002; Omon, 2002). Direct contact with soil containing dipterocarp tree roots under 'a mother tree' in the nursery assists in the subsequent growth of dipterocarps used in enrichment planting of natural forest (Yasman, 1995; Alexander *et al.*, 1992). Survival of dipterocarp seedlings proved to be more dependent on EcM presence than on light intensity and soil properties (Yasman, 1995).

After its introduction from Brazil at the end of the 19th century, the rubber tree *Hevea brasiliensis* became a major component of the local economy of the lowland forest zone in North Sumatra (Tengwall, 1945), compatible with trees from the local flora in agroforest or 'jungle rubber' forms of land use (Gouyon *et al.*, 1993; Joshi *et al.*, 2003; Murdiyarso *et al.*, 2002). The extensive management allows other trees to grow naturally from the seed bank and from newly dispersed seeds. The plant diversity in rubber agroforests is high, while the tree composition differs only in detail from that of the natural forest (Rasnovi, 2006; Beukema *et al.*, 2007).

Enrichment planting with valuable timber species in a rubber agroforest (RAF) context is an option that has yet to be explored. It has potential to meet the challenge of satisfying local demands for timber, but it is not yet widely practiced. RAF allows for a diverse floristic composition and creates the appropriate microclimate for late successional species, such as members of the Dipterocarpaceae. However, *H. brasiliensis* itself is not ectomycorrhizal, nor are many of the trees that grow in RAF with it, and this raises the concern that the effort to plant with dipterocarps may be constrained by the absence of EcM fungi in RAF soil. In this study *Shorea selanica* and *S. lamellata* seedlings were transplanted into rubber gardens of different age and condition, in order to:

1. Assess whether fungal inoculant increased the survival, growth, nutrient uptake and ectomycorrhiza formation of *S. selanica* and *S. lamellata* seedlings in the first 2 years after out-planting.
2. Assess the effect of RAF site history (time since conversion of rainforest to rubber agroforest) and condition on seedling survival, growth and nutrient uptake.
3. Identify constraints and other factors affecting growth of the two *Shorea* species tested in the RAF system.

Material and methods

Study site

Five rubber gardens were studied. They differed in the age of the rubber trees and in the history of cultivation. Specifically, rubber gardens of 1, 5 and >10 years, derived directly from forest, and rubber gardens of 1 and 5 years, derived from mature RAF, were selected based on Landsat 7 ETM satellite imagery and on the interpretation of local land use changes in the past two decades. We used the ICRAF series of data readings from satellite imagery for Jambi from 1973-2002 (Ekadinata and Vincent, 2005) as well as later data from 2004. A remnant natural mixed dipterocarp forest (MDF) in Tebo district (belonging to the Silvagama education forest, Gadjahmada University), was used as a 'forest control' site. Geo-coordinates for each site were obtained using a Garmin GPS.

The Bungo and Tebo Districts are located in Jambi Province, Indonesia (101°52'-102°20' E and 1°30' – 1°48' S, 50-250 m a.s.l.), and lie on the undulating to flat basin areas of the Batang Bungo, Batang Pelepat and Batang Tebo rivers (Fig. 1), which are tributaries of the Batang Hari river. The area was selected as benchmark for the global 'Alternatives to Slash and Burn' program and details of land use and land use change are documented (van Noordwijk *et al.*, 1995; Murdiyarso *et al.*, 2002). The mean annual precipitation (in 2000-2006) in Tebo was 2893 mm (cf. Sepunggur - ICRAF's climate station), while in Bungo district it was 3014 mm (cf. Muara Kuamang – ICRAF's climate station) with a pronounced dry season from May to September. During the study period of 22 months, the total amount of precipitation was 3678 mm and 5341 mm (in Sepunggur and Muara Kuamang, respectively). Rainfall above 700 mm month^{-1} occurred in January and April 2005 in Muara Kuamang; rainfall around or below 100 mm month^{-1} data in the June – August period of both years.

The soils at the Bungo and Tebo sites are very deep, well drained, and very acid, and they have a low soil fertility status. Dominant soil types according to United States Department of Agriculture (USDA) soil classification are Oxisol, Kandiudox and Tropofluvent (van Noordwijk *et al.*, 1995).

Seed germination and media preparation

Seeds from two species of dipterocarps were collected in the Arboretum of the Forest Research and Development Agency (FORDA), Bogor, Indonesia, in late September 2004. *Shorea lamellata* Foxw grows in undulating land and low hills and has medium size nuts (ca. 10-12 x 9-11 mm) (Newman *et al.*, 1996; Ashton, 1982); its wood is classified in the timber trade as a white light hardwood meranti. *Shorea selanica* Bl grows naturally in lowland forest on well drained land with fertile soils and also has medium size nuts (ca. 15 x 8 mm) (Ashton, 1982); its wood belongs to the group of red light meranti.

The seeds of *S. lamellata* and *S. selanica* were sown in sterilized mixed coco peat and rice husk (1:1) that had been autoclaved (121 °C for 30 min). After the seeds had germinated (about 4 weeks after sowing), the seedlings (two leaves and cotyledons present) of both *Shorea* species were transferred into a nursery at Babeko village, Bungo District,

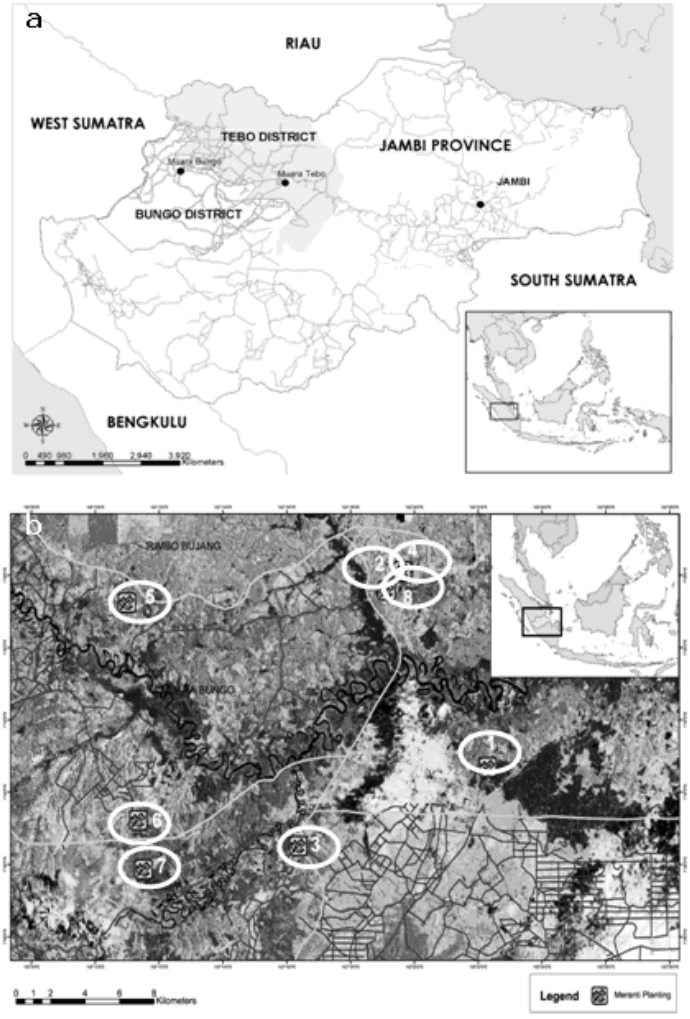

Fig. 1 a. Study area in the lowland of the Bungo and Tebo districts, Jambi Prov., Indonesia. b. Land cover map based on satellite imagery of Landsat 7 ETM: (1) Original mixed dipterocarp forest; (2&6) rubber gardens 1 to 2 yrs old, derived from forest; (4&5) 5 yr old rubber gardens derived from forest; (3) >10 yr old rubber garden derived from forest; (7) 1 yr old rubber gardens derived from RAF; (8) 5 yr old rubber gardens derived from RAF.

Media sterilisation and EcM fungi inoculation

The medium in which the seedlings were planted had been prepared prior to seedlings transfer. We used the top soil of rubber gardens mixed with rice husk (2:1). The mixed medium was sterilized chemically, using Basamid G (Dazomet 97%, Hoechst GmbH., Frankfurt, Germany) in a dosage of 100 g m^{-3} medium, in a closed plastic container. The medium was incubated for 2 weeks.

Seedlings were transferred into the sterilized medium in plastic bags of 1000-1200 cm^3 each. A day after transplanting, a single 0.4 g tablet containing spores of the EcM fungus *Scleroderma columnare*, produced by the Laboratory of Forest Microbiology, Forest and Nature Conservation Research and Development Centre, Bogor, Indonesia (Turjaman *et al.*, 2005;

Turjaman *et al.*, 2006b), was inoculated into the potted seedling near the roots. The seedlings were watered regularly and maintained in the nursery for three months. Control seedlings were not inoculated. One thousand and twenty seedlings of each species were grown and distributed over the plots in the research sites with 6 different histories (see below).

Experimental design

Experimental plots were set up according to a split plot block randomized design in a factorial experiment (Gomez and Gomez, 1984; Steel and Torrie, 1960), with tree species as plots and inoculation levels as subplots. The histories of the six sites were:

(1) History 1 (L1 or Forest): natural mixed diptercarp forest (MDF), dominated by Dipterocarpaceae species, such as *Dipterocarpus crinitus, Shorea bracteolata, S. palembanica, S. acuminata* and *S. gibbosa*;

(2) History 2 (L2 or ExFo_1): rubber age 1 to 2 yrs, site derived from forest; (3) History 3 (L3 or ExFo_5): rubber age 5 yr, site derived from forest;

(3) History 4 (L4 or ExFo_10): rubber age >10 yr, site derived from forest;

(4) History 5 (L5 or ExRAF_1): rubber age 1 yr, site derived from previous RAF; at least 40 years since clearance from natural forest; and

(5) History 6 (L6 or ExRAF_5): rubber age 5 yrs, site derived from previous RAF and about 50 years since clearance from natural forests.

At each site two experimental plots of about 1 ha each were selected to serve as replicates. Within each plot four quadrats of 240 m^2 were established; the quadrats were at least 15 m apart. In each quadrat 80 seedlings were transplanted, either from *S. lamellata* or from *S. selanica*. In one of the quadrats of each species (randomly selected), seedlings inoculated in the nursery were used, while in the remaining plots uninoculated seedlings were used. The seedlings were planted between the rubber trees at distances of 3 to 5 m away from those trees.

Plot management

Eight farmers from 5 different villages, viz. 4 farmers in the Bungo district (Babeko, Dusun Danau, Muara Kuamang and Koto Jayo villages); and 4 farmers in the Sei Srumpun village (Tebo district), collaborated in the study (Figure 1). The farmers managed the plots according to their normal practice; areas around the planting holes were cleared at planting time in January 2005 and the planting rows were weeded twice a year after that.

Field assessment

Survival and growth of planted seedlings

Height and stem diameter at 5 cm above ground level of the seedlings were recorded immediately at planting time. Survival and growth were monitored every 6 months for 2 years and the probability of survival over each measurement interval was estimated from the survival fractions.

Light availability

Light availability in the study plots was measured using a Lux meter (Extech light meter) at midday on a single occasion in February 2007. Each subplot was measured once; therefore each block consisted of 4 measurements. It was expressed as the ratio of the light in the quadrat to the light in an open area (in%).

Occurrence of animal disturbance and Imperata cylindrical

The intensity of animal disturbance, mainly due to wild pigs (*Sus scrofa*) on planted seedlings, was recorded on a single occasion, as was of the degree to which *I. cylindrica* had invaded the quadrats. Disturbance by animals was ranked into two numerical classes: 0 (no disturbance), or 1 (disturbed). Presence of *I. cylindrica* was ranked as 0 (none), 1 (rare) or 2 (abundant).

Harvest

Twelve months after planting, 552 seedlings of the two *Shorea* species were harvested by systematic sampling (this amounted to ca. 20% of the seedlings surviving in each quadrat). The number of leaves was counted. Total biomass (including leaves, stems and roots) of seedlings was recorded after drying the plant samples at 70 °C for 48 hours. The concentrations of total N and P in the leaves was determined by means of the Kjeldhal and Bray1 methods, respectively, in the laboratory of Soil Chemistry, Centre for Soil and Agroclimate Research (CSAR) in Bogor, Indonesia.

Prior to drying, the roots were washed under tap water to separate them from soil. We randomly selected root tips from about 20% of the harvested seedlings and these were spread into Petri dishes. The number of root tips and roots with EcM were counted under a dissecting microscope and expressed as a fraction of the whole sample. Confirmation of EcM colonization was obtained by examining a cross section of the root tips using a compound microscope and recording the presence of a mantle and a Hartig net (Brundrett *et al.*, 1995). The morphotype of the EcM was recorded according to Agerer (1987-1998).

Soil analysis

Soil samples were collected randomly from the top soil (0-15 cm depth) in each quadrat and mixed to obtain a composite for every block. In total, 12 soil samples were analyzed in the laboratory of Soil Chemistry, CSAR, in Bogor, Indonesia. The soil samples were analyzed for texture (sand, silt, clay), pH (in a 1:25 soil : solution extract with water or 1 N KCl), PBray1, Corg (Walkley and Black), Ntotal (Kjeldhal), exchangeable K, Ca, Mg, Na (exchanged with 1N NH4-acetate solution pH 7) and exchangeable Al and H (exchanged with a 1 N KCl solution). The effective cation exchange capacity (ECEC) was obtained by summation of these cations. Initial soil conditions were assessed in May 2005. The reference organic C (C-ref) content for forest soils was calculated using a regression equation derived from a large data set for Sumatra (van Noordwijk *et al.*, 1997):

$$C_{ref} = (SampleDepth\_cm/7.5)^{-0.42} * EXP(1.333 + 0.00994 * clay\% + 0.00699 * silt\% - 0.156 * pH(KCl) + 0.000427 * elevation\_masl) \quad \text{(Eq. 1)}$$

Data analysis

Basic statistical analyses were conducted using GenStat 9th Edition for windows (VSN International Ltd., U.K.). Data were checked for homogeneity of variance and normality by analysis of the residual. Data on survival, growth in height and stem diameter and number of leaves were log10 transformed, while EcM colonization was log10 transformed after adding 1 unit. The N and P concentrations of the shoots were subjected to analysis of using the GLM procedure of Statistica ver.6 (StatSoft Inc., U.S.A.).

Absolute growth rates (AGR) of height and stem diameter were calculated as the increment between two consecutive measurements six month apart. The relative growth rate of stem diameters (RGRD) was calculated as:

$$RGRD = \log_e D_2 - \log_e D_1 \quad \text{(Eq. 2)}$$

where D_1 and D_2 are stem diameters measured at times 1 and 2, which were 6 month apart.

The relative height growth rate (RGRH) was calculated as:

$$RGRH = \log_e H_2 - \log_e H_1 \quad \text{(Eq. 3)}$$

where $H_1$and H_2 are height at times 1 and 2, again 6 month apart.

Four specific contrasts (Table 1) where tested in the ANOVA within the 5 degrees of freedom of the 'land history/condition' factor: 1) reflecting the time (on a logarithmic scale,

scaled back to achieve a mean of zero), 2) reflecting the current light level, 3) the primary texture-related soil variables and 4) the first (ex Forest) or second (ex RAF) agroforest cycle. Occurrence of animal disturbance and *I. cylindrica* were used as covariates for tree response parameters.

Table 1 Specific contrasts of land history for (A) soil properties and (B) relative growth rates in height and diameter (RGRH and RGRD, respectively) of *Shorea lamellata* and *S. selanica* 2 years after planting at each site.

Contrasts	A. Contrast of soil properties			B. Contrast of growth (RGRH and RGRD)	
Land history	1. Time since conversion level	2. Current light level	3. 'Sand minus clay' factor	1. Time since conversion level	2. Current light level
1 = Forest	-2.51	-0.53	0.77	-9	-4
2 = ExFo_1	-0.51	0.57	1.3	-6	4
3 = ExFo_5	0.19	-0.23	0.05	1	-2
4 = ExFo_10	0.49	-0.13	1.01	3	-1
5 = ExRAF_1	1.15	0.37	-5.6	5	3
6 = ExRAF_5	1.19	-0.05	2.47	6	0
Sum	0	0	0	0	0

Note: Land history codes refer to previous vegetation ('ex forest', 'ex RAF') and age of rubber trees at time of planting of *Shorea* (1, 5 and 10 years)

Results

Light availability and soil properties

Light availability varied among the six study sites. The lowest light intensity occurred in the forest (2.7%), while the highest was found in site type L2, forest-derived plots with 1 yr old rubber seedlings (49.4%).

All sites had very acid soils of low nutrient content, with the highest pH (as measured in KCl extracts) of 4.1 recorded for site L2.2 (1-yr-old rubber seedlings in RAF derived from forest – plot 2) and the lowest value of 3.5 for the forest (L1). The textures ranged from sandy clay loam to clay. Sand content generally was high (49-71%), except in both plots of site type L5 (1-yr-old rubber seedlings in RAF derived from earlier RAF), where values ranged from 16-22%.

Table 2 analyzes the soil properties for the three contrasts, to test for possible confounding factors in subsequent interpretation of tree growth. The relative soil C content (C/Cref) was close to 1.0 in the forest (L1) at 0.92±0.01 and was lower in RAF plots derived from forest 0.66±0.04 or in second-generation RAF plots 0.63±0.10. The TimeSinceConversion contrasts were associated, as expected, with a decline in the C/Cref ratio (but not with organic C without correction for texture via the reference value) and with an increase in pH (as measured in KCl extracts) and an associated decrease in exchangeable Al^{3+}. They were also associated with a decrease in the fraction of the soil made up by silt and ECEC/Clay.

The contrast CurrentLightLevel was associated with decrease in silt and ECEC/clay and with an increase in pH (in KCl extracts) but not with a decrease in exchangeable Al^{3+} or with relative Al saturation. The soil texture contrast termed the 'sand minus clay' factor was negatively associated with the organic C and N content of the soil as well as with available P and exchangeable Mg^{2+} and K^{+}. It was not associated with C/Cref or soil pH. The total Fe content and the exchangeable Ca^{2+} content in the soil varied considerably among sites (0.9 to 5.8%, and 0.2 to 2.6 $cmol^{+}kg^{-1}$, respectively) but neither factor was associated with any of the three contrasts. The forest and forest-derived plots had high Al saturation (mean 72%). Second generation rubber agroforest plots tended towards lower aluminum saturation (Fig. 2).

Table 2 Soil properties (grand means and standard errors of the mean [S.E.M.]) and their association with the three contrasts described in Table 1A. as evaluated by the ratio of the total contrast and the mean, the slope of the regression line and the percentage of variance accounted for in a linear regression line (r^2).

			1) TimeSinceConversion			2) Current light level			3) 'Sand minus clay' factor		
Variable	Grand Mean	S.E.M.	Contrast1/ mean	Slope	r^2	Contrast2/ mean	Slope	r^2	Contrast3/ mean	Slope	r^2
Sand (%)	54.5	5.3	-0.1	-3.3	0.06	0	-14.9	0.11	0.8	6.4	1
Silt (%)	7.7	0.9	-0.4	-1.9	0.69	-0.1	-4.4	0.32	0.2	0.2	0.03
Clay (%)	37.8	5.5	0.2	5.2	0.14	0.1	19.4	0.17	-1.2	-6.6	0.99
pH (H_2O)	3.6	0.07	0.02	0.05	0.09	0	-0.05	0.01	0.02	0.01	0.01
pH (KCl)	3.8	0.05	0.04	0.09	0.41	0.01	0.31	0.44	-0.01	0	0
Organic C (%)	2.4	0.25	-0.1	-0.15	0.05	0.01	0.24	0.01	-0.72	-0.24	0.63
C/Cref	0.69	0.05	-0.18	-0.08	0.38	-0.01	-0.05	0.01	-0.21	-0.02	0.11
N	0.16	0.02	-0.08	-0.01	0.04	0.02	0.02	0.02	-0.68	-0.02	0.62
C/N	14.5	0.2	-0.03	-0.24	0.22	-0.002	-0.21	0.01	-0.05	-0.11	0.2
$P_{available}$ (Bray1, ppm P_2O_5)	15.5	2.8	-0.02	-0.16	0	0.04	4.2	0.03	-0.97	-2.15	0.4
Exchangeable cations ($cmol^+kg^{-1}$)											
Ca	0.83	0.2	-0.03	0	0	-0.04	-0.25	0.02	-0.11	-0.11	0.22
Mg	0.48	0.08	0.35	0.11	0.27	0.06	0.2	0.08	-0.06	-0.06	0.36
K	0.1	0.01	0.2	0.01	0.17	0.06	0.04	0.16	-0.01	-0.01	0.57
Na	0.01	0.01	-0.72	-0.01	0.2	0.12	0.01	0.06	0	0	0
Al3+	2.1	0.24	-0.34	-0.45	0.54	-0.05	-0.75	0.13	-0.01	-0.01	0
H+	0.43	0.03	-0.18	-0.05	0.41	-0.02	-0.05	0.05	0.003	0.003	0.01
ECEC	3.9	0.32	-0.16	-0.39	0.24	-0.03	-0.8	0.09	-0.19	-0.19	0.24
ECEC/Clay	0.12	0.01	-0.34	-0.03	0.56	-0.09	-0.07	0.42	0.01	0.01	0.34
Al Saturation	0.65	0.05	-0.13	-0.05	0.17	-0.001	-0.01	0	0.02	0.02	0.13
Fe total (%)	2.4	0.5	0.04	0.07	0	0.13	2.2	0.3	-0.31	-0.31	0.26

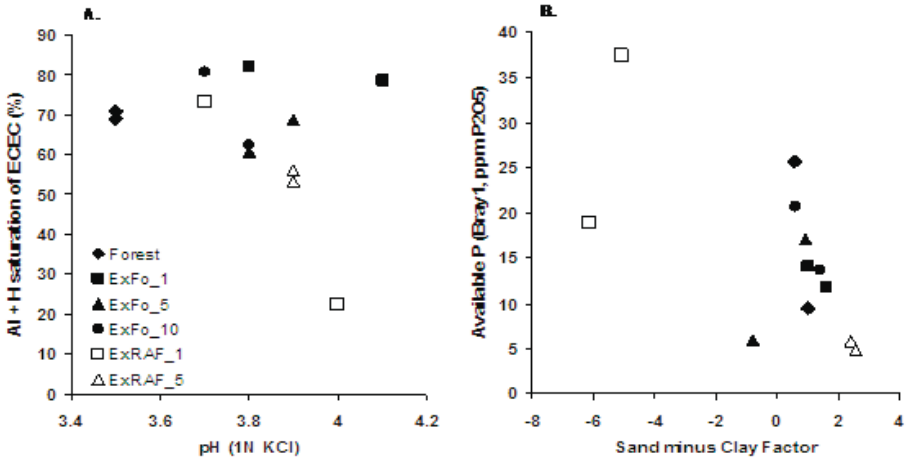

Fig. 2 (A) Relationship between pH and Al saturation in the top soil of the 6 plots types; (B) Relationship between the 'sand minus clay' factor and Pavailable at all sites. ExFo_1 yr old rubber trees, plot derived from forest; ExFo5: 5 yr old rubber trees, plot derived from forest; ExFo10: >10 yr old rubber trees, plot derived from forest; ExRAF_1: 1 yr old rubber trees, plot derived from RAF; ExRAF_5: 5 yr old rubber trees, plot derived from RAF.

Survival rate

Mortality was high between 6 and 18 months for both *S. selanica* and *S. lamellata*. During two years of observation, the survival rate of both seedlings inoculated with EcM was higher than that of the uninoculated seedlings, except for *S. lamellata* planted in L6 (ExRAF_5: RAF 5 years old derived from forest). After two years, the highest survival rate was seen in inoculated *S. lamellata* and *S. selanica* planted in L4 (ExFor_10: RAF >10 years old derived from forest), while the lowest survival rate was seen with the uninoculated *S. selanica* and the inoculated *S. lamellata* planted in L6 (ExRAF_5).

The ANOVA (Table 3) and means (Table 4) showed that the *Shorea* species used did not affect the survival rate, while EcM inoculation yielded a significant effect only at the third period of measurement. The history/condition of the plots affected the survival of the two *Shorea* species. The interaction of EcM inoculation (E), history/condition of land (L) and species (T) was significant only at the fourth period of measurement.

Table 3 Variance ratios (F) of the data on survival and relative growth rates of height and stem diameter (RGRH and RGRD) for four observation periods for the factors Tree species (T), EcM inoculation (E), land use history/condition (L), the two primary contrasts in L (L1 and L2) and their interactions (only columns with significant interactions are shown).

Response Variable		Tree (T)	EcM (E)	History/ condition of land (L)	L1	L2	TxE	ExL	TxL1	TxL2	TxExL
Survival fraction (%)	1	1.5	1.7	3.1*			0.62	0.06	0.26		0.38
	2	3.1	6.5	7.3***			1	0.67	1		0.92
	3	0.16	19.7*	3.2*			1.4	0.33	0.32		2.4
	4	0.2	0.5	8.6***			10.3	4.5**	4.5**		4.0*
RGRH (cm month^{-1})	1	915.3*	16.6	9.7*	0.46	0.33	15.8	4.3**	0.54		0.91
	2	17.4	29.7*	44.6**	<0.001***	<0.001***	2.8	0.68	2.4		2.7*
	3	24	0.79	5.9**	0.33	0.13	0.5	1	1.4		0.03
	4	17.2	0.52	3.0*	0.59	0.36	0.28	1.2	0.97		0.42
RGRD (mm month^{-1})	1	37	7.2	7.6***	0.27	0.16	0.09	3.0*	1.1	0.151	1.6
	2	0.07	21.7*	38.8***	0.002**	<0.001***	0.05	0.32	1.4	0.22	0.29
	3	2.2	0.22	9.0***	0.08	0.05*	0	0.54	0.63	0.02*	0.36
	4	288.2*	0.02	0.81	0.94	0.83	2.6	1.7	1.1	0.71	0.3
No. of leaves		1.8	22.2*	9.7*			1.66	0.73	0.58		1.2
DW_leaves (g)		7.9	20.5*	12.0***			2.6	0.74	0.61		1.7
DW_ stems (g)		39.9	11.9	20.8***			1.1	0.77	0.84		1.1
DW_roots (g)		0.74	20.0*	29.7***			1.5	1	1.2		1.8
S:R ratio		62.3	33.3*	2.6			14.4	0.79	0.25		0.53
EcM colonization (%)		87.5	32.2*	0.82			1.8	1.4	2.3		0.21
df of F		(1,1)	(1,2)	(5,20)	(1,20)	(1,20)	(1,2)	(5,20)	(5,20)	(1,20)	(5,20)

Asterisk denotes significant value: * at the $p < 0.05$, ** at the $p < 0.01$, *** at the $p < 0.001$. Analyses were made on log-transformed data for survival fraction, RGRH, RGRD, number of leaves, DW_leaves, DW_stems, DW roots, and S:R ratio and on 1+log-transformed for EcM colonization

Table 4 Means of tree response parameters for the main effects to the land history (L) and inoculation (EM) factor were identified. L1: mixed dipterocarp forest, L2: 1 yr old rubber trees, plot derived from forest, L3: 5 yr old rubber trees, plot derived from forest; L4: >10yr old rubber trees, plot derived from forest; L5: 1 yr old rubber trees, plot derived form RAF; L6: 5 yrs old rubber trees, plot derived from RAF.

Parameter	Time	*S. lamellata*	*S. selanica*	L1	L2	L3	L4	L5	L6	EcM (-)	EcM (+)
Survival	1	5.8	5.7	6.1	4.9	5.8	6	6	5.7	5.7	5.8
fraction (%)	2	4.5	4.6	4.4	3	4.6	6.1	4.8	4.3	4.4	4.6
	3	4.9	4.8	4.4	5.3	4.8	5.4	5.7	3.3	0.3	5.8
	4	6.3	6.5	7	7.9	4.9	5.9	6.9	5.5	6.3	6.4
RGRH	1	0.12	0.16	0.05	0.22	0.19	0.16	0.13	0.1	0.12	0.16
(cm month^{-1})	2	0.23	0.25	0.06	0.3	0.17	0.18	0.54	0.2	0.21	0.27
	3	0.15	0.14	0.06	0.23	0.15	0.12	0.19	0.13	0.14	0.15
	4	0.13	0.1	0.08	0.13	0.11	0.13	0.11	0.12	0.11	0.12
RGRD	1	0.18	0.23	0.14	0.28	0.27	0.23	0.15	0.17	0.2	0.21
(mm month^{-1})	2	0.15	0.16	0.04	0.18	0.08	0.07	0.43	0.12	0.14	0.17
	3	0.13	0.11	0.05	0.17	0.13	0.08	0.18	0.13	0.12	0.12
	4	0.07	0.06	0.05	0.08	0.07	0.07	0.07	0.06	0.06	0.06
No. of leaves		17.5	17.5	5.6	23.2	16.5	9.3	37.3	13.3	11.7	23.3
DW_leaves (g)		4.9	4.8	1.3	6	4.8	2.8	10.1	4	3.5	6.2
DW_stems (g)		7.4	5.7	1.5	6.9	5.4	3.6	18	4.1	4.8	8.3
DW_roots (g)		4.5	4.1	1.5	4.3	3.7	3.4	10.2	2.9	3.5	5.1
S:R ratio		2.6	2.5	2.2	3	2.5	1.7	2.6	3.2	2.3	2.7
EcM colonization (%)		4.7	4.1	4.2	4.5	4.7	3.6	4	5.5	4.7	4.2

Relative growth rate of height (RGRH) and stem diameter (RGRD)

Tree growth varied primarily between sites (L), with few differences between the tree species (T) and effects of inoculation (E) (Tables 3, 4). Contrasts L1 and L2 contributed most to the L effect and the ex-Forest versus ex-RAF contrast were not statistically significant. The longer the time since forest clearing and the higher the light availability, the higher RGRH and RGRD in period 2 (1 yr after planting). Interaction between land history and tree species was statistically significant only in 2 out of 16 comparisons. No statistically significant effect was noted for interaction between land history and the impact of EcM inoculation on tree growth.

A statistically significant interaction between EcM inoculation and land history/condition was found at the first measurement of RGRH and the last of RGRD, but the trend seen was not in the direction hypothesized: the positive inoculation effect was smallest rather than largest in the plots with the longest history of cultivation since forest clearing. The three-way interaction of species, EcM inoculation and land history/condition was significant for RGRH only at the second period of measurement.

S:R (Shoot:Root) ratio, number of leaves, and biomass

Seedlings planted at site L5 (1 yr old rubber on a plot derived from RAF) had the highest number of leaves and biomass. After one year, the S:R ratio, the dry weight of leaves and roots and EcM colonization were affected by EcM inoculation, while history of land or associated light levels significantly affected number of leaves and biomass, e.g. dry weight (DW) of leaves, stems and roots. No interaction effect was shown for all response variables. The effect of EcM inoculation on the S:R ratio varied among sites and between species. EcM inoculation did

not always increase the S:R ratio. Inoculation with EcM increased the number of leaves and biomass (DW of leaves, stems and roots) of *S. lamellata* and *S. selanica* planted at all sites, except for *S. lamellata* at L6.

Concentration of Nitrogen and Phosphorus

ANOVA results showed that history/condition of sites and the interaction between site history/ condition and EcM inoculation significantly effected shoot nutrient (N and P) concentration. EcM inoculation alone did not affect shoot N and P concentration of the two *Shorea* species. In L5, N and P concentration of uninoculated seedlings was higher than that of inoculated seedlings (Table 5). Seedlings in L6 had the lowest N concentration while those in L4 had the lowest P concentration.

Table 5 Shoot nitrogen and phosphorus concentration of two *Shorea* species uninoculated and inoculated with EcM in series of RAF at 12 MAP. L1: mixed dipterocarp forest, L2: 1 yr old rubber trees, plot derived from forest, L3: 5 yr old rubber trees, plot derived from forest; L4: >10yr old rubber trees, plot derived from forest; L5: 1 yr old rubber trees, plot derived form RAF; L6: 5 yrs old rubber trees, plot derived from RAF.

	Concentration of elements (%)			
	N		P	
Site	Ecm not inoculated	EcM inoculated	Ecm not inoculated	EcM inoculated
L1	1.7 ± 0.1 d	1.4 ± 0.3 ab	0.07 ± 0.01 pq	0.06 ± 0.01 p
L2	1.6 ± 0.3 cd	1.6 ± 0.1 cd	0.08 ± 0.01 q	0.09 ± 0.01 qr
L3	1.5 ± 0.2 bc	1.7 ± 0.2 d	0.06 ± 0.01 p	0.07 ± 0.02 pq
L4	1.6 ± 0.0 cd	1.6 ± 0.1 cd	0.05 ± 0.01 p	0.05 ± 0.01 p
L5	1.7 ± 0.3 d	1.7 ± 0.2 d	0.11 ± 0.02 r	0.10 ± 0.02 r
L6	1.2 ± 0.2 a	1.3 ± 0.2 a	0.06 ± 0.01 p	0.08 ± 0.02 q

Letters denote significant differences in performance among EcM inoculation treatments for two *Shorea* species at the $p < 0.05$ level, based on LSD test. Treatments followed by the same letter are not significantly different.

EcM colonization

The effect of EcM inoculation on EcM colonization varied among treatments. EcM colonization of inoculated seedlings was not always higher than uninoculated seedlings. At the age of 12 MAP, uninoculated *S. selanica* planted at L1 had no EcM colonization, while uninoculated *S. selanica* planted at L6 had high EcM colonization. EcM colonization of uninoculated *S. lamellata* planted at L1 showed moderate EcM colonization in the forest plot. We found five EcM morphotypes on *S. selanica* and *S. lamellata* roots. Morphotype T1, featuring simple monopodial to regularly pinnate branching and brownish mantle colors, was very common in seedlings planted at L1, L2, L3, L4 and L6. Morphotype T5, showing irregular clumpy branches, black mantle colour, and black emanating hyphae, was very common in seedlings planted at L5. The frequency of each morphotype in relation to the main plot contrasts is shown in Fig. 3. Inoculation did not lead to a systematic increase of any of the EcM morphotypes. The 'sand minus clay' factor was associated with an increase in morphotype 1 and 3 and a decrease in morphotype 5. The prevalence of morphotype 5 increased with 'current light' and increasing 'time since forest conversion.'

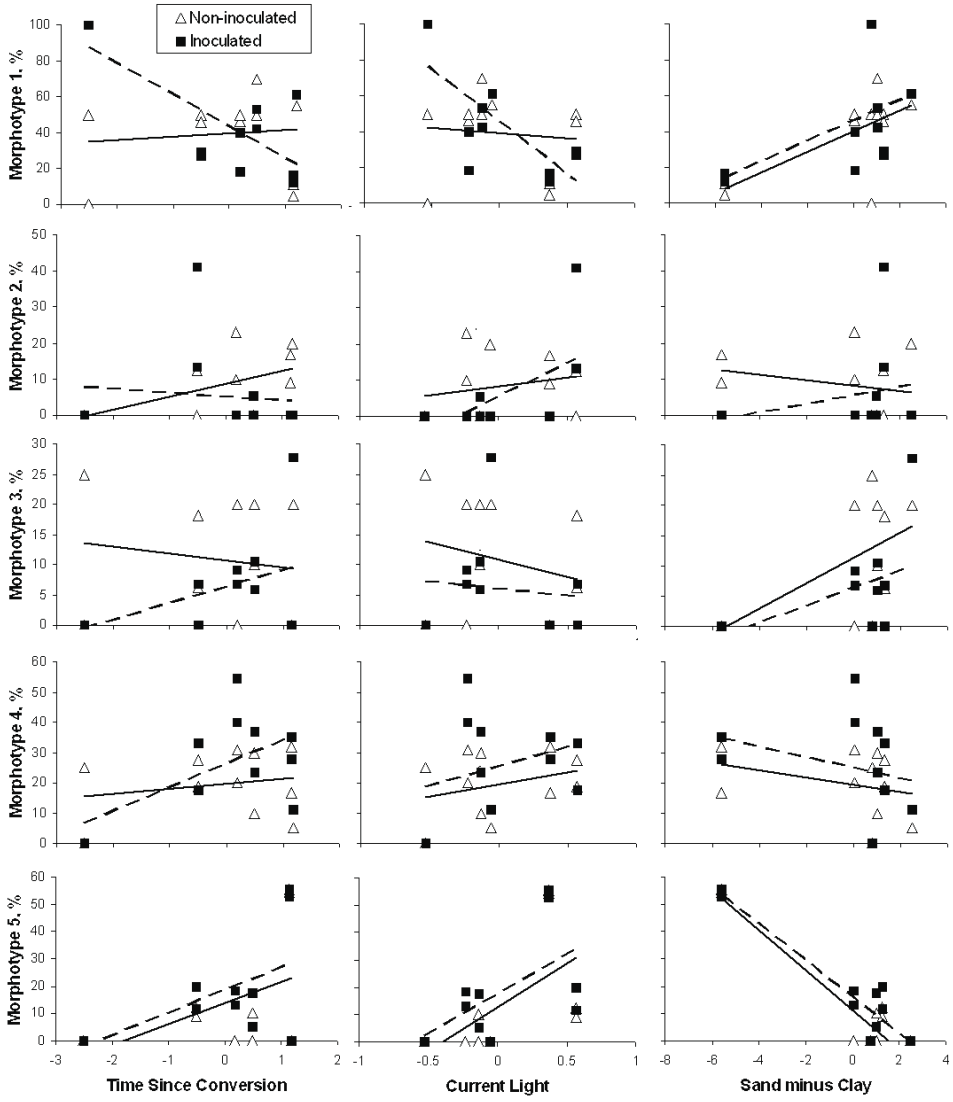

Fig.3 Morphotypes of EcM in relation to three contrasts of the land history/condition for inoculated and non-inoculated trees of the two *Shorea* species; T1: monopodial regular pinnate, mantle colour: white brownish; T2: simple unramified, mantle colour: white brownish; T3: irregular dichotom, mantle colour: white and brownish; T4: Monopodial irregular pinnate, mantle colour: white and brownish; T5: Irregular coralloid, mantle colour: black and black emanating hyphae.

Discussion

The limited response of survival and growth to inoculation and the abundance of EcM of different morphotypes in all locations where *S. lamellata* and *S. selanica* were outplanted was unexpected and very different from what has been reported so far for early growth of dipterocarp species (Smits, 1994; Priadjati, 2002; Tennakoon *et al.*, 2005; Turjaman *et al.*, 2005, 2006b, 2007). Our results challenge, at least for lowland Sumatra, the concept that lack of EcM is a primary constraint on the use of dipterocarps for enrichment planting.

Land history and light condition effects

Our results indicated that early survival of *S. lamellata* and *S. selanica* was influenced by land history more than by EcM inoculation. The 'current light conditions' factor had more impact on survival and growth than did 'land history since forest conversion.' The two *Shorea* species grew very slowly in the forest plot (L1), but they did manifest a positive response to inoculation in this environment. The canopy of L1 was dense, and light availability was low; these conditions may be unsuitable for the growth of the test species. Seedlings of both species grew better in the open areas, such as L2 and L5 (1 year old rubber plantation derived from forest and RAF, respectively). Previous studies have shown growth of dipterocarp seedlings (mainly *Shorea*) to be enhanced in well illuminated conditions (Tennakoon *et al.*, 2005; Brearley *et al.*, 2007) and to show a positive correlation with gap distance (Otsamo, 2000).

Regarding EcM colonization, land history did not have a significant effect. Both *Shorea* species planted in the relatively exposed conditions of RAF generally showed higher EcM colonization than those planted in the L1 forest site. Previous reports have shown better mycorrhization for several species of dipterocarp seedlings when they are planted under high light conditions in an open canopy rather than under closed canopy (Ingleby *et al.*, 1998; Tennakoon *et al.*, 2005; Brearly, 2007). Gehring (2004) found a similar effect of light intensity on mycorrhization of *Chrysophyllum* (Apocynaceae) seedlings. This suggests that higher levels of photosynthesis in more open areas support mycorrhization while a dense forest canopy limits carbohydrate availability in tree seedlings and thus also limits EcM formation and function (Ingleby *et al.*, 1998; Bücking and Heyser, 2001).

High light availability in RAFs did not decrease EcM colonization or the number of EcM types. Ingleby *et al.* (1998) similarly found that under a relatively open canopy, *S. parvifolia* seedlings had higher EcM colonization and more diverse EcM than those found under a closed canopy. They found that many EcM types present in a more open canopy were not encountered on seedlings under a closed canopy. Our result showed that the mycobiont of morphotype 5 (e.g. ramification irregular pinnate and coralloid; mycorrhizae and emanating hyphae black) appeared to be well adapted to growth on seedlings experiencing high light availability in more open areas. A high frequency of morphotype 5 was observed in L5 and L2, but not in L1. We suspect that the mycobiont of morphotype 5 occurs indigenously in the soils of Bungo and Tebo districts. According to Agerer (1987-1998), we suspect the morphotype 5 was belonging to *Cenococcum geophillum*. *C. geophillum* is a multi-stage fungi which is more abundance in jack pine stands (Visser, 1995) and *Quercus ilex* stands (de Román and de Miguel, 2005) following wildfire. *Cenococcum* forms thick-walled sclerotia (Agerer, 1987-1998). This form, as viable resting structures enable them to survive in the soil after slashing-burning has been applied by farmers during land preparation to open the rubber garden.

EcM inoculation effect

Positive effects of EcM inoculation in the nursery were observed mainly at the end of the first year. Beyond that point, EcM inoculation had no effect on the growth and survival of either test species. Similar results for endomycorrhizal inoculation were reported by Murniati (2002), who found positive effects of nursery-stage inoculation on initial survival but then detected no influence on subsequent growth of four endomycorrhizally associated tree species planted in *Imperata* grassland in East Kalimantan. Jones and Smith (2004) concluded that mycorrhiza formation does not always increase plant fitness (e.g. survival and growth), but this depends on the specific plant and fungal genotypes and abiotic and biotic environments.

Our study showed no effect of EcM inoculation on growth and survival in the second year. No effect of EcM inoculation could be related to rainfall in this study. Precipitation in the Bungo and Tebo Districts in the second year (2006) was lower than in 2005 (Appendix 1). As reported by Valdes *et al.* (2005) and Swaty *et al.* (2004), drought reduced EcM colonization on pine trees (*Pinus oaxacana* and *P. edulis*) and altered EcM species community composition on the roots of *P. edulis* (Swaty *et al.*, 2004). They suggested that the reduction of EcM

colonization that occurs during drought could be beneficial for the plant, as it could reduce the carbon cost of EcM fungi during times when photosynthesis is limited because of limited water availability.

In contrast to findings of Turjaman *et al.* (2006a, 2006b, 2007), inoculation of dipterocarps with EcM in the nursery stage did not increase shoot N and P tissue concentration or total uptake in our experiment. Jones and Smith (2004) suggested that transfer of P from the fungus to the plant does not necessarily mean that the plant will accumulate more P or is able to grow faster, because the fungus gains a high proportion of the P, while there is no overall increase of P in the plant. Site L5, which had the highest available P content in the soils, yielded the highest P concentration in the shoots for both uninoculated and inoculated seedlings. In any event, phosphorus relations may have little influence in general on the degree of mycorrhization. Indeed, the fact that addition of phosphate fertilizer does not reduce EcM colonization (Brearly *et al.*, 2007; Baxter and Dighton, 2005) and EcM richness (Baxter and Dighton, 2005) has been cited as evidence that the EcM symbiosis is an obligate relationship, i.e., one not abolished by ideal nutrient conditions and therefore one not necessarily stimulated by nutrient deprivation.

EcM colonization of uninoculated *S. lamellata* at all sites and uninoculated *S. selanica* at some sites was relatively high compared with inoculated seedlings. This showed that EcM fungi persisted in the soil after the land use change from forest to RAF, allowing indigenous colonization of *S. lamellata* and *S. selanica* planted in the RAF. The same type of inoculum used in our study (i.e., *S. columnare*) was shown to be effective following nursery inoculation (Turjaman *et al.*, 2005, 2006b), and other types of *Scleroderma* spp. inoculum were shown to be effective elsewhere (Suhardi, 2000; Turjaman *et al.*, 2007). We can not exclude that other inoculum types could have been more effective. However, the diversity of morphotypes on seedling planted in RAF sites in our study appears to reflect the abundance of indigenous mycorrhizal inoculum, rather than the ineffectiveness of the nursery inoculation.

Plot management

In the establishment of smallholder rubber plantings in Jambi, the most significant practical problem noted was damage by vertebrate pests, such as wild pigs, (*Sus scrofa*), monkeys (*Presbytis melalophos nobilis*) and sheep (*Ovis aries*) (Williams *et al.* 2001). Wild pigs damaged *Shorea* planted in the RAF sites but there was no evidence of damage in MDF. When we used animal disturbance and presence of *I. cylindrica* as covariates, it did not affect the results.

We conclude that in lowland Sumatra or at least in Jambi, RAF provides suitable sites for enrichment planting with dipterocarp trees. Inoculation of EcM inoculum in the nursery provides a small increase in seedling survival rate but is not essential, since EcM inoculum potential persists in the soil after natural forest was changed to RAF. *S. selanica* and *S. lamellata* can be selected for use in enrichment planting, afforestation and reforestation in Sumatra under conditions where there is a partially open canopy.

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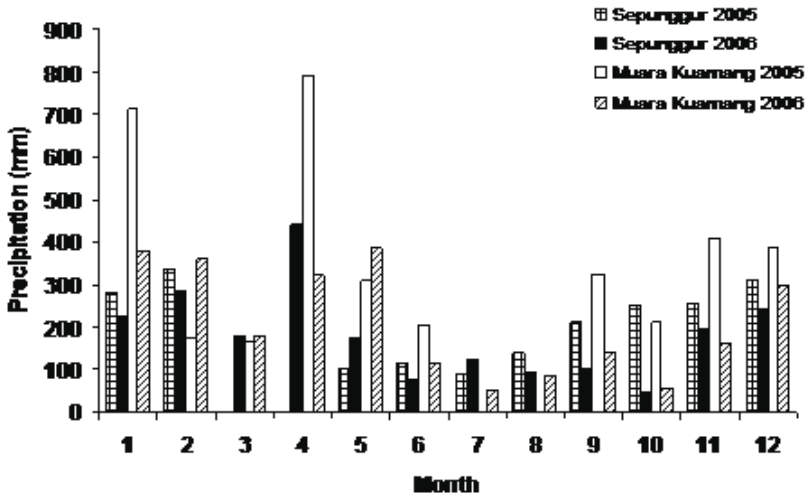

Appendix 1 Precipitation in climate station Sepunggur and Muara Kuamang, Bungo district, Jambi province, in 2005-2006.

CHAPTER 6

Molecular identification of mycorrhizal fungi of dipterocarp seedlings in Indonesian rubber agroforests

with G.S. de Hoog and R.C. Summerbell

Abstract

Little is known about ectomycorrhiza (EcM) communities in disturbed ecosystems, for example, after change from natural forests to other types of land use. In the presence study, sequencing of ribosomal DNA (rDNA) was applied to identify EcM fungi on root tips of several species of Dipterocarpaceae from three different experiments. In the first experiment, the EcM inoculum potential in the soil from seven land use types was studied, based on colonization of nursery-grown plants. In the second, EcM inoculum surviving heat and drought treatment of the soil was characterized. In the third, root tips for analysis were harvested in situ in rubber agroforest (RAF) with different histories. The first experiment yielded three genera of EcM fungi: *Tomentella, Laccaria* and an unidentified member of the Sclerodermataceae. In addition, an endophytic *Curvularia* was obtained. The second experiment yielded three genera of EcM fungi: *Tomentella, Laccaria*, and *Inocybe,* as well as four species of endophyte fungi: *Pestalotiopsis* sp., *Fusarium* sp., *Muscodor* sp., and *Cosmospora vilior*. The field experiment yielded 5 genera of EcM fungi: *Tomentella, Pisolithus, Clavulina, Sebacina* and *Sistotrema*. Isolation of mycorrhizal only yielded Ascomycetous fungi in experiment 1 and there was no overlap with the results of direct sequencing. EcM fungal community in dipterocarp seedlings comprise numerous species. Finally, this suggests EcM propagules persists in the soil after forests change to other land use type, such as RAF.

Keywords: *cloning, ectomycorrhizae, endophyte,* Hopea, *Internal Transcribed Spacer, ribosomal DNA,* Shorea*, Sumatra,* Vatica

Introduction

Tropical rain forests in Indonesia are dominated by trees belonging to the family Dipterocarpaceae, which produce a high economic value of timber. Some species also produce non-timber forest products, such as dammar resin and illepe nuts. Dipterocarp trees have been widely documented as forming symbiotic relationships with ectomycorrhizal fungi (EcM) (Ogawa, 1992; Smits, 1994; Lee *et al.*, 1997; Tedersoo *et al.*, 2007). The fungal species involved include diverse Basidiomycetes and Ascomycetes (Harley, 1972; Smith and Read, 1997). EcM fungi play important roles in nutrient and biogeochemical cycle in forests ecosystems (Smith and Read, 1997; Whitfield, 2007). Many reports have shown that EcM fungi increase the growth of dipterocarp seedlings (Tennakoon *et al.*, 2005; Turjaman *et al.*, 2005, 2006b, 2007; Brearley *et al.*, 2007), to suppress root diseases (Machon *et al.*, 2006) and increase drought tolerance (Palátová, 2002). They also play an essential role in forest succession (Janos, 1980; Allen *et al.*, 1995). Some EcM fungi are edible and may serve as a food source (Watling and Lee, 2007; Vikineswary *et al.*, 2007).

The diversity of ectomycorrhizal fungi in Indonesia has been partly characterized in undisturbed forests of East Kalimantan only, based on morphological sporocarp identification. Members of Russulaceae, Amanitae and Boletaceae were the three most common groups of EcM fungi recorded (Smits, 1994; Ogawa, 1992; Yasman, 1995). These groups , however, are known as late-stage EcM fungi in the succession of species colonizing trees as they become progressively older (Mason, *et al.*, 1983; Fleming, 1983). High deforestation rates in Indonesia have not only reduced the diversity of trees, but have also affected the diversity of fungal symbionts, since late stage mycobionts require mature trees. Forest disturbances, such as logging and fires, were shown to decrease the diversity of EcM fungi (Ingleby *et al.*, 1998; Grogan *et al.*, 2000; Nurjanto and Suhardi, 2001; Tata, *et al.*, 2003) and to shift the species composition of belowground EcM to decrease the proportion of Basidiomycota observed in relation to the proportion of Ascomycota (Jones *et al.*, 2003; Heinonsalo, 2004).

Current understanding of dipterocarp reforestation in Indonesia is constrained by a lack of knowledge about levels of symbiotic fungal inoculum after forest degradation; hence, in attempts to plant dipterocarps in disturbed habitats, prior inoculation with EcM propagules in the nursery may or may not be necessary. We do not know how the diversity and composition of EcM fungi in the soil may be affected by change from forest to other land use types, such as rubber agroforestry (RAF). To date, no study on the composition of below-ground EcM fungal communities in Indonesia has been reported.

During the past decades, polymerase chain reaction (PCR) and subsequent sequencing have been routinely used to identify EcM fungi. For some EcM mycobionts, sequences of the Internal Transcribed Spacer (ITS) regions have been published, allowing identification to species level. The identities of DNA types not recognized through this means can be estimated from examining the list of closely related taxa seen in BLAST searches of GenBank (Bruns *et al.*, 1998; Horton and Bruns, 2001). When comprehensively applied, the molecular approach also provides a clear understanding of the complexity of mycorrhizal communities and populations (Martin and Slater, 2007). Using a PCR and direct sequencing approach, we identified EcM fungi on roots of several Dipterocarpaceae. These studies were linked to those presented in chapters 3, 4 and 5. A subsample of the EcM roots analysed in chapter 3 were cultured in vitro and also examined in PCR analysis.

Our objective was to analyze the identities of EcM species occurring in three experiments, two experiments involving nursery cultivation and one coordinated with a planting trial in the field. Individual EcM roots of Dipterocarpaceae were analysed in each case. Fungal DNA was characterized by direct DNA extraction and ITS-PCR amplification from segments of mycorrhizal dipterocarp roots.

Methods

Plant material

Plant materials used for this study were collected during the course of three experiments, as described above and in earlier chapters. The number of samples used in molecular analyses is shown in Table 1.

In experiment 1, *Shorea lamellata* and *S. selanica* seedlings were planted in pots containing soils derived from different land use types, and were then cultivated in the nursery until being harvested 10 months after planting (MAP), (details given in chapter 3);

In experiment 2, cuttings were taken from potted nursery plants of *S. selanica*, *S. leprosula, S. platyclados, Hopea odorata* and *Vatica sumatrana*. Soils used in potting were treated by exposing them to a temperature of 150 °C for 3 hours and by drying them. Untreated soil was used as a control. Cuttings were examined at 10 MAP (detail information in chapter 4);

In experiment 3, *S. lamellata* and *S. selanica* seedlings were out-planted in rubber gardens of different land use histories, at 12 MAP (detail information in chapter 5).

Morphotyping

Root segments colonized by EcM were washed under tap water to remove soil debris and then washed with sterile water. After morphotyping as described in the previous chapters, up to 5 root segments of each morphotype, 1 cm in length, were taken from each root system and placed separately in 1.5 ml centrifuge tubes containing silica gel. They were labeled and stored in the freezer at -4 °C. These samples were transferred to the Centraalbureau voor Schimmelcultures (CBS), Fungal Biodiversity Centre, Utrecht, the Netherlands for further analysis.

Isolation into pure culture

Isolation into pure culture was established using subsequent EcM root tip samples from experiment #1 (chapter 3). A total of 132 EcM root samples were surface sterilized to allow culture of internally growing mycota. The washed roots were serially surface sterilized on 20 times 5 min in sterile water, and finally immersed in 100 µg/ml mercuric chloride ($HgCl_2$) for 3 min and then rinsed thoroughly in sterilized water. Five root tips from the same sample were plated on one plate with modified Hagem agar (5 g glucose, 5 g Malt Extract, 0.5 g NH_4NO_3, 0.5 g KH_2PO_4, 0.5 $MgSO_4.7H_20$, 1.0 g yeast extract, 20 g agar in 1000 ml deionized H_2O; amended with 100 mg/l tetracycline) and incubated at 25 °C in the dark (Summerbell, 1985). Mycelia that emerged from the roots were subcultured and maintained on Malt Extract Agar (MEA) for subsequent analysis. Putative EcM fungi were subcultured on Modified Melin Norkrans (MMN) agar (Marx, 1969).

For molecular identification, a total of 181 isolates of root colonizing fungi were extracted using the same method as described for direct sequencing from root tips, amplified and sequenced using forward primer ITS1 and ITS4 with same condition as mentioned above.

DNA extraction

DNA extraction from EcM root tips and from fungal isolates followed the protocol of Möller *et al.* (1992) with modifications. The internal transcribed spacer (ITS) region of the fungal nuclear ribosomal DNA (rDNA) was sequenced for all samples in Table 1. The starting material for extraction consisted of about 1 cm^2 of mycelium taken from 1 wk old culture on MEA, or 3 to 5 root segments of each morphotype from a single sampling code was transferred to a 2 ml screwcapped FastPrep tube containing glass beads (Sigma G9143) and 400 µl TE-extraction buffer (100 mM Tris, 40 mM Na-EDTA, pH adjusted to 9.0). Samples were homogenized for two times 3 min with TissueLyser (Qiagen Inc., Valencia, United State of America, USA). To this mixture, 120 µl of 10% sodium dedocyl sulfate SDS and 10 µl Proteinase K were mixed, and the tubers were vortexed. The mixture was incubated in a water bath at 55 °C for 30 min. The

mixture was then homogenized for two times 3 min with TissueLyser. The salt concentration was increased by adding 120 µl 5 M NaCl solution. The mixture was combined with 1/10 volume cetyltrimethylammonium bromide (CTAB) buffer 10%, followed by incubation at 55 °C for 60 min. Material was homogenized with TissueLyser for two times 3 min. One volume of mixture solution of cholorofom and isoamylalcohol (with ratio of 1:24, v/v) (SEVAG) was added and mixed gently by hand. After centrifugation at 14,000 rpm, 4 °C, for 5 min, the top layer was transfered into a new and sterilized Eppendorf™ tube. The sample was added with 225 µl 5M NH_4-acetate and mixed gently by inverting the tubes. After incubation for 30 min at 0 °C, the mixture was centrifuged. The supernatant was transfered into a clean Eppendorf tube and 0.55 of volume ice-cold isopropanol (ca. 510 µl) was added. The mixture was incubated at -20 °C for 60 min, followed by centrifugation at 4 °C at 14,000 rpm for 5 min. The supernatant was decanted and the pellet was washed with 1 x 1000 µl ice-cold 70% ethanol. The pellet was air-dried in a vacuum dryer (DNA 110 Speed Vac, GMI Inc., Minnesota, USA), for 10 min. The powder was resuspended in 100 µl Tris-EDTA buffer (pH 8.0). The DNA concentration was quantified with a NanoDrop™ 1000 spectrophotometer (Thermo Scientific, Wilmington, USA). The DNA samples were kept at -20 °C until use.

Table 1 Number of sample, number of morphotype, number of RFLP pattern and number of sample and clone used for sequencing

Experiment	Treatments	Hosts	Morphotype no.	Sample no.	RFLP patterns	Sample & Clone no.
1 (chapter 3)	6 land use types, i.e. SF, RAF, RM, OP, AC, IG	*S. lamellata*	5	32	n.a.	Direct sequencing: 13 samples,
		S. selanica	5	31		Cloning: 17 clones from 1 sample
2 (chapter 4)	Heat 150 °C and control (0 °C)	*S. selanica* *S. leprosula* *S. platyclados* *H. odorata* *V. sumatrana*	4 4 4 4 4	10 10 10 10 10	10	115 clones from 10 samples
3 (chapter 5)	Ex_forest 1yr, Ex_ forest 5yr, Ex_forest 10yr, Ex_RAF 1yr, Ex_RAF 5yr, forest ; inoculated by *S. columnare* at nursery stage or not inoculated	*S. lamellata* *S. selanica*	5 5	101 106	19	48 clones from 7 samples

Note: n.a. is data not available

DNA amplification

PCR was performed in 25 µl of a reaction mixture containing 2.5 µl 10x NH_4 buffer, 2.5 µl dNTP Mix, 0.1 µl 5 U BioTaq™ (Bioline GmbH., Luckenwalde,, Germany), 1 µl of 10 pmol forward and reverse primers, and 1 µl of 10 to 100 ng rDNA. The fungus-specific forward primer ITS1-F (5′-CTTGGTCATTTAGAGGAAGTAA-3′; Gardes and Bruns, 1993) was used for experiment heat and drying treatment (experiment #2). Universal primer ITS1 (5'-TCCGTAGGTGAACCTGCGG-3'; White *et al.*, 1990) was used for experiments EcM inoculum potential (MIP) from different land use and in-situ experiment in the field (Experiment #1 and #3). Universal backward primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3'; White *et al.*, 1990) was used for experiment #1 and #2. A fungus-specific reverse primer NL6C (5'-CAAGTGTTTCCCTTTCAACA-3'; Egger, 1995) was used for experiment #3. The amplifications were performed in an Gene Amp PCR system

9700 thermal cycler (Applied Biosystems, Foster City, CA) with the following parameters: initial denaturation at 94 °C for 2 min, subsequently 30 cycles, consisting of denaturation at 94 °C for 30 sec, annealing at 52 °C for 30 sec, extension at 72 °C for 70 sec, with final extension at 72 °C for 8 min.

DNA bands were visualized with ethidium bromide. For experiment #1, if only one DNA band was present per sample (confirming that all DNA came from one source), the product was used for sequencing. For experiments #2 and #3, all samples were subjected for RFLP analysis.

RFLP analysis

RFLP analysis was conducted for experiments #2 and #3, in order to select root-tip samples to be used for cloning and sequencing. PCR products were digested with restriction enzymes *Alu*I, *Hha*I and *Hinf*I (New England BioLabs Inc., Ipswich, MA). For each sample, 8 µl of PCR product was used with 9.4 µl sterile MilliQ water (Milipore Ltd., UK), 2 µl endonuclease buffer, 0.2 µl bovine serum albumine (New England BioLabs Inc., Ipswich, MA) and the three restriction enzymes to final concentrations of 10 U μl^{-1} each. Fragments were separated on 2% Ultrapure™ agarose (Invitrogen Ltd., Paisley, UK) in 1x TAE (Tris-acetate-EDTA, 40 mM Tris-acetate and 1 mM EDTA, pH 8.5) buffer, at 100 V for 15 min and subsequently at 150 V for 3 hr.

Cloning and sequencing

The PCR products from samples of experiment #1, i.e. fungal isolation from root tips, were sequenced using 1 µl template DNA (1 to 10 ng), 3 µl dilution buffer, 1 µl BigDye® Terminator (Applied Biosystems, Foster City, CA, USA) and 1 µl of 4 pmol primer in a 10 µl total volume with ultrapure water, as follows: initial denaturation at 95 °C for 2 min, then 30 cycles of denaturation at 95 °C for 10 sec, annealing at 50 °C for 5 s, extension at 60 °C for 2 min, cooling down to 8 °C.

In case where amplicons were too similar in size after electrophoresis, separation was accomplished by cloning using TOPO TA® kits (Invitrogen, Paisley, United Kingdom, UK) for experiments #1 and #3, and using pGEM-T (Easy) vector cloning kit (Promega Inc., Madison, USA) for experiment #2. One sample was cloned to 20 bacterial colonies on Luria Bertani Agar (Sigma Aldrich®, USA) amended with 50 µg ml^{-1} ampicillin (Fluka 10044), 2% X-gal (Promega V3941) and 0.1M IPTG (Isopropyl-D-thiogalactoside; Sigma I-6758). Cloning was done following protocol available from the kit. PCR was then performed on 10 to 20 bacterial colonies from each ligation using primers M13 forward (5'-GTAAAACGACGGCCAGT-3') and M13 reverse (5'-GGAAACAGCTATGAC-3'). Sequencing PCR conditions were performed using 1 µl of template DNA (1 to 10 ng), and reaction mix solution mentioned above, as follows: initial denaturation at 94 °C for 3 min, then 28 cycles of denaturation at 93 °C for 1 min, annealing at 52 °C for 1 min, extension at 72 °C for 2 min, with final extension at 72 °C for 3 min, and cooling down to 8 °C.

Sequencing products were purified with Sephadex G-50 Fine (GE Healthcare, Uppsala, Sweden) and analyzed by using an ABI Prism 3730 DNA analyzer (Applied Biosystems).

Molecular identification

The sequences were adjusted by using software programme SeqMan II (DNAStar Inc.). The identity of the sequences, i.e. nearest neighbors, was determined using GenBank (Altschul *et al.*, 1997) and some dedicated databases at CBS.

Results

Detection of taxa by sequencing from ectomycorrhizal roots

The initial PCR products obtained from root tips produced 1 to 4 distinct amplicons, ranging in size from 0.5 to 1 kb. Restriction digestion of all amplicons revealed 10 distinct RFLP patterns

from experiment of heating and drying treatment and 19 distinct RFLP patterns from experiment of out-planted dipterocarp in RAF. Numbers of samples used for cloning varied from 1 to 10, due to different efficiencies of two cloning methods used in the experiment, viz. TOPO-TA and pGEM®-T (Easy). Results showed that cloning with pGEM®-T (easy) yielded more positive clones than TOPO®-TA (Table 1). Numbers of positive clones, i.e. bacterial colonies that carried the fungal ITS sequence, varied from 6 to 17 clones (Table 1). The cloned PCR products yielded 1 to 5 distinct sequences from each ligation.

Thirteen DNA samples from experiment of MIP yielded a single band. SeqMan analysis confirmed that all DNAs originated from a single source. Eleven taxa of nearest neighbors of EcM fungi were found (Table 2). Only a single sample with a thick band yielded 17 clones, having five taxa as nearest neighbors (Table 2). *Tomentella* appeared to be the most frequent genus, with 62% including 13 taxa, followed by *Laccaria* (3 species) and *Curvularia* and unidentified Sclerodermataceae (1 species each) (Table 2).

Table 2 Identification of EcM and endophyte species-groups direct from ectomycorrhizal roots of *S. lamellata* and *S. selanica* 10 months after planting at different soil source (experiment #1). Study names are based on general taxonomic affinities of GenBank sequences showing the BLAST matches. Asterisk denotes endophytic fungus.

Host	Type of LU	Nearest Neighbour	Accession no.	Similarity (%)	Sample / Clone no.
S. lamellata	SF	*Tomentella* sp.4	U83482.1	89	1
	RAF	*Tomentella* sp.4	U83482.1	90	2
	RM	*Laccaria amethystina*	AF539737.	92	3_clone40
		Laccaria bicolor	DQ367906.	92	3_clone38
		Laccaria sp.1	AJ534899	92	3_clone9
		Tomentella sublilacina	AF323111	90	3_clone16; 4
		Tomentella sp.1	AJ534914.1	90	3_clone1;3_ clone18; 3_clone19; 3_clone2; 3_clone24; 3_clone28; 3_clone29; 3_clone4; 3_clone44; 3_clone5;
	AC	*Tomentella* sp.4	U83482.1	87	5
	OP	*Tomentella ramosissima*	U83480.	87	6
	IG	*Curvularia inaequalis* *	AM924157.	91	7
		Tomentella viridula	AF272914.	90	8
S. selanica	SF	*Laccaria amethystina*	AF440665.	92	9
	RM	Sclerodermataceae sp.	AM412304	92	10
		Tomentella sp.3	U92537.1	89	11
		Tomentella sp.6	EF538421.	89	12
	IG	*Tomentella atramentaria*	DQ974772.	89	13
		Tomentella sublilacina	AF323111	89	14

Fifty DNA samples from experiment #2 in the nursery yielded 10 distinct RFLP patterns. Sequencing of 115 clones from 10 ligations revealed different taxa and species groups of fungi; about 42% of the clones was identified as EcM and endophytic fungi (Fig. 1a). The most frequent EcM genus was *Tomentella* with 5 species, followed by *Inocybe* (4 species) and *Laccaria* (2 species). Four endophytic fungi were found at low frequency, such as *Pestalotiopsis* sp., *Fusarium* sp., *Muscodor* sp., and *Cosmopora* sp. (Table 3).

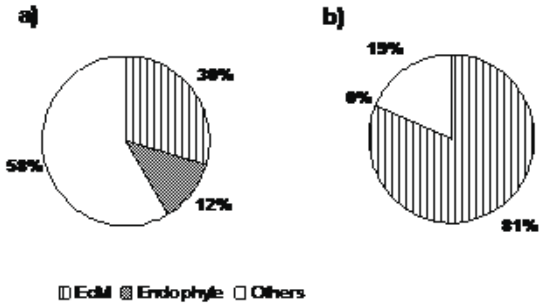

Fig. 1 Percentage of molecular identification on EcM and endophyte fungi colonized dipterocarp species based on two experiments. a) Potted cuttings of five dipterocarp species using preheated and dried soil in nursery (percentage of 115 clones) .b) *In situ* (field) study on two species of Dipterocarpaceae seedlings planted in RAF (percentage of 48 clones).

Table 3 Identification of EcM and endophyte species-groups from EcM roots of *S. platyclados, S. selanica, S. leprosula, V. sumatrana* and *H. odorata* (experiment #2). Study names are based on general taxonomic affinities of GenBank sequences showing the BLAST matches. Asterisk denotes endophytic fungus.

Hosts	Temperature (°C)	Nearest Neighbour	Accession no.	Similarity (%)	Clone code
Shorea platyclados	0	*Inocybe rufofusca*	EU326156.1	84	EMD_CloneR149.16
		Laccaria montana	EU486434.	92	EMD_CloneR147.10; 147.11
		Tomentella ellisii	AF272913.	94	EMD_CloneR147.1r
	150	*Tomentella* sp.1	AJ534914.1	88	EMD_CloneR122.10f; 122.6
Shorea selanica	0	*Inocybe intricata*	AM412271.1	83	EMD_CloneR139.14
		Pestalotiopsis sp.1 *	AF409994.1	99	EMD_CloneR139.10
		Inocybe sp.1	AM412271.	98	EMD_CloneR139.7
	150	*Laccaria montana*	EU486434.1	92	EMD_CloneR113.7; 113.1; 113.10; 113.11; 113.12; 113.13; 113.15; 113.16
		Laccaria sp.1	AJ534899.	92	EMD_CloneR113.2f
Shorea leprosula	150	*Fusarium* sp.6 *	AB277550.	100	EMD_CloneR101.1
		Tomentella cinerascens	U83483.	94	EMD_CloneR101.17r; 101.18r; 101.19r
		Tomentella sp.7	EF218827.1	92	EMD_CloneR101.12; 101.9; 101.10; 101.13
		Tomentella sp.1	AJ534914.1	91	EMD_CloneR101.5
Vatica sumatrana	0	*Inocybe rufofusca*	EU326156.1	84	EMD_CloneR131.4
	150	*Inocybe rufofusca*	EU326156.	84	EMD_CloneR109.11
		Inocybe tenebrosa	AM882899	86	EMD_CloneR109.13
		Laccaria sp.1	AJ534899.	92	EMD_CloneR110.15
		Muscodor sp. *	EF564150.1	90	EMD_CloneR109.14
		Tomentella sp.8	EF218834.1	92	EMD_CloneR110.9; 110.12
		Cosmospora vilior *	AY805574.	91	EMD_CloneR106.4
Hopea odorata	0	*Laccaria montana*	EU486434.1	92	EMD_R145

Two hundred and seven DNA samples from experiment #3 in the study area in Jambi yielded 19 distinct RFLP patterns. Sequencing of 48 clones from 7 ligations, about 81% of total clone was identified as EcM and endophyte fungi (Fig. 1b), consisted of 19 taxa of EcM fungi (Table 4). *Tomentella* is the most frequent genus, consisted of 6 species, followed by *Pisolithus* (2 species), *Clavulina, Sebacina* and *Sistotrema* (1 species each).

Comparison of detected taxa occured in dipterocarp seedlings within three experiments were shown in Table Appendix.

Table 4 Identification of EcM and endophyte species-groups from EcM roots of *S. lamellata* and *S. selanica* 1 yr after planted in different history of rubber garden (experiment #3). Study names are based on general taxonomic affinities of GenBank sequences showing the BLAST matches. Asterisk denotes endophyte fungus.

Hosts	Type of LU	Nearest Neighbour	Accession no.	Similarity (%)	Clone code	Note
S. lamellata	Ex_forest_5	*Tomentella* sp.1	AJ534914.	89	EMF_Clone_78.1	i
	Ex_forest_10	*Tomentella* sp.1	AJ534914.	89	EMF_Clone_81.3; 81.19; 81.3; 81.15; 81.8	ni
		Tomentella sp.2	DQ974783.1	88	EMF_Clone_81.8	ni
		Tomentella sp.1	AJ534914.	91	EMF_Clone_28.3	i
		Tomentella sp.3	U92537.1	91	EMF_Clone_28.2; 28.1	i
S. selanica	Ex_forest_10	*Clavulina* sp.	DQ974711.1	85	EMF_Clone_21.7	i
		Thelephoraceae sp.	U83468.	96	EMF_Clone_141.14	i
		Tomentella sp.1	AJ534914.	93	EMF_Clone_141.7	i
		Tomentella sp.4	U83482.1	91	EMF_Clone_141.6	i
		Tomentella sp.3	U92537.1	90	EMF_Clone_21.3	i
		Tomentella sp.2	DQ974783.1	88	EMF_Clone_21.19; 21.11; 21.1; 21.16; 21.11;	i
		Tomentella sp.5	AM412297	91	EMF_Clone_141.6	i
		Tomentella sp.1	AJ534914.	89	EMF_Clone_78.1	i
		Sebacina vermifera	DQ983816	94	EMF_Clone_141.13	I
		Sistotrema alboluteum	AY463467.2	86	EMF_Clone_21.17	i
	Ex_RAF_1	*Pisolithus indicus*	AY756113.	95	EMF_Clone_139.8	ni
		Pisolithus indicus	AY756113.	82	EMF_Clone_ 139.1	ni
		Pisolithus sp.1	AB106875	100	EMF_Clone_139.11; 139.6	ni
	Ex_RAF_5	*Tomentella* sp.5	AM412297	99	EMF_Clone_140.3; 140.2; 140.18; 140.11	i
		Tomentella sp.5	AM412297	98	EMF_Clone_140.7; 140.4; 140.21; 140.20; 140.16; 140.6; 140.4; 140.7	i

note: i = inoculted with *S. columnare*, ni = not inoculated

Detection of taxa by sequencing from fungal isolates

Sequencing 181 isolates from ectomycorrhizal roots of *S. selanica* and *S. lamellata* yielded 23 taxa belonging to the Ascomycota. Isolated fungi could be attributed to 13 genera,

while one unidentified species remained. *Fusarium* predominated among the 23 taxa, consisted of 8 species, 7 of which were endophytic fungi (Table 5). No EcM basidiomycetous fungus could be isolated from root tips. Percentage of ecological groups are listed in Fig. 2

Table 5 Identification of species-groups from isolation of EcM roots of *S. lamellata* and *S. selanica* 10 months after planting at different soil source (experiment #1). Study names are based on general taxonomic affinities of GenBank sequences showing the BLAST matches. Asterisk denotes endophytic fungus..

Hosts	Type of LU	Nearest Neighbor	Accession no.	Similarity (%)	Sample no.
S. lamellata	SF	Apiosporaceae sp.	DQ117959.	99	dH 17170
		Aspergillus nomius	DQ467992	99	dH 17173
		Bipolaris sp.	EU668994.	100	dH 17269
		Cylindrocladiella lageniformis	AY793449.	99	dH 17177
		Cylindrocladiella parva	AF261745.1	100	dH 17193
		Fusarium culmorum *	AM262427.	99	dH 17182
		Fusarium oxysporum *	EU625403.	99	dH 17174
		Fusarium sp.1 *	AY433806.	99	dH 17190; dH 17188_ *Fusarium* sp.1
		Pestalotiopsis neglecta	EF055208	99	dH 17200
	RAF	Ascomycetes fungi *	AF284133.	90	dH 17180_ sterile endophyte
		Fusarium oxysporum *	EU625403.	98	dH 17183
		Fusarium sp.2 *	DQ657851.	98	dH 17196_ *Fusarium* sp.2
		Fusarium sp.3 *	DQ682582.1	99	dH 17185_ *Fusarium* sp.3
	RM	*Fusarium oxysporum* *	EU625403.	99	dH 17197
		Trichoderma spirale	DQ083014.	99	dH 17203
		Cochliobolus lunatus	AF071339.	98	dH 17175
	IG	*Calonectria ilicicola*	AF493963.	99	dH 17178
		Cylindrocladiella parva	AF261745.1	100	dH 17194
	AC	*Fusarium* sp.7 *	DQ480359	100	dH 17187
S. selanica	Sterilized soil	*Cylindrocladiella lageniformis*	AY793449.	99	dH 17246
		Trichoderma asperellum	AF414341.	98	dH 17295
	SF	*Nectria* sp.1	DQ779785.1	94	dH 17341
		Calonectria kyotensis	AF261740.	99	dH 17249
		Cylindrocladiella camelliae	AF261746.	100	dH 17318
		Cylindrocladiella peruviana	AY793466.1	100	dH 17247
	RAF	*Cylindrocladium gracile*	AF261735	100	dH 17251
		Fusarium sp.4	EF423517.1	99	dH 17254
		Phoma multirostrata	CBS database		dH 17250; dH 17293; dH17320
	RM	*Bionectria* sp.	AY236956.	99	dH 17290
		Fusarium sp.5 *	DQ682577	99	dH 17252_ *Fusarium* sp.
		Nectria sp.1	DQ779785.1	94	dH 17341
	IG	*Fusarium solani*	DQ094667.	99	dH 17291

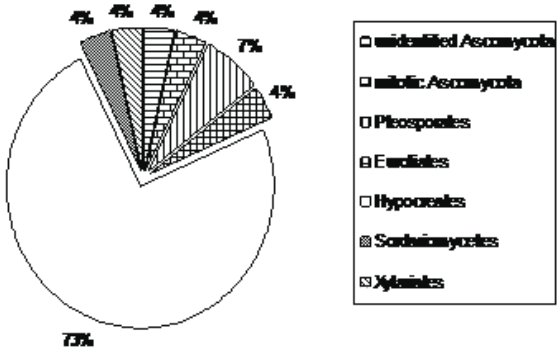

Fig. 2 Percentage of identification fungal species of root colonizing fungi from mycelia isolation

Discussion

The data on identity of EcM and endophyte fungi colonizing roots of dipterocarp seedlings were based on bait seedlings planted in a glass-house and in the field (*in-situ* test). ITS amplification and direct sequencing provide novel identity of below-ground ectomycorrhizal fungi on dipterocarps. Although a specific primer ITS-1F (Gardes and Burns, 1993) used to amplify ITS of EcM roots, also non-EcM fungi were detected (Fig. 1a).

Direct sequencing of EcM and culturing from EcM root tips resulted in different pictures of the composition of fungal assemblages associated with root tips. Most root-colonizing fungi from mycelial isolations were members of the Ascomycota, while root-colonizing fungi detected from direct sequencing of EcM root tips were members of the Basidiomycota. Similar differences in flora were also reported by Allen *et al.* (2003) and Sýkorová *et al.* (2007). Most EcM fungi are known to be unculturable in artificial media (Anderson and Cairney, 2004). Other reports, however successfully isolated EcM fungi from Ericacea and Coniferaceae (Smith *et al.*, 1995) and from black spruce (Summerbell, 1985; Summerbell, 2005a). The type of surface sterilization applied in the experiment apparently was less effective to prevent growth of contaminants from dipterocarp roots.

Molecular techniques are advantageous to identify of EcM fungi when starting from root materials (Horton and Burns, 2001; Anderson and Cairney, 2004; Martin and Slater, 2007). However, the number of samples of root tips and clones is one of limitations to study fungal diversity (Taylor, 2002; Anderson and Cairney, 2004). Sampling intensity has small effect on species richness in a mycorrhizal community, but particularly affects species composition (Koide *et al.*, 2007).

All EcM fungi on dipterocarp seedlings from nurseries and from an *in situ* study in the field are Basidiomycota (Table Appendix). Nursery experiment on MIP yielded three groups of EcM fungi, namely *Tomentella, Laccaria* and one unidentified Sclerodermataceae fungus. From preheated and dried soil experiment, three genera of 'early stage' EcM fungi were detected, such as *Tomentella*, *Laccaria* and *Inocybe*. *Tomentella* and *Laccaria* were found in two experiments in the nursery; they are well known as 'early stage' of EcM fungi (Visser 1995; Gherbi *et al.*, 1999; Cripps, 2001). *Inocybe* is also well known as early stage fungi, which colonize with seedlings in nursery and young stands (Nara *et al.*, 2003; Ryberg *et al.*, 2008).

We also detected endophytic fungi from experiment in the nursery. MIP experiment yielded one taxa of endophytic fungus, e.g. *Curvularia inaequalis*. It has been reported as endophyte fungus in grass *Dactylis glomerata* (Sánchez Márquez *et al.*, 2007). Heating and drying experiment yielded four taxa of endophytic fungi. *Fusarium* sp. 6 was reported as an endophyte on *Lygeum spartum* (Graminae) (Maciá-Vicente *et al.*, 2008). *Cosmopora vilior* has

been reported as an endophyte on *Picea abies* (Menkis *et al.*, 2004); *Pestalotiopsis* sp. 1 was an endophyte on medicinal plants (Jeewon *et al.*, 2003) and *Muscodor* sp. 1 was an endophyte on *Garcinia* (Phongpaichit *et al.*, 2006). Endophytic fungi are defined as fungi colonizing living plant organ without causing any diseases or negative effect (Jumponen and Trappe, 1998), and may present in broad range of host plants and present in ectomycorrhizal roots (Jumpponen and Trappe, 1998; Summerbell, 2005b).

Thelephoroid fungi dominated Dipterocarps from nursery experiment of MIP and heating and drying treatment circumscribe, apparently due to airborne reinoculation. It was not possible to eliminate contamination through the air-borne route, even though the potted plants were maintained in the glass-house. *Tomentella* sp. 1 was found among the three experiments. *Tomentella* sp. 2 and *Tomentella* sp. 5 were found in the field only. Thelephoroids also dominated dipterocarp seedlings in field planting in Jambi (Sumatra). The order Thelephorales encompassing ectomycorrhizal species in temperate, boreal and tropical forests and across continents (Amornpitak *et al.*, 2006; Tedersoo *et al.*, 2007; Yorou, 2008).

The *in-situ* test (field experiment) yielded 19 RFLP patterns, while heating and drying experiment yielded 10 RFLP patterns (Table 1). This suggested that EcM fungi in the soil from nursery experiment was less diverse than from *in-situ* experiment in the field. Identification of EcM fungi from the *in-situ* test in RAF in Jambi (Sumatra) showed that 81% of identified taxa were EcM fungi (Fig. 2), with Thelephoraceae as the dominant group, and members of Pisolithaceae, Clavulinaceae, Sebacinaceae and Corticiaceae at lower frequencies. Although number of clone used for molecular analysis was less than represent number of RFLP patterns, however, our data suggested that EcM fungi in RAF were more diverse than presumed initially. Few reports are available on the association of fungi of the Sebacinaceae and Corticiaceae with dipterocarp trees from South East Asia, particularly from Indonesia. Several taxa of Clavulinaceae, Sebacinaceae and Cortinariaceae were found to colonize *Pakaraimea dipterocarpacea* (Dipterocarpaceae) (Moyersen, 2006). *Sebacina vermifera* has been reported to form orchid mycorrhiza (Warcup, 1988; Desmukh *et al.*, 2006). Inoculation of *S. vermifera* increased growth of *Nicotiana attenuata* (Solanaceae) (Barazani, 2005). *Sebacina alboluteum* and some species of *Sistotrema* (members of the corticioid group) have been reported as EcM fungi (Larsson *et al.*, 2004; Nilsson *et al.*, 2006).

To date, identification of mycorrhizal fungi in Indonesia has mostly been based on sporocarp surveys, which gave different picture of EcM fungal species composition on above-ground and below-ground. From field observations at different sites, it can be concluded that EcM fungi occurring in mature RAF, i.e. rubber trees age more than 20 years after planting, are particularly members of *Scleroderma, Russula, Geastrum* and coralloid mycorrhiza (data not shown). Direct sequencing from EcM root tips provided information about diversity of below-ground EcM fungi across different land use types.

Mycelial isolation yielded different taxa from and consists of several orders. Our data showed about 97% of fungal isolates were found with high similarity to the Blast search sequence (Table 5). Eight endophytic fungi were identified as *Fusarium* from mycelial isolation. Most of them reported colonized medicinal plants, such as *Garcinia* (Phongpaichit *et al.*, 2006), *Dioschorea* (Xu *et al.*, 2008). *Fusarium* sp. 3 and sp. 5 colonized coffee plants (Posada *et al.*, 2007), which may act as biocontrol agent. Further investigation on interaction between EcM and endophytic fungi is necessary to be studied, since less endophyte fungi reported from the tropics compare to EcM fungi.

In conclusion, EcM fungal community in dipterocarp seedlings comprise numerous species. From experiment in nursery, early stage of EcM fungi were detected, while from *in-situ* experiment in RAF, early stage of EcM fungi and some other EcM type of Pisolithaceae, Sebacinaceae and Corticiaceae were encountered. This suggests EcM propagules persists in the soil after forests change to other land use type, such as RAF. For practical implication, reforestation of dipterocarp particularly in Sumatra, is not constrained by whether or not 'nursing' trees present in the area for reforestation.

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Appendix Fungi identified from root tips Dipterocarpaceae from three experiments, namely mycorrhizal inoculum potential study in nursery, treatment of heating and drying soil in nursery and *in-situ* test in rubber agroforests with different history, in Jambi, Indonesia. Asterisk denotes endophytic fungus.

Taxa	Family / Order	MIP_ nursery	Heat and Dry treatment_ nursery	*In-situ* test
Tomentella sp.1	Thelephoracea	+	+	+
Tomentella sp.2	Thelephoracea			+
Tomentella sp.3	Thelephoracea	+		+
Tomentella sp.4	Thelephoracea	+		+
Tomentella sp.5	Thelephoracea			+
Tomentella sp.6	Thelephoracea	+		
Tomentella sp.7	Thelephoracea		+	
Tomentella sp.8	Thelephoracea		+	
Thelephoraceae_unidentified	Thelephoracea			+
Tomentella sublilacina	Thelephoracea	+		
Tomentella ramosissima	Thelephoracea	+		
Tomentella viridula	Thelephoracea	+		
Tomentella atramentaria	Thelephoracea	+		
Tomentella ellisii	Thelephoracea		+	
Tomentella cinerascens	Thelephoracea		+	
Laccaria sp.1	Tricholomataceae	+	+	
Laccaria amethystina	Tricholomataceae	+		
Laccaria bicolor	Tricholomataceae	+		
Laccaria montana	Tricholomataceae		+	
Sclerodermataceae_unidentified	Sclerodermataceae	+		
Inocybe sp.1	Cortinariaceae		+	
Inocybe rufofusca	Cortinariaceae		+	
Inocybe intricata	Cortinariaceae		+	
Inocybe tenebrosa	Cortinariaceae		+	
Clavulina sp.	Clavulinaceae			+
Pisolithus indicus	Pisolithaceae			+
Pisolithus sp.1	Pisolithaceae			+
Sistotrema alboletum	Corticiaceae			+
Sebacina vermifera	Sebacinaceae			+
Curvularia inaequalis *	Pleosporales	+		
Pestalotiopsis sp.1*	Xylariales		+	
Fusarium sp.6 *	Hypocreales		+	
Muscodor sp. *	Xylariales		+	
Cosmospora vilior *	Nectriaceae (Hypocreales)		+	

CHAPTER 7

General discussion and summary

General discussion and summary

In tropical lowland forests trees and fungi are strongly linked, through mycorrhiza (EcM) formation. In South East Asia, west of the Wallace Line, such forests are dominated by trees of the Dipterocarpaceae family. The trees and mycorrhizal fungi intermingle through the wood-wide web, where roots of the trees provide carbon to the fungus and in return mycelia transfer soil nutrients to the host trees (Smith and Read, 1997). However, as part of the rapid deforestation and forest transformation, mixed dipterocarp forests have been replaced by other vegetation and land use types. Increase of tree cover and return to forest-like conditions is desirable, but faces many constraints. Poor soils in the tropics, especially after the litter layer has been burnt and/or eroded, can limit a successful reforestation. Low pH, low available P in the soil and heavy soil texture due to high clay contents may hinder late succession trees, such as Dipterocarpaceae, to regenerate successfully. Inoculation with fungal symbionts at the nursery stage was strongly recommended to achieve accelerated growth of dipterocarps (Priadjati, 2002; Turjaman *et al.*, 2006b). In Indonesia research on the community of EcM in dipterocarp forests and other land use types derived from forests has been limited, and optional regimens of soil regeneration still remain an open question.

In Sumatra, considerable areas of the lowland tropical forest have been turned into Rubber Agroforest (RAF). Despite the rapid deforestation of Sumatra, the area of RAF has remained constant during the past decade (Ekadinata and Vincent, 2005); the most recent data, however, suggest that RAF is now also converted to more intensive tree crop systems. RAF is a land use type in which rubber trees are planted, while allowing the spontaneous establishment of forest tree species, with selective retention of the useful trees, such as Dipterocarpaceae and species of which the fruits are eaten. As a result the farmer gets the benefit from tapping rubber and collecting other forest products including timber. It is clear that RAF is important for the conservation of biodiversity, and that conservation strategies need to incorporate these systems in landscape-scale efforts to regenerate the landscape.

This thesis is a combination of basic and applied research. It focuses on the role and identity of EcM fungi in dipterocarps and their relation with land use change in Jambi, Sumatra. As part of the basic research, the effect of land use change on diversity of vegetation (chapter 2) and EcM inoculum available to dipterocarp trees (chapter 3) were analysed. The effect of soil heating and drying treatments on EcM propagules in the nursery was investigated (chapter 4) and the EcM fungi on dipterocarps were identified (chapter 6). The applied research dealt with enrichment planting with dipterocarp species in RAF (chapter 5). This study does not discuss policy, timber marketing and other options for enrichment planting with trees in RAFs, but provides backgrounds for such discussions in future.

Species richness in RAF

Rubber agroforest (RAF) is common in Sumatra. Land use change from forest to another land use type affected plant diversity in Jambi. Our research showed that in RAF there is a good regeneration of species compared to secondary forest. Species richness of the seedlings strata in RAF was higher than in forest. Species richness of the saplings and trees stratum, however, was higher in forests than in RAF. The diversity index for all strata is higher in forest than in RAF. Human activity in RAF management, such as thinning and weeding, has strong effects on species richness. Very few trees dependent on EcM fungi, which belong to the family of Dipterocarpaceae, Fagaceae and Gnetaceae, were encountered in the RAF. However, the relative distribution of early and late successional species as evident from the wood density distribution showed no difference between RAF and secondary forest (chapter 2).

Farmers allow natural regeneration from seed banks and from dispersal agents, which is reflected in a higher species richness in the seedlings stratum. Eight to ten years after rubber planting, or 5-6 years for clonal rubber, farmers come back to the garden to tap the latex. Farmers then start to thin and fell unnecessary trees. Tree species which are not economically beneficial may be thinned. Selective thinning was, based on interviews with farmers, done

to minimize competition for soil nutrients and light, in order to raise productivity of latex. This activity reduces species richness and vegetation diversity in RAF. On the other hand, farmers allow trees with edible parts to grow. Apart from the rubber trees, farmers maintain other valuable trees in RAF, which produce timber, food, spices, dye, medicine and fodder. The relative abundance of trees with edible parts in RAF was significantly higher than in forest (chapter 2).

Role of EcM inoculation

The Indonesian National Standardization Agency (*Badan Standarisasi Nasional*) strongly recommends EcM fungi inoculation on seedlings of forest tree species, as stated in the Indonesia National Standard (*Standar Nasional Indonesia, SNI*) number 01-7198-2006, to produce vigorous seedlings. However, our results suggest that inoculation with EcM fungus *Scleroderma columnare* in the nursery stage yielded only a small positive effect on survival and no effect on growth of *S. lamellata* and *S. selanica*, as sufficient inoculum potential exists in all conditions tested (chapter 5). In contrast to other results (Turjaman *et al.*, 2006b, 2007), our results showed that inoculation of dipterocarps with EcM in the nursery stage did not increase total N and P uptake of *S. lamellata* and *S. selanica*. The effect of EcM inoculation on early survival, especially in forest soils, suggests that the avoidance of root-born diseases may be the main benefit (chapter 5).

EcM inoculum potential in the soils and identification

Mycorrhiza in the forest soils interlink with common mycorrhizal networks, the so-called wood-wide web (Peter, 2006). Our study in the nursery tested disturbed soils in containers, and thus relied on the effectiveness of spore survival and dispersal. The small differences in EcM colonization between soils derived from a wide range of land use types that we reported, may indicate that survival of spores and colonization potential are not sensitive to the history of the site (chapter 3). EcM fungi on *S. lamellata* and *S. selanica* planted in soil derived from different land use types belong to the genera of *Tomentella, Laccaria* and an unidentified genus of the Sclerodermataceae. In addition, an endophytic *Curvularia* was obtained (chapter 6).

Drying and heating the soil simulated fire impact on EcM inoculum in the soil. It showed little effect on EcM colonization on five dipterocarp species in the nursery (chapter 4). Molecular identification of mycorrhiza on five dipterocarps species planted in treated soils showed three genera of EcM fungi, *Tomentella, Laccaria*, and *Inocybe,* as well as four species of endophytic fungi: *Pestalotiopsis* sp., *Fusarium* sp., *Muscodor* sp., and *Cosmospora vilior* (chapter 6). Rapid recolonization of the heated soil in the experiment may imply sufficient availability of EcM fungi for dipterocarps under field conditions, where fire as a tool for land clearing is commonly used in Sumatra.

The field experiment in rubber gardens with different histories showed that EcM inoculum persists in the soil after forest was changed to RAF (chapter 5). Molecular identification of mycorrhiza on *S. selanica* and *S. lamellata* planted in RAF revealed 5 genera of EcM fungi: *Tomentella, Pisolithus, Clavulina, Sebacina* and *Sistotrema* (chapter 6). None of fungi was identified as *S. columnare,* which was inoculated to *S. lamellata* and *S. selanica* seedlings in the nursery stage. This indicated that indigenous EcM fungi dominated on the dipterocarp seedlings planted in the field, regardless of nursery inoculation (chapter 5).

Tomentella was the prevalent genus of EcM fungi colonizing dipterocarp seedlings in the nursery stage and in the *in-situ* test 1 year after the seedlings have been planted in the field. *Tomentella* sp.1 was encountered in all three experiments, and this suggested that it is widely distributed.

The extraction of DNA from mycorrhizal root tips and identification of fungi provided a radically different perspective on the fungi involved than other methods that rely on aboveground sporocarp surveys of known EcM species in the vegetation and thus miss species such as *Tomentella*. In line with other experience, our efforts to transfer EcM fungi to culture media were unsuccessful.

Relation between aboveground plant and belowground EcM fungi diversity

Late successional trees, like Dipterocarpaceae and Fagaceae, and trees belonging to the Gnetaceae, are known to be dependent on EcM fungi. They occur with low abundance in RAF (chapter 2). Dipterocarpaceae have host specificity to EcM fungi (Yasman, 1995; Priadjati, 2002). Host trees of EcM fungi play a role as mother-trees for seedlings, since many seedlings of Dipterocarpaceae germinate within the root zone of the 'mother-tree' (Yasman, 1995). This shows the importance of mycorrhizal networks in the soils for plant regeneration. In contrast, our results showed that lack of trees that are dependent on EcM fungi in RAF and in non-forested land does not necessarily imply the absence of EcM inoculum belowground in the ecosystem.

Fundamental relationships exist between the aboveground (plant) diversity and the belowground biodiversity (including microbes, macro- and microfauna) concerning aspects of root quality, water balance and microclimate. *Vice versa*, the belowground diversity affects plant growth by enhancing nutrient uptake efficiency and the protection against diseases and rhizovores (van Noordwijk and Swift, 1999; Hooper *et al.*, 2000). Links between roots and fungi are initially mediated by root exudates produced by diverse trees, which induce microbial symbionts, such as mycorrhiza and endophytic fungi, to colonize the roots (Bais *et al.*, 2006). The application of host plants, like *S. lamellata* and *S. selanica*, as bait for EcM inoculum potential in the soils from other land use types showed considerable EcM colonization as compared to forest soils (chapter 3).

Kernaghan (2005) stated that plant diversity is positively correlated with EcM fungal diversity, having a positive feed back mechanism. Our results based on RFLP patterns showed that the EcM community on dipterocarps in a nursery study, using 5 dipterocarp species, yielded a lower diversity than that from *in-situ* planted seedlings in the field (chapter 6). The presence of dipterocarp trees in the rubber garden may restore mycorrhizal networks in non-forested land (chapter 5). Sýkorová *et al.* (2007) concluded that cultivation bias yielded a different diversity of arbuscular mycorrhizal fungi (AMF) in four different plant species, since the succession of the AMF community occurs in the nursery stage. Succession of EcM fungi also occurs in the primary succession following disturbance, such as fire (Visser, 1995).

Our results suggested that the reduction of plant diversity in *Imperata* grassland, compared to RAF and forest, did not cause a critical loss of ecosystem functioning with respect to inoculum of fungal symbionts. This finding potentially is of great value for reforestation efforts with dipterocarps or other fast-growing trees associated with EcM fungi, such as *Acacia* and *Eucalyptus*.

Practical implications on sustainable forestry management in Indonesia

While these studies were going on, the basic premise of restoration of logged over natural forest through enrichment planting became a fiction. Using the existing logging roads and with adequate protection from security forces and local power brokers, the forest disappeared by illegal logging, including many of the research plots. The forestry authorities who want to secure the restoration of forests were not able to provide control. Scarcity of timber for local consumption in Indonesia, particularly in Sumatra, is gradually increasing, due to lack of timber from natural forests and forest plantations.

An alternative may exist for such restoration where it is done by farmers on land that they control, and with trees that they expect to be able to harvest in future. Dipterocarp planting in RAF is challenging, since farmers indeed have interest to plant timber trees in their rubber garden (van Noordwijk *et al.*, 2004; Tata *et al.*, 2008a). Regulation on timber extraction and timber marketing from plantings of smallholder farmers is hindering the farmer to plant dipterocarp species (Tomich and Lewis, 2001; Suyanto *et al.*, 2004), and this may reduce the benefits they can expect from their land. Farmers are not sure whether or not forestry authorities and rules intended to stop illegal logging.

Rubber farmers lack knowledge on dipterocarp planting and have limited access to find sources of timber seeds and seedlings (Tata *et al.*, 2008a). However, several guidelines

about dipterocarp planting and EcM inoculation techniques have been published at a national level (Anonymous, 2004; Santoso *et al.*, 2006; Tata *et al.*, 2008b). If farmers intend to plant and manage the trees, however, the nursery technology will have to be simple, and each component will need to be scrutinized. With such techniques in hand, further studies need to focus on growth estimation, biomass measurement and suitability of various sizes and shapes of gaps for enrichment planting.

To conclude, this thesis suggests no biological constraint to enrichment planting with Dipterocarpaceae in Sumatra, Indonesia. The summary of research findings is shown in a poster in Fig. 1.

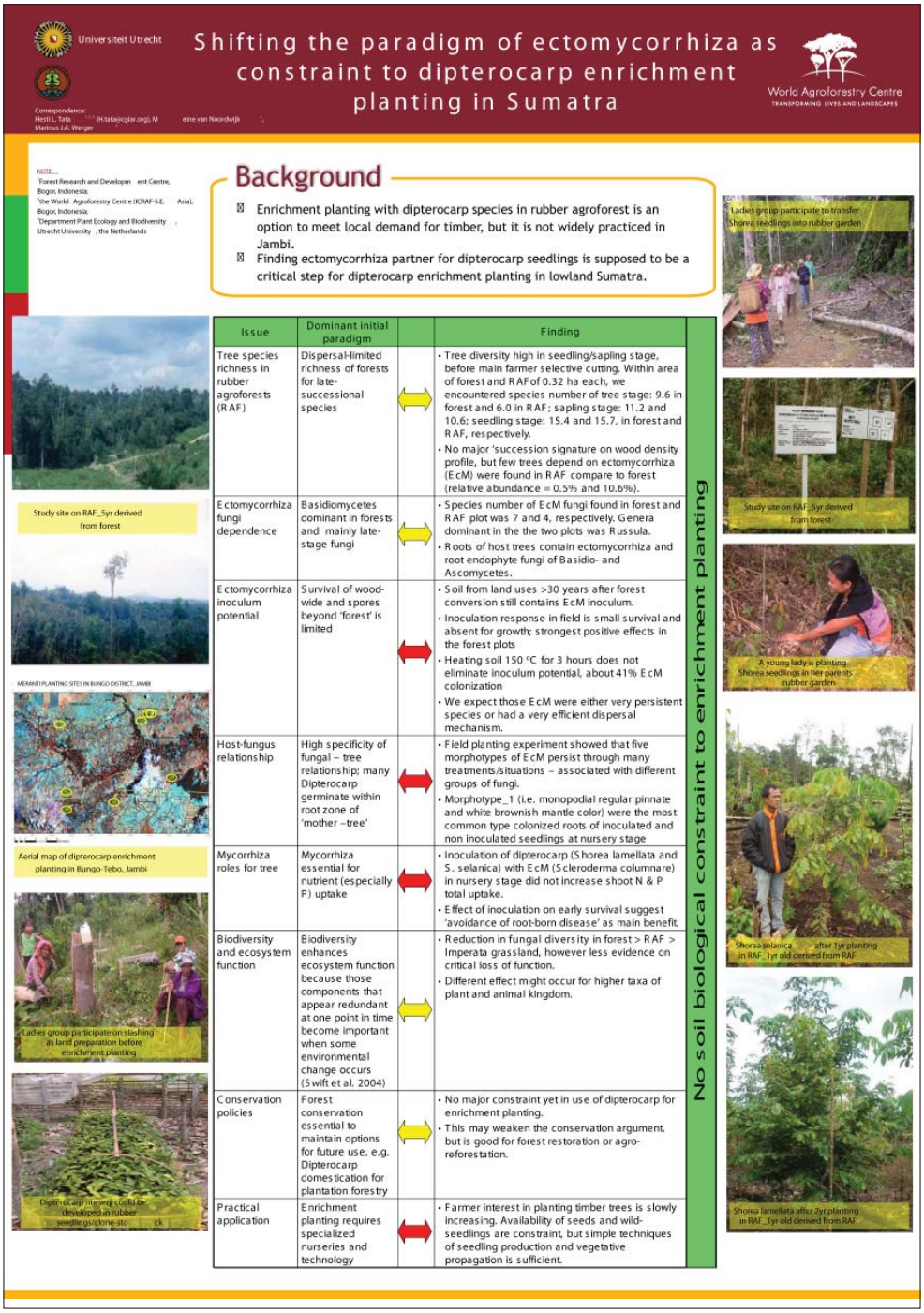

Fig. 1 Research findings on the study of 'mycorrhizae on dipterocarps in rubber agroforests (RAF) in Sumatra'; (Poster presented at the Science Meeting of World Agroforestry Centre, Nairobi, March 3rd - 7th, 2008).

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Abstract

Mycorrhizae on dipterocarps in rubber agroforests (RAF) in Sumatra

In tropical lowland forests trees and fungi are strongly linked, through ectomycorrhiza (EcM) formation. In Sumatra, such forests are dominated by trees of the Dipterocarpaceae family. However, as part of the rapid deforestation and forest transformation, mixed dipterocarp forests have been replaced by other vegetation and land use types. Considerable areas of the lowland tropical forest in Sumatra have been turned into Rubber Agroforests (RAF). RAF is a land use type in which rubber trees are planted, while allowing the spontaneous establishment of forest tree species.

The method presented in this thesis includes four main elements. Firstly, vegetation analysis in seven types of land use. Secondly, study in nursery using dipterocarps species as bait plant to assess the effect of land use change on EcM inoculum and to analyze effect of soil heating and drying on EcM propagule. Thirdly, *in-situ* experiment in RAF with different history was done to assess whether fungal inoculant increased the survival, growth, nutrient uptake and EcM formation on *Shorea selanica* and *Shorea lamellata*. Finally, molecular techniques of PCR and sequencing have been applied to identify EcM fungal symbiont on dipterocarps. The study has been conducted in Jambi province, Sumatra, Indonesia. *Ex-situ* experiment has been conducted in the nursery of Forest Research and Development Agency, Bogor, Indonesia. Identification of EcM fungi was done in Fungal Biodiversity Centre (CBS), Utrecht, the Netherlands.

The result on vegetation analysis showed human activity in RAF management has strong effects on species richness. Very few trees dependent on EcM fungi were encountered in the RAF. Our study in the nursery shows the small differences in EcM colonization between soils derived from a wide range of land use types, may indicate that survival of spores and colonization potential are not sensitive to the history of the site. Rapid recolonization of the heated soil in the experiment may imply sufficient availability of EcM fungi for dipterocarps under field conditions, where fire as a tool for land clearing is commonly used in Sumatra. *In-situ* experiment in the RAF showed that lack of trees that are dependent on EcM fungi in RAF and in non-forested land does not necessarily imply the absence of EcM inoculum belowground in the ecosystem. EcM inoculum persists in the soil after forest was changed to RAF.

Molecular identification of EcM fungi showed that *Tomentella* was the prevalent genus of EcM fungi colonizing dipterocarp seedlings in the nursery stage and in the *in-situ* test 1 year after the seedlings have been planted in the field. None of fungi was identified as *Scleroderma columnare,* which was inoculated to *S. lamellata* and *S. selanica* seedlings in the nursery stage. This indicated that indigenous EcM fungi dominated on the dipterocarp seedlings planted in the field, regardless of nursery inoculation.

The implementation of this study is potentially of great value for reforestation efforts with dipterocarps in RAF in collaboration with rubber farmers. This reforestation approach will ensure the successfulness of the reforestation managed sustainably by farmer, where they will benefit from them in the future.

Keywords: agroforestry, Dipterocarpaceae, ectomycorrhizae, Indonesia, Internal Transcribed Spacer, mycorrhiza inoculum potential, rubber agroforests, Scleroderma columnare, Shorea, *tropical forests*.

Samenvatting

Mycorrhizae op Dipterocarpaceae in rubber agroforests (RAF) in Sumatra

In tropisch laagland regenwoud bestaat er een sterke band tussen bomen en paddestoelen (fungi) door de vorming van ectomycorrhiza (EcM) rond de boomwortels. In Sumatra worden zulke bossen gedomineerd door bomen die behoren tot de familie Dipterocarpaceae. Als gevolg van snelle ontbossing en omvorming tot productiebos zijn de gemengde dipterocarpe wouden door andere typen vegetatie en landgebruik vervangen. Grote delen van het tropische laaglandbos zijn in rubber productiebos omgezet, de zgn. Rubber Agroforests (RAF). RAF is een type grondgebruik waarin rubberbomen worden aangeplant, maar voor het overige spontane bosgroei wordt toegestaan.

Het onderzoek dat in dit proefschrift wordt gepresenteerd bestaat uit vier hoofdelementen. Ten eerste is de vegetatie ingedeeld in 7 typen landgebruik. Vervolgens werden jonge dipterocarpe soorten in de kwekerij gebruikt om de mate van mycorrhizavorming, en de invloed van verschillende typen landgebruik daarop, vast te stellen. Tevens werd het effect van verhitting en uitdrogen van de grond op mycorrhiza vorming onderzocht. Vervolgens werd een *in-situ* experiment in RAF-percelen, die verschilden in voormalig landgebruik, uitgevoerd om vast te stellen of *Shorea selanica* en *Shorea lamellata* planten met EcM inocula een betere overleving, groei, voedselopname en EcM vorming vertoonden. Tenslotte werden moleculaire technieken met PCR en sequentiëring toegepast om de EcM symbionten op dipterocarpe wortels te identificeren. De veldstudie werd in de provincie Jambi op Sumatra, Indonesië, uitgevoerd en de e*x-situ* experimenten in de kwekerij van de Forest Research and Development Agency in Bogor, Indonesië. Identificatie van de EcM-vormende fungi werd aan het Centraalbureau voor Schimmelcultures, Utrecht, uitgevoerd.

Het onderzoek naar de vegetatie toonde aan, dat de mate van menselijke activiteit bij RAF management veel effect op soortenrijkdom heeft. Slechts weinig boomsoorten die van EcM afhankelijk zijn kwamen in de RAF voor. Ons onderzoek in de kwekerij toonde de kleine verschillen in EcM-kolonisatie tussen een breed scala van typen landgebruik aan, hetgeen er op wijst dat overleving van sporen en hun kolonisatiepotentie niet gevoelig zijn voor de gebruiksgeschiedenis van de grond. Snelle herkolonisatie van de verhitte grond uit ons experiment wijst op voldoende beschikbaarheid van EcM-fungi voor dipterocarpen onder veldcondities in Sumatra, waar afbranden van het bos een veelgebruikt middel is om land open te leggen voor plantages en landbouwgrond. *In-situ* experimenten toonden aan, dat de afwezigheid van EcM-afhankelijke boomsoorten in de RAF en in ontboste landerijen, niet betekent dat EcM inocula afwezig zijn in de ondergrond van het ecosysteem. EcM inocula houden in de grond stand nadat het oorspronkelijke bos is omgezet in RAF.

Moleculaire identificatie van EcM-fungi toonde aan dat *Tomentella* het belangrijkste genus was van EcM-fungi in de jonge dipterocarpe planten in de kwekerij alsmede in de *in-situ* test een jaar nadat de jonge planten in het veld waren uitgepoot. Geen enkele stam werd gedetermineerd als *Scleroderma columnare*, welke eerder was gebruikt om *S. lamellata* en *S. selanica* kweekplanten in de kwekerij te inoculeren. Dit betekent dat in het veld locale EcM-fungi de wortels van de jonge dipterocarpe planten meteen bezetten, en daarmee een grotere rol spelen dan de inocula uit de kwekerij.

De resultaten van deze studie kunnen van groot belang zijn voor de herbebossingsprogramma's met dipterocarpen in RAF in samenwerking met rubberboeren. Deze lokale benadering van herbebossing draagt bij tot het succes van duurzame herbebossing door de boeren, die daar in de toekomst profijt van zullen hebben.

Keywords: agroforestry, Dipterocarpaceae, ectomycorrhizae, Indonesia, Internal Transcribed Spacer, mycorrhiza inoculum potential, rubber agroforests, Scleroderma columnare, Shorea, t*ropical forests*

Rangkuman

Mikoriza pada dipterokarpa di wanatani karet di Sumatra

Pepohonan suku Dipterocarpaceae yang mendominasi hutan tropis dataran rendah Asia Tenggara, yaitu di bagian barat garis Wallacea memiliki hubungan erat dengan fungi, melalui pembentukan mikoriza. Pohon-pohon dan fungi mikoriza saling berhubungan melalui "*wood-wide web*", dimana akar-akar pohon menyediakan karbon dalam bentuk karbohidrat sederhana bagi fungi dan sebaliknya miselium-miselium fungi menyalurkan hara tanah dan air ke pohon inang (Smith dan Read, 1997).

Tingginya laju deforestasi dan transformasi hutan mengakibatkan hutan campuran dipterokarpa berubah menjadi tipe penggunaan lahan lain dan tipe vegetasi lain. Salah satu perubahan penggunaan lahan dari hutan campuran dipterokarpa adalah wanatani karet. Sejak puluhan tahun yang lalu, sejumlah area hutan tropis dataran rendah di Sumatra, telah berubah menjadi wanatani karet. Ekadinata dan Vincent (2005) menyebutkan bahwa selama dekade terakhir area hutan di kabupaten Bungo khususnya, berkurang sangat cepat, sebaliknya luasan area wanatani karet tetap stabil. Akan tetapi, dari terkini dari observasi langsung di lapangan mengungkapkan bahwa wanatani karet dewasa ini juga telah dikonversi menjadi sistem pertanian yang lebih intensif.

Wanatani karet adalah tipe penggunaan lahan dengan pananaman karet sebagai tanaman pokok dan membiarkan jenis-jenis tanaman lain tumbuh secara spontan, terutama jenis-jenis tanaman penghasil buah yang dapat dimakan, penghasil kayu dan jenis-jenis lain yang berguna bagi kehidupan sehari-hari. Pada sistem wanatani, petani mendapatkan keuntungan dari menyadap karet, selain itu juga mengumpulkan hasil-hasil hutan lainnya, termasuk kayu. Wanatani karet berperan penting dalam konservasi keragaman hayati, sehingga strategi konservasi tersebut perlu untuk disambungselaraskan dalam upaya meregenerasi bentangan alam (*landscape*) berskala luas melalui peningkatan jumlah tegakan dan pemulihan kondisi pada hutan yang terganggu.

Namun, upaya ini mengalami beberapa kendala antara lain: (a) tanah tropis pada umumnya miskin unsur hara, terutama setelah lapisan seresah terbakar dan/atau mengalami erosi, sehingga menghambat keberhasilan rehabilitasi lahan dan reboisasi hutan; (b) tanah yang masam; dan (c) rendahnya kadar $P_{tersedia}$ dalam tanah; serta (d) tekstur tanah yang berat karena tingginya kandungan liat, dapat menghambat regenerasi jenis-jenis pepohonan dalam kelompok suksesi akhir, seperti Dipterocarpaceae.

Inokulasi fungi yang bersimbiosis dengan dipterokarpa pada tahap persemaian, sangat dianjurkan untuk meningkatkan pertumbuhan dipterokarpa (Priadjati, 2002; Turjaman *et al.*, 2006b). Namun sampai saat ini, penelitian mengenai komunitas ektomikoriza di hutan dipterokarpa dan lahan lain yang berasal dari hutan di Indonesia sangat terbatas, sehingga pilihan rencana mengenai regenerasi vegetasi dan biota tanah masih menjadi pertanyaan terbuka.

Tesis ini merupakan kombinasi dari penelitian dasar dan aplikasi, dengan fokus utama adalah peran dan identitas fungi ektomikoriza pada dipterokarpa serta hubungannya dengan perubahan lahan di Jambi, Sumatra. Sebagai bagian dari penelitian dasar, beberapa penelitian seperti efek perubahan lahan terhadap keragaman vegetasi (bab 2) dan ketersediaan inokulum ektomikoriza dalam tanah terhadap dua jenis dipterokarpa (bab 3) telah dianalisa. Selain itu telah diselidiki pula pengaruh perlakuan pengeringan dan pemanasan tanah dengan oven terhadap propagul ektomikoriza dalam tanah dengan menggunakan lima jenis dipterokarpa di persemaian (bab 4) dan identifikasi ektomikoriza fungi dengan menggunakan teknik molekular DNA, yaitu Polymerase Chain Reaction (PCR) dan sekuensing (bab 6). Penelitian aplikasi berhubungan dengan penanaman pengayaan dengan jenis-jenis dipterokarpa di wanatani karet (bab 5). Hal-hal yang berkaitan dengan kebijakan pemerintah, pemasaran kayu dan pilihan jenis tanaman lain dalam pengayaan jenis di kebun karet tidak dibahas dalam tesis ini, akan tetapi menjadi latar belakang dalam diskusi.

Kekayaan jenis di wanatani karet

Perubahan penggunaan lahan dari hutan menjadi tipe lain mempengaruhi keragaman jenis tanaman, salah satu contoh adalah wanatani karet yang umum dijumpai di Jambi, Sumatra. Penelitian ini menunjukkan bahwa regenerasi jenis anakan di wanatani karet lebih baik dari pada di hutan sekunder. Kekayaan jenis anakan di wanatani karet lebih tinggi dari pada di hutan, akan tetapi kekayaan jenis pancang dan pohon (termasuk tihang) di hutan lebih tinggi dibandingkan dengan di wanatani karet. Index keragaman jenis untuk semua strata di hutan lebih tinggi dari pada di wanatani karet. Aktifitas manusia sebagai bagian dari pengelolaan wanatani karet, seperti pembersihan gulma dan penjarangan, berpengaruh negatif terhadap kekayaan jenis tumbuhan. Sangat sedikit jenis-jenis pohon yang bersiombiosis dengan fungi ektomikoriza, seperti Dipterocarpaceae, Fagaceae dan Gnetaceae, yang dijumpai di wanatani karet. Akan tetapi, tidak terdapat perbedaan distribusi relatif jenis-jenis tumbuhan yang umum dijumpai pada awal suksesi dan akhir suksesi antara hutan dan wanatani karet (bab 2). Penggolongan jenis tumbuhan berdasarkan bobot jenis kayu (*wood density*) menunjukkan bahwa jenis tumbuhan yang biasa tumbuh pada awal suksesi memiliki bobot jenis kayu rendah dan bersifat tumbuh cepat (*fast growing*), sedangkan jenis tumbuhan yang biasa tumbuh pada akhir suksesi memiliki bobot jenis kayu tinggi dan tumbuh lambat (*slow growing*).

Petani karet memiliki peran dalam terjadinya regenerasi alami dari bank benih (*'seed banks')* dalam tanah dan dari agen pemencar biji, yang tercermin dari tingginya kekayaan jenis tingkat anakan. Delapan sampai sepuluh tahun setelah penanaman karet, atau 5-6 tahun untuk karet klon, petani karet kembali ke kebunnya untuk menyadap karet. Pada tahap ini, petani mulai melakukan penjarangan dengan menebas pohon-pohon yang sekiranya tidak menguntungkan secara ekonomi. Berdasarkan wawancara, penjarangan secara selektif dilakukan oleh petani dengan alasan untuk mengurangi persaingan hara tanah dan cahaya dengan tanaman lain sehingga produktivitas getah karet (*latex*) meningkat. Kegiatan ini mengurangi kekayaan jenis dan keragaman vegetasi di wanatani karet. Namun sebaliknya, petani membiarkan pepohonan yang menghasilkan bagian yang dapat dimakan (baik oleh manusia maupun ternak) untuk tetap tumbuh. Selain karet, petani juga memelihara jenis-jenis yang bermanfaat bagi kehidupan di wanatani karet, seperti pohon-pohon penghasil kayu, penghasil makanan, bumbu, pewarna, obat-obatan dan pakan ternak. Kelimpahan relatif pohon-pohon dengan bagian yang dapat dimakan di wanatani karet lebih tinggi dari pada di hutan (bab 2).

Peran inokulasi ektomikoriza

Badan Standarisasi Nasional merekomendasikan inokulasi ektomikoriza fungi pada tanaman kehutanan untuk menghasilkan bibit yang berkualitas baik, seperti disebutkan dalam Standar Nasional Indonesia (SNI) nomor: 01-7198-2006. Hasil penelitian dalam tesis ini menunjukkan bahwa inokulasi dengan fungi ektomikoriza *Scleroderma columnare* terhadap bibit *Shorea lamellata* dan *S. selanica* pada tahap persemaian hanya memberikan sedikit pengaruh positif terhadap daya tahan hidup dan tidak berpengaruh terhadap pertumbuhan tinggi dan diameter. Dalam tanah di wanatani karet dengan sejarah lahan dan umur tanaman karet yang berbeda yang menjadi lokasi penanaman *S. lamellata* dan *S. selanica* di Jambi, masih cukup mengandung inokulum ektomikoriza (bab 5). Berlawanan dengan laporan oleh Turjaman *et al.* (2006b, 2007), hasil penelitian kami menunjukkan bahwa inokulasi dipterokarpa dengan ektomikoriza fungi di persemaian tidak meningkatkan penyerapan unsur hara N dan P pada bibit *S. lamellata* dan *S. selanica*. Pengaruh inokulasi fungi ektomikoriza terhadap daya tahan hidup awal, terutama pada bibit yang ditanam di hutan sekunder, menunjukkan bahwa manfaat utama dari inokulasi adalah meningkatkan daya tahan terhadap penyakit akar (bab 5).

Potensial inokulum ektomikoriza dalam tanah dan identifikasinya

Ektomikoriza dalam tanah hutan berhubungan sangat erat satu sama lain melalui jejaring mikoriza, yang disebut dengan *'wood-wide web'* (Peter, 2006). Hasil penelitian kami yang

dilakukan di rumah kaca menguji tanah dalam kondisi terganggu di dalam pot plastik, sehingga studi ini berdasarkan pada efektivitas daya tahan hidup spora dan pemencaran spora fungi ektomikoriza. Rendahnya perbedaan kolonisasi ektomikoriza antara tanah yang berasal dari berbagai tipe penggunaan lahan yang berbeda mengindikasikan bahwa daya tahan hidup spora dan kolonisasi ektomikoriza tidak sensitif terhadap sejarah lahan (bab 3). Dari hasil identifikasi molekular berdasarkan sekuen *Internal Transcribed Spacer* (ITS) rDNA, menunjukkan bahwa fungi ektomikoriza yang berkolonisasi dengan *S. lamellata* dan *S. selanica* yang ditanam dalam tanah dengan tipe penggunaan lahan yang berbeda termasuk dalam genus *Tomentela, Laccaria* dan satu genus yang tidak teridentifikasi dari suku Sclerodermataceae. Selain itu, diidentifikasi juga satu endofit *Curvularia* (bab 6).

Percobaan pemanasan tanah dengan suhu yang berbeda dan pengeringan mensimulasikan dampak kebakaran terhadap inokulum mikoriza dalam tanah. Hasil penelitian menunjukkan bahwa pemanasan dan pengeringan tanah kurang berpengaruh terhadap kolonisasi ektomikoriza pada stek pucuk 5 jenis dipterokarpa setelah 10 bulan di rumah kaca (bab 4). Identifikasi molekular fungi ektomikoriza berdasarkan sekuen ITS rDNA menunjukkan terdapat 3 genus fungi ektomikoriza, yaitu: *Tomentella, Laccaria,* dan *Inocybe*, dan juga 4 jenis fungi endofit: *Pestalotiopsis* sp., *Fusarium* sp., *Muscodor* sp., dan *Cosmospora vilior* (bab 6). Cepatnya ektomikoriza mengkolonisasi kembali tanaman inang pada tanah yang mendapat perlakuan pemanasan hingga 150 ^{o}C selama 3 jam, mengindikasikan bahwa propagul ektomikoriza dalam tanah tersedia dalam jumlah yang cukup di lapangan. Seperti yang telah diketahui di Sumatra, api umum digunakan sebagai alat untuk membuka lahan. Ketersediaan inokulum ektomikoriza alami dalam tanah di Sumatra dapat mendukung pertumbuhan jenis-jenis Dipterocarpaceae.

Percobaan penanaman dua jenis *Shorea* di wanatani karet dengan sejarah lahan dan umur karet yang berbeda menunjukkan bahwa inokulum ektomikoriza tetap bertahan hidup di dalam tanah setelah hutan berubah menjadi wanatani karet (bab 5). Identifikasi molekular mikoriza pada bibit *S. selanica* dan *S. lamellata* yang di tanam di wanatani karet mengungkapkan terdapat 5 genus fungi ektomikoriza, yaitu: *Tomentella, Pisolithus, Clavulina, Sebacina* and *Sistotrema* (bab 6). Tidak ada fungi ektomikoriza yang teridentifikasi sebagai *S. columnare*, yang telah diinokulasikan kepada bibit kedua jenis *Shorea* di persemaian. Ini mengindikasikan bahwa indigenus fungi ektomikoriza mendominasi bibit dipterokarpa yang ditanam di lapangan, tanpa memperdulikan inokulasi ektomikoriza di persemaian (bab 5).

Tomentella merupakan fungi yang umum dijumpai mengkolonisasi bibit dipterokarpa di persemaian dan pada percobaan *in-situ*, 1 tahun setelah penanaman bibit di lapangan. *Tomentella* sp.1 dijumpai di semua percobaan, dan mengindikasikan bahwa jenis tersebut memiliki penyebaran yang luas.

Ekstraksi DNA dari ujung akar dipterokarpa yang bermikoriza dan identifikasi molekular ektomikoriza memberikan perspektif yang sangat berbeda terhadap komunitas fungi ektomikoriza pada dipterokarpa dibandingkan metode lain yang berdasarkan survei dan pengamatan tubuh buah (*sporocarp*), sehingga melewatkan pengamatan jenis *Tomentella*. Upaya untuk mengisolasi fungi ektomikoriza ke media kultur (dalam hal ini media Hagem dan MMN) dari akar dipterokarpa tidak berhasil (bab 6), karena fungi ektomikoriza pada umumnya tumbuh lebih lambat dari pada fungi non-ektomikoriza yang mengkolonisasi permukaan akar.

Hubungan antara keragaman pohon di atas permukaan tanah dan fungi ektomikoriza

Pohon-pohon pada suksesi akhir, seperti Dipterocarpaceae dan Fagaceae, dan beberapa jenis Gnetaceae, diketahui berasosiasi dengan fungi ektomikoriza. Ketiga suku pohon tersebut terdapat di wanatani karet dengan kelimpahan yang rendah (bab 2). Dipterocarpaceae memiliki hubungan inang-spesifik terhadap fungi ektomikoriza (Yasman, 1995; Priadjati, 2002). Pohon inang ektomikoriza fungi memiliki peran penting sebagai 'pohon-ibu' bagi anakan, karena banyak benih Dipterocarpaceae berkecambah dan tumbuh di dekat zona perakaran

'pohon-ibu' (Yasman, 1995). Hal ini menunjukkan pentingnya jejaring mikoriza dalam tanah bagi regenerasi tanaman. Hasil penelitian kami menunjukkan bahwa kurangnya pohon yang berasosiasi dengan fungi ektomikoriza di wanatani karet dan di lahan bukan hutan tidak menunjukkan hilangnya inokulum ektomikoriza dalam tanah di ekosistem tersebut.

Hubungan mendasar antara keragaman jenis pohon (di atas permukaan tanah) dengan keragaman jenis biota di bawah permukaan tanah (termasuk mikroba, makro- dan mikro-fauna) dipengaruhi oleh aspek kualitas perakaran, keseimbangan air dan iklim mikro. Demikian pula sebaliknya, keragaman jenis di bawah permukaan tanah mempengaruhi pertumbuhan tanaman dengan meningkatkan efisiensi penyerapan unsur hara dan perlindungan terhadap penyakit akar dan pemakan akar (van Noordwijk and Swift, 1999; Hooper *et al.*, 2000). Awal hubungan antara akar dan fungi dimediasi oleh eksudat akar yang dihasilkan oleh berbagai jenis tanaman, yang menginduksi mikroba simbion, seperti mikoriza dan fungi endofit, untuk mengkolonisasi akar (Bais *et al.*, 2006). Penggunaan tanaman inang, seperti *S. lamellata* dan *S. selanica*, sebagai 'umpan' bagi potensial inokulum ektomikoriza fungi dalam tanah selain tanah dari hutan menunjukkan kemampuan kolonisasi ektomikoriza pada bibit *Shorea* yang sebanding dengan potensial inokulum mikoriza yang terdapat pada tanah hutan (bab 3).

Kernaghan (2005) menyatakan bahwa keragaman tanaman berhubungan positif dengan keragaman jenis fungi ektomikoriza, dan memiliki mekanisme umpan balik yang positif. Penelitian kami di rumah kaca menggunakan 5 jenis dipterokarpa yang didasarkan pada pola *'restriction fragment length polymorphism'* (RFLP), menunjukkan bahwa komunitas ektomikoriza pada dipterokarpa menghasilkan keragaman jenis yang lebih rendah dibandingkan dengan percobaan *'in-situ'*, dengan penanaman 2 jenis bibit *Shorea* di lapangan (bab 6). Dengan penanaman bibit *Shorea* di wanatani karet dapat memulihkan jejaring mikoriza dalam tanah di lahan tak berhutan (bab 5). Sýkorová *et al.* (2007) menyimpulkan bahwa bias perbedaan sistem kultivasi (penanaman) antara persemaian dan penanaman di lapangan menghasilkan perbedaan keragaman fungi arbuskular mikoriza (FAM) pada empat jenis tanaman yang berbeda. Hal ini diakibatkan oleh terjadinya suksesi komunitas FAM terjadi pada tahap persemaian. Suksesi fungi ektomikoriza juga dilaporkan terjadi pada suksesi primer setelah bencana alam, seperti kebakaran hutan (Visser, 1995).

Hasil penelitian kami menunjukkan bahwa rendahnya keragaman jenis vegetasi di padang ilalang (*Imperata cylindrica*), dibandingkan dengan wanatani karet dan hutan), tidak menyebabkan kehilangan fungsi ekosistem yang parah, terutama inokulum simbion fungi. Kesimpulan ini berpotensi menyumbang nilai penting bagi upaya reboisasi hutan dan rehabilitasi lahan dengan jenis-jenis Dipterocarpaceae atau jenis-jenis pohon cepat tumbuh lain yang berasosiasi dengan fungi ektomikoriza, seperti *Acacia* dan *Eucalyptus*.

Implikasi praktis terhadap pengelolaan hutan secara lestari di Indonesia

Ketika penelitian ini sedang dilakukan, pernyataan yang mendasari penalaran restorasi hutan alam setelah pembalakan melalui pengayaan jenis seolah menjadi cerita fiksi. Akibat penebangan liar, hutan dan termasuk plot-plot penelitian di dalamnya lenyap dalam seketika, walaupun kawasan hutan telah dilindungi di sepanjang jalan *logging* dan perlindungan yang ketat dari petugas keamanan, polisi, dan penguasa lokal (yang bertindak sebagai makelar). Petugas penanggung jawab kehutanan yang ingin melindungi restorasi hutan tidak mampu mengontrol keadaan. Salah satu pemicu pembalakan liar adalah meningkatnya kelangkaan kayu untuk konsumsi penduduk lokal di Indonesia, terutama di Sumatra, karena berkurangnya produksi kayu dari hutan alam dan hutan tanaman.

Salah satu alternatif untuk merestorasi hutan adalah penanaman pohon oleh petani di lahan mereka yang dapat mereka kelola sendiri untuk dipanen kayunya di masa depan. Namun penanaman jenis-jenis Dipterocarpaceae di wanatani karet menghadapi beberapa tantangan (van Noordwijk et al, 2004; Tata *et al.*, 2008a). Peraturan yang berlaku tidak mendukung penebangan dan pemasaran kayu yang ditanam petani penggarap di lahan mereka. Hal ini menyurutkan minat petani untuk menanam kayu dari jenis-jenis dipterokarpa (Tomich dan Lewis, 2001; Suyanto *et al.*, 2004).

Meski beberapa petunjuk tenis mengenai penanaman dipterokarpa telah diterbitkan pada skala nasional (Anonymous, 2004; Santoso *et al.*, 2006; Tata *et al.*, 2008b), petani karet masih memiliki pengetahuan yang terbatas mengenai penanaman jenis-jenis Dipterocarpaceae. Mereka juga memiliki akses yang terbatas untuk mendapatkan benih dan bibit jenis pohon kayu (Tata *et al.*, 2008a). Jika petani berkeinginan untuk menanam dan mengelola pohon kayu, sebaiknya menerapkan teknologi persemaian yang sederhana, dan tiap tahap hendaknya dijelaskan secara rinci. Dengan metode yang tersedia, penelitian lanjutan sebaiknya difokuskan pada pendugaan pertumbuhan, pengukuran biomasa serta kesesuaian ukuran dan bentuk celah (*gap*) untuk pengayaan jenis di kebun karet dengan pohon dipterokarpa.

Kesimpulan singkat dari tesis ini adalah biologi tanah tidak menjadi kendala dalam usaha pengayaan jenis dengan jenis-jenis Dipterocarapceae di Sumatra, Indonesia.

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... Enjoy your achievements as well as your plans. Keep interested in your own career however humble; it is a real possession in the changing fortunes of time.

...

And whether or not it is clear to you, no doubt the universe is unfolding as it should. Therefore, be at peace with God, whatever you conceive Him to be. And whatever your labors and aspirations, in the noisy confusion of life, keep peace in your soul. With all its sham, drudgery, and broken dreams, it is still a beautiful world. Be cheerful. Strive to be happy.

"Desiderata" by Max Ehrmann (1927).

List of Publications and Posters[1]

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Curriculum Vitae

Made Hesti Lestari Tata (Hesti) was born in May 25th, 1970, in Denpasar, Indonesia. She spent her childhood in Denpasar and moved to Bandung in 1984. She graduated from senior high school in 1988 in Bandung. She obtained her bachelor degree (*Sarjana Sains*) at the Biology department, Faculty of Mathematics and Science, Bogor Agricultural University, in Bogor in 1993. In 1994 she started working as junior researcher in the Ministry of Forestry at the Forest Research Institute Samarinda in Samarinda, East Kalimantan. In 1995 she joined Tropenbos-Kalimantan Programme as a counterpart in Botany group and transferred to Wanariset Samboja, Balikpapan. In 1996 she transferred to the Forest and Nature Conservation Research and Development Centre (FNCRDC) in Bogor and joined with Silviculture group.

In 1998 she was awarded a fellowship from Tropenbos-Kalimantan programme to pursue Master degree in the post-graduate study of Forestry, Bogor Agricultural University, Bogor. She worked in a collaborative research project on the impact of forest fire in Sungai Wain protection forest in East Kalimantan. Her master thesis entitled "Effect of forest fire on the survival of ectomycorrhizal dipterocarp fungi (A case study in Sungai Wain protection forest, East Kalimantan)". After completion of her master in 2001, she returned to FNCRDC, Bogor.

In 2003 she was awarded a DELTA scholarship Utrecht University to pursue Ph.D. study at the Department of Plant Ecology. She started at Utrecht University in January 2004. Her Ph.D. proposal entitled "Diversity of ectomycorrhiza and root endophyte fungi at different landuse type sites in Jambi (Sumatra, Indonesia)" was accepted and awarded a Ph.D. scholarship from NUFFIC (the Netherlands Organization for International Cooperation in Higher Education) which started in January 2005. She worked under the supervision of Prof. Dr. M.J.A. Werger (Utrecht University), Prof. Dr. G.S. de Hoog (Fungal Diversity Centre – CBS), Dr. Meine van Noordwijk (World Agroforestry Centre – ICRAF) and Dr. Richard Summerbell (Sporometrics, Inc.). Eventually she completed her dissertation entitled: "Mycorrhizae on dipterocarps in rubber agroforests (RAF) in Sumatra".

She is a member of several professional organizations, such as the International Society of Tropical Forester (ITSF), the International Mycorrhizae Society (IMS), The British Mycological Society (BMS), the Asia Pacific Association of Forestry Research Institution (APAFRI) and the Indonesian Mycorrhiza Society (AMI). She was awarded several fellowships, such as: a Netherlands Fellowship Programme for regular course in 2002; a travel grant from Freezailah fellowship – International Tropical Timber Organization (ITTO) in 2003 and a travel grant from The British Mycological Society in 2006.

After completion of her Ph.D. in December 2008, she will return to Indonesia and continue her career as a scientist at the Forest Research and Development Agency, the Ministry of Forestry.

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