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Growth response of *Pterocarpus soyauxii* and *Lophira alata* seedlings to host soil mycorrhizal inocula in relation to land use types

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ABSTRACT
Deficiency in mycorrhizal inoculum in soils due to land use types (LUT) can be alleviated by inoculum addition. Inoculum effects may depend both on quantity and on quality of inoculum applied. A greenhouse bioassay was carried out to determine the effect of host soil mycorrhizal inoculum on mycorrhizal colonization, carbon allocation and partitioning of seedlings of two native timber species of Cameroon humid forest, raised on surface soils (0-20 cm) collected from early secondary forests, LUT derived from slash-and-burn agriculture and selective logging. Host soil mycorrhizal inoculum was collected from the root zones of con-specific tree species. Mycorrhizal inoculation effect (MIE) was estimated as percent difference of average total biomass between seedlings grown on inoculated and non-inoculated soils. Six months-old seedlings of *Pterocarpus soyauxii* and *Lophira alata* were mainly colonized by members of the Glomaceae and Gigasporaceae, respectively, as shown by molecular typing. They generally performed poorly in soils with indigenous inoculum. But addition of soil inoculum from *P. soyauxii* trees favored nodulation, significantly increased mycorrhizal colonization and total biomass but decreased root-to-shoot ratios, resulting in large and positive MIE, irrespective of LUT. In contrast, host soil inoculum of *L. alata* did not affect fractional mycorrhizal colonization but significantly increased total biomass, resulted in high carbon allocation to roots, in low and sometimes negative MIE. Therefore, seedlings' responses to mycorrhizal inoculum depend on host soil inoculum and that could be critical for successful rejuvenation of tropical trees.
Key words. Arbuscular mycorrhiza-host soil inoculum-land use types-*Pterocarpus soyauxii*-*Lophira alata*-Cameroon

INTRODUCTION

Current rates of deforestation and timber exploitation in the humid tropics suggest that sustained production of highly priced timbers will require alternative forest management scheme. Such design includes plantation forestry and artificial regeneration through quality seedlings in enrichment planting of logged-over forest management units or former agricultural farms. Recruitment and establishment of seedlings in natural forests and artificial woodlands are vital for forest structure and diversity.

Virtually, seedlings of all rain forest trees are arbuscular mycorrhizal (AM) and many of them strongly depend on, and are highly responsive to mycorrhizal fungi [1-4]. However, the availability of mycorrhizal fungal inoculum might not always be sufficient and suitable to guarantee optimal seedling growth under the prevailing soil physical, chemical and biological conditions, thereby, suggesting that quality of mycorrhizal propagules in substrates or soils may determine seedling's fitness.

In particular, land use practices may affect the abundance of mycorrhizal fungal inoculum. It was noted scarce native mycorrhizal fungi in nurseries in East Africa for most grown tree species [5]. Degraded tropical savannas of Venezuela also lacked enough autochthonous mycorrhizal inoculum to enhance seedling survival and growth [6]. In general, agricultural practices did not negatively affect quantity and quality of mycorrhizal fungal propagules [7, 8]. On the opposite, logging practices such as mechanical clearing, creation of forest roads, skid trails and landings, erosion and compaction have been shown to always be detrimental to mycorrhizal inoculum [4, 9, 10, 11]. Consequently, growth of seedlings could be enhanced by addition of ample mycorrhizal fungal propagules to nursery substrates.

Influence of mycorrhizal inoculum on seedlings' growth of distinct host plants has been little

investigated in the tropics. This may be partly due to production constraints of large quantities of efficient and effective mycorrhizal inocula for field application. This impediment is likely to be partly alleviated if small quantities of soil could harbor adequate mycorrhizal inoculum to enhance growth and establishment of seedlings in soils deprived of effective mycorrhizal fungi. Such low-input technology, successfully applied in African nurseries for exotic, ectomycorrhizal pines inoculation with natural soil and humus from established plantations [12], could also be valuable for silviculture of indigenous tree species, irrespective of whether they form ectomycorrhiza or arbuscular mycorrhiza. Introduced mycorrhizal inocula in phosphorus-deficient sandy soils improved growth of *Parkia biglobosa, Tamarindus indica* and *Ziziphus mauritiana* but to various degrees [13]. A consortia of indigenous AM fungi differently and significantly increased mycorrhizal root colonization and plant biomass of five fodder crops [14]. Both *Dicorynia guianensis* and *Eperua falcata* seedlings differed in mycorrhizal response as a combination of high seed mass, phosphorus reserve and quality of mycorrhizal inoculum [15]. Thus, preferential associations rather than ecological specificity *sensu stricto* may be more common in AM symbiosis. AM fungi may have different colonization strategies, varying both with the fungus and host plants [16]. Mycorrhizal colonization effect may also vary with successional status of host tree species [3]. and seed reserves [17]. In tropical rain forests, there may be an overwhelming set of interactions between host plants and mycorrhizal fungi [18-20].

The aims of this investigation were to determine how varied mycorrhizal colonization, above- and below-ground biomass of seedlings of two major native timber species of Cameroon humid forests grown in soils with different inherent inoculum potential of AM fungi caused by slash-and-bum agriculture and selective logging, and to assess their responsiveness to inoculation with host soil mycorrhizal inoculum taken from the root zones of con-specific tree species.

MATERIALS AND METHODS

Site and vegetation description

The soils used for the bioassays belonged to the Ebom series. Ebom (3°06'N; 10°44'E) is a rural community, on a rolling landscape (350-500 m a.s.l), located 114 km east of Kribi (2°57'N; 9°55'E), in the southern coastal zone of Cameroon. Annual rainfall varies between 1500 and 2000 mm with two maxima in May and October; average monthly temperatures fluctuate between 23°C and 27°C. Relative humidity is generally high, above 80% [7]. Soils are moderately heavy clayey (60-80% clay), strongly acid (pH water 4.7), with low to medium organic matter content and very poor in available phosphorus (0.005 μg P in H_2O ml^{-1} soil). They are classified in the FAO system as Xanthic Ferralsol [21]. Within the Ebom area, forests are under intense human influence of both fallow-rotational shifting cultivation and selective logging [21, 22].

Five land use types (LUT) were selected, *viz.* food crop fields locally called "Afub Owondo", fallows of *Chromolaena odorata* (Asteraceae), thereafter referred to as fallow, skid trails, bare landings, and landings re-colonised by the pioneer, facultative mycorrhizal tree, *Musanga cecropioides* (Moraceae), thereafter, referred to as re-vegetated landings. In addition, early secondary forest stands were also included as they constitute the first phases of forest recovery following fallow-rotational shifting cultivation practices. These stands are very dense due to the abundance of climbers, young saplings, juveniles, and undergrowth vegetation. Fields of food crops were chosen after slashing the undergrowth vegetation, felling the existing trees, removing surface debris and burning dried biomass. Well-vegetated fallows with shrubs of 3-5 m high of about three to four years old were selected. Fields and fallow are LUT derived from agricultural practices while skid trails and both types of landings are LUT resulting from selective logging; re-vegetated landings constitute the first phase of forest recovery following selective logging practices.

Timber species

Two native, large tree species, which provide highly valued timber and which are among the most frequently harvested trees nationwide, were chosen: *Pterocarpus soyauxii* (Fabaceae) and *Lophira alata* (Ochnaceae), locally known as Padouk and Azobe, respectively. *P. soyauxii* (Taub.) is commercially popular, being harvested for exports in Gabon, Equatorial Guinea, and Cameroon where it is well-renowned for the local wood transformation industry. Its seeds are flat,

circular (diameter about 1.5 - 2 cm) and papery (0.1 g). Seedlings develop a finely branched whitish root system devoid of root hairs but with numerous large pinkish nodules. *L. alata* (Banks ex Gaertn.) is harvested in Ghana, Gabon, Equatorial Guinea, and Cameroon. Its seeds are bulging and elongated, weighing about 1.0 g. Seedlings grow rapidly and produce abundant dark red roots which are coarsely branched and devoid of root hairs.

Collection of soil samples and host soil mycorrhizal inoculum

Surface soils cores (0 - 20 cm) were collected from three random and independent spots in each land use type, bulked into about 20 kg composite of soil and fine root samples.

The host soil mycorrhizal soil inoculum used for inoculum addition was a mixture of fresh soil and fine root samples (0 – 10 cm) taken at four cardinal points around and in the vicinity of the stem base of five widely spaced mature seed-bearer trees of *P. soyauxii* and *L. alata.* These inocula will be thereafter referred to as *Pterocarpus* and *Lophira* inoculum, respectively.

Collected soil samples and mycorrhizal inocula were placed, separately, in air-filled polyethylene bags, taken to Kribi, and kept under greenhouse benches to preserve soil from direct sun heat. Abundance of each type of mycorrhizal propagules in soils of all LUT and early secondary forest was determined by three methods (Table 1).

Attempt to identification of spore types from host soil inoculum from *Pterocarpus* and *Lophira* trees in trap culture was carried out from DNA extraction from pool of spores using DNeasy Plant Mini Kit (QIAGEN), followed by PCR amplification of partial LSU rDNA region, cloning, restriction fragment length polymorphism analysis and sequence alignment and phylogenetic analysis [23, 24].

Mycorrhizal inoculation

Three days after field collection of soil and inoculum samples, half of potted bags were thoroughly mixed with 50 g (d.w. basis) portions kg^{-1} soil of either *Pterocarpus* or *Lophira* inoculum. Non-inoculated unsterile soils received similar amounts of steam sterilized host soil inoculum and 50 ml kg^{-1} soil of filtered soil inoculum leaching to insure similar microbial activity (Hetrick et al., 1988). Steam sterilization was achieved at 100°C for 1 hour. The sterilized soil was left to stand for five days on greenhouse benches before use.

For each plant species, there were 36 experimental units composed of six land use types (LUT), two inoculation treatments and three replicates. In addition, a triplicate control was prepared to assess growth and mycorrhizal colonization effect of host rhizospheric soil inoculum. Plastic bags were placed on greenhouse benches in a randomized complete block design.

Seed collection and treatment

Seeds were collected around seed-bearer mother trees in Ebom. Bulging and firm *L. alata* seeds were soaked overnight in cool water before manual scarification; flat seeds of *P. soyauxii* did not require a pre-treatment. Seeds of both plants were sterilized in 70 % alcohol for 1 min, rinsed three times with sterile water, and placed in washed and sterilized sea sand. One one-week-old pregerminated seed was then placed in five kg (dry weight basis) portions of potted soils. Plants were grown without nutrient amendment. Water was added as needed to maintain potted soils at water holding capacity. Plants were raised under natural light in a shaded house in Kribi.

Assessment of seedling growth and mycorrhizal colonization

Six months after transplanting, shoots were separated from the root systems, dried at 70°C for 72 hours and 24 hours, respectively. Then, shoot and root dry weights were taken; total biomass and root-to-shoot ratio were derived. Nodules on roots of *P. soyauxii* were counted and grouped in three categories: category 1: 1 - 5 nodules, 2: 6 - 10; 3: above 10.

The response to host soil mycorrhizal inoculation was assessed as mycorrhizal inoculation effect (MIE). MIE were calculated as follows:

$$MIE = 100 (a - b/a)$$

where a and b were the average total biomass of seedlings grown in soil with host soil and indigenous inoculum added, respectively.

Fractional colonization by AM fungi was estimated by scoring the presence or absence of mycorrhizal fungal structures in at least 100 intersection points between root fragments and gridlines of a petri dish, under a dissecting microscope at 40x [7]. after clearing, bleaching

(only of roots of *L. alata),* staining with fuchsin acid and destaining in a lacto-glycerol solution [22]. 10 to 20 short root fragments were randomly chosen, mounted under glass and cover glass, gently squashed and examined under a light microscope at 25 – 40 X to confirm mycorrhizal structures such as arbuscules, vesicles, hyphal coils, internal hyphae, and/or auxiliary bodies attached to external hyphae.

Statistical analyses

Statistical analyses were performed using the SAS package [26]. All data were tested for normality and homogeneity of variances. As variances were unequal, fractional colonization data were arcsin square root transformed and total biomass dry weight data square root transformed. A two-way analysis of variance (ANOVA) with land use types and mycorrhizal inoculum as independent variables was performed using the general linear model. Average means were separated by Duncan's multiple range tests. Pearson's correlation coefficients were calculated between fractional mycorrhizal colonization, total biomass of seedlings and root-to-shoot ratio. Mycorrhizal inoculation effect (MIE) was based on average total biomass of seedlings with indigenous inoculum and seedlings with host soil mycorrhizal inoculum added, thus, no statistical tests of MIE were executed.

RESULTS

Pterocarpus soyauxii

Identification of spores from both *Pterocarpus* soil inocula showed mostly *Glomus* species including *Glomus constritum*, *G. manihotis, G. coranatum* and the most abundant small size and hyaline *Glomus* sp (Table 1).

In the presence of indigenous inoculum, few nodules were observed in soils from all land use types (LUT) but the re-vegetated landings, *Pterocarpus* soil inoculum increased the number of nodules in soils from all LUT; the highest nodule number was recorded in soils from food crop fields and re-vegetated landings (Table 1).

Mycorrhizal colonization of roots of *P. soyauxii* seedlings consistently showed abundant intra-radical hyphae, variously shaped vesicles, few arbuscules and hyphal coils; a small number of auxiliary bodies on extra-radical hyphae were also observed.

Fractional mycorrhizal colonization (FMC) of roots of *P. soyauxii* seedlings was very highly significantly affected by LUT and by inoculum addition but not by their interaction (Table 2). Before inoculation, FMC was the highest in soils from re-vegetated landings and food crop fields. It substantially increased after addition of *Pterocarpus* inoculum in soils from all LUT; it was still the lowest in soils of bare landing, though (Table 4). FMC was positively and very highly correlated to total biomass: $r = 0.875$, $n = 36$; $P < 0.0001$.

Total biomass was significantly affected by LUT and very highly significantly affected by addition of *Pterocarpus* inoculum but not by their interaction (Table 2). In soils taken under *P. soyauxii* seed-bearer trees, total biomass averaged 11.6 g per seedling. In the presence indigenous inoculum, total biomass was lower in soils from all LUT; the lowest total biomass was observed in soils from bare landings. Addition of *Pterocarpus* inoculum significantly increased total biomass in soils from all LUT but the re-vegetated landings; fallow soils yielded the highest total biomass with similar level of magnitude as the control (Figure 1A). There was no correlation between total biomass and root-to-shoot ratio.

Root-to-shoot ratio of *P. soyauxii* seedlings was very highly significantly affected by addition of *Pterocarpus* inoculum but neither by LUT nor by their interaction (Table 2). Mycorrhizal inoculation generally decreased root-to-shoot ratio with the exception of seedlings grown in soils from food crop fields and re-vegetated landings (Table 3). Root-to shoot ratio was negatively correlated with FMC: $r = 0.374$; $n = 36$; $P < 0.0248$.

Mycorrhizal inoculation effect (MIE) on *P. soyauxii* seedlings was large and positive, irrespective of LUT; the highest MIE was recorded in soils from bare landing and the lowest in soils from re-vegetated landings (Table 4).

Table 1: Variation in inoculum potential of indigenous arbuscular mycorrhizal fungi in relation to land use types and changes in nodule number before and after addition of soil inoculum ITom *Pterocarpus soyauxii* seed-bearer trees

Land use types	Spore number[1]	MPN[2]	Root colonization [3]	Nodule number[4]	
				Indigenous inoculum	Tree-specific inoculum
Food crop fields	28b	29	31a	1	3
Fallow	44a	55	39a	1	2
Secondary forest	21b	16	23b	1	2
Skid trails	9c	0	16c	0	2
Bare landing	5c	0	3d	0	2
Re-vegetated landing	8c	6	25b	2	3
Some AM spore species identified from the root zones of soil inocula of both tree species				*G. constritum* *G.manihotis* *G.coronatum* *Glomus* sp	*Gi.decipiens* *Gi.margarita* *Gi.gigantae* *Sc.nigra*

Different letters indicate significant differences at p < 0.05%.

[1]Number of spores.g^{-1} dry soil assessed by the wet decanting and sugar centrifugation method [7]. [2]Number of infective propagules.g^{-1} dry soil assessed by a four-fold dilution series with five replicates a local variety of cowpea *(Vigna unguicu/ata)* (Fabaceae) as bait plant [38]. [3]Root colonization (percent mycorrhizal root length colonized) of a local variety of cowpea in intact soil cores, replicated five times and grown for 30 days under greenhouse conditions in Kribi, South Cameroon. [4]Nodule numbers in roots of seedlings of *Pterocarpus soyauxii* defined as: 1: 1 - 5 nodules per seedling; 2: 6 - 10 nodules per seedling; 3: > 10 nodules per seedling.

G: *Glomus*; Gi: *Gigaspora*; Sc : *Scutellospora*

Fallow refers to fallow of *Chromolaena odorata;* Re-vegetated landing refers to bare landing re-colonized by the pioneer tree *Musanga cecropioides.*

Table 2: General linear model procedure of arc sin transformed fractional mycorrhizal colonization, square root transformed total biomass and root-to-shoot ratio (RIS ratio) of six months-old *Pterocarpus soyaux;* and *Lophira alata* seedlings in relation to land use types (LUT) and addition of host-tree specific inoculums

Sources of variation	Fractional colonization			Total biomass		R/S ratio	
	df	F	p	F	p	F	p
			Pterocarpus soyauxii				
LUT	5	10.4	<0.0001 **	2.84	0.0176*	2.52	0.057ns
Inoculum	1	68.5	<0.0001 **	29.1	<0.00001**	11.7	0.00023**
LUT x Inoculum	5	1.34	0.281ns	1.20	0.375ns	2.40	0.0684ns
			Lophira alata				
LUT	5	4.14	0.0075**	24.7	<0.0001**	4.15	0.0074**
Inoculum	1	1.96	0.175ns	9.04	0.0061**	7.57	0.0111*
LUT x Inoculum	5	0.53	0.749ns	6.70	0.0005**	0.58	0.716ns

ns: non significant at p<0.05%; * significant (p<0,05%) and ** highly significant (p< 0.01%).

Lophira alata

Lophira soil inoculum was dominated by AM fungi belonging to Gigasporaceae family, particularly, *Gigaspora decipiens, Gisgaspora margarita, Gigaspora gigantae* and *Scutellospora nigra* (Table 1). Mycorrhizal colonization of roots *L. alata* seedlings was inconsistently extra-radical with large and thick-walled hyphae, frequently carrying numerous spiny auxiliary cells; internal colonization was low without arbuscules, vesicles and hyphal coils.

Fractional mycorrhizal colonization of *L. alata* was very highly significantly affected only by LUT but neither by inoculum addition nor by their

interaction (Table 2). The highest fractional mycorrhizal colonization (FMC) was recorded from re-vegetated landings and was of the same level of magnitude as that of food crop fields. However, it was the lowest in soils from bare landings, low in fallow soils, intermediate in soils from successional forest and skid trails (Table 4). FMC was not correlated with total biomass.

Total biomass of *L. alata* seedlings was very highly significantly influenced by both independent variables and their interaction (Table 2). In soils taken under *L. alata* seed-bearer trees, total biomass was 12.2 g per seedling. In the presence of indigenous inoculum, total biomass of *L. alata* seedlings was significantly lower in soils from skid trails, bare and re--vegetated landings than in soils from fields and fallow. Addition of *Lophira* inoculum significantly increased total biomass in soils from all LUT but fallow and re-vegetated landings (Figure 1B). Total biomass was negatively correlated with root-to-shoot ratio: $r = 0.466$; $n = 36$; $P < 0.0041$.

Root-to-shoot ratio of *L. alata* seedlings was very highly affected only by inoculum but neither by LUT nor by their interaction (Table 2). In soils from most LUT but skid trails, root-to-shoot ratio was increased after addition of *Lophira* soil inoculum (Table 3).

Mycorrhizal inoculation effect on *L. alata* seedlings was generally low and positive; it was however negative in soils from fallow and re-vegetated landings (Table 4). There was no correlation between root-to-shoot ratio and FMC.

Table 3: Variation in root-to-shoot ratio (RIS ratio) of six months-old seedlings of *Pterocarpus soyauxii* and *Lophira alata* seedlings in relation to land use types (LUT) and addition of host-tree specific inoculum

Land use types/ Tree species/ Mycorrhizal inoculum	*Pterocarpus soyauxii*		*Lophira alata*	
	Indigenous	Tree-specific	Indigenous	Tree-specific
Food crop fields	0.667	0.461	0.319	0.385
Fallow	0.534	0.545	0.347	0.380
Secondary forest	0.902	0.666	0.332	0.406
Skid trails	0.726	0.321	0.681	0.554
Bare landing	0.728	0.439	0.663	0.672
Re-vegetated landing	0.471	0.478	0.529	0.567

NB: Root-to-shoot data were based on average root and shoot biomass. Hence, statistics were not applied.

Table 4: Fractional mycorrhizal colonization (FMC) after addition of *Pterocarpus* and *Lophira* inocula and mycorrhizal inoculation effect (MIE) based on total biomass of six months-old seedlings of *Pterocarpus soyauxii* and *Lophira alata* seedlings in relation to land use types

Tree species	*Pterocarpus soyauxii*		*Lophira alata*	
Land use types	FMC	MIE	FMC	MIE
Food crop fields	52.7b	+55	34.0 ns	+ 18
Fallow	69.4 a	+79	12.7 ns	- 42
Secondary forest	58.4 ab	+ 56	16.7 ns	+24
Skid trails	54.0b	+41	16. ns 7	+ 14
Bare landing	26.3 c	+ 81	2.00 ns	+20
Re-vegetated landing	72.7 a	+23	36.7 ns	- 31

Different letters indicate significant differences at $p < 0.05$. ns: no significant differences at $p < 0.05$. Fallow refers to fallow of *Chromolaena odorata;* Re-vegetated landing refers to bare landing re-colonized by the pioneer tree *Musanga cecropioides.*

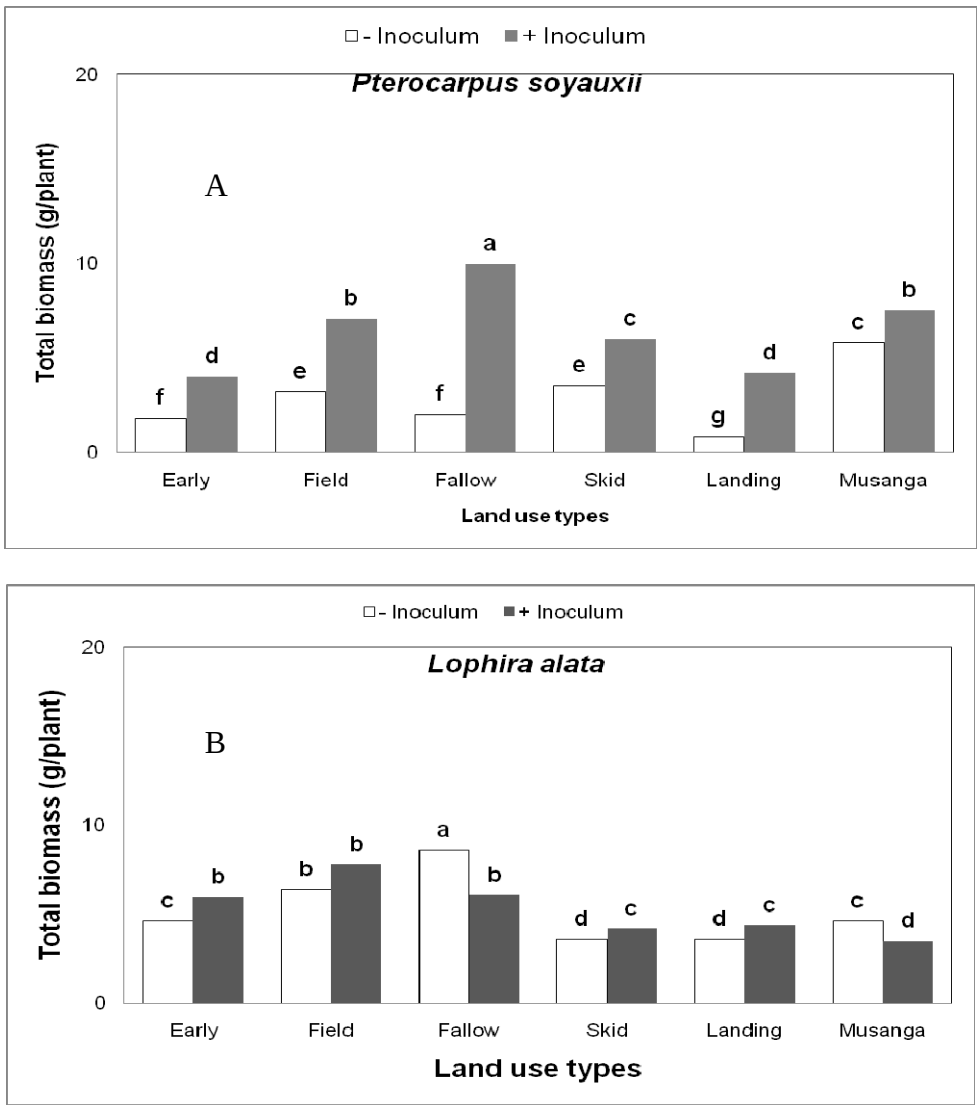

Figure 1: Total biomass of six months-old seedlings of *Pterocarpus soyauxii* (A) and *Lophira alata* (B) in relation to land use types (LUT) and addition of host-tree specific inoculum.
Different letters indicate significant differences at $p < 0.05$

DISCUSSION

Initial evidence of the effect of host soil mycorrhizal colonization of seedlings of both tree species was provided by dissimilar morphological characteristics of colonizing AM fungi. Mycorrhizal colonization of roots of *P. soyauxii* seedlings showed abundant inter- and intra-hyphal mycelia, profuse vesicles, few arbuscules and hyphal coils, typical of the *Arum-type* mycorrhizas [27]. The presence of variously shaped vesicles in roots of *P. soyauxii* seedlings strongly indicates mutual associations with AM fungi of the Glomacean family. On the opposite, the presence of numerous spiny auxiliary cells on external hyphae attached to roots of *L. alata* seedlings and the large size of internal hyphae points out to colonization mainly by members of the Gigasporaceae. In another investigation, seedlings of both *Dicorynia guianensis* and *Eperua falcate* exhibited the *Paris-type* AM colonization [15].

AM fungi may differ in their colonization strategies depending both on the type of fungal propagules and the host plants. Isolates of *Glomus* and *Acaulospora* were both able to colonize host plants from spores, colonized root fragments and extra radical hyphae while those of *Gigaspora* and *Scutellospora* colonized only from colonized root fragments, to a lesser extent, though [28]. However, regulating mechanisms of particularly effective host-mycorrhizal fungus interactions remain to clarify.

Attempt to identify spores from tree-specific inocula used in this study clearly indicates that different AM fungal taxa could preferentially associate with different tree species and elicit enhanced growth. Seedling performance of common *Pulsatilla* species grown with native AM fungal communities from grassland compared with forest inoculum was more vigorous than that of rare *Pulsatilla* species [29]. A large degree of selectivity of the effect of mycorrhizal colonization has also been recently noted on tropical tree species. In Guyana rain forest, seedlings of *Dicorynia guianensis* and of *Eperua falcate* (both members of the Caesalpiniaceae) grown in soil with roots of mature trees of the former trees species had dissimilar growth response, with increased and decreased growth of the first and the second, respectively [15]. Host tree selectivity of AM fungi has also been observed for *Erythrophloeum ivorensis* (Caesalpiniaceae) where members of the Gigasporaceae proliferated [19]. If preferential associations of AM fungi with various tree species are widespread in tropical rain forests, it could to some extent contribute to the explanation for the lack of success in artificial regeneration attempts of indigenous timber species even in the presence of large quantity of mycorrhizal inoculum.

Our results demonstrate that addition of host tree-specific inoculum, just like addition of grass inoculum, can yield substantially larger seedlings of timber species [4]. *Pterocarpus* and *Lophira* soil inocula significantly increased carbon build up and allocation of seedlings but to different extent. Similar results were recorded by earlier workers [4, 15, 29, 30, 31]. Various factors could influence mycorrhizal responses of seedlings, such as light demand and seed size. A positive correlation between mycorrhizal colonization and growth performance was recorded in seedlings of *Dicorynia guianensis* raised under 14% of full sunlight, showing a greater mycorrhizal efficiency [32]. Responses in growth and biomass allocation to AM colonization were higher under small gap light intensities and resulted in larger plants with small carbon allocation to roots [31]. In this study, total biomass of seedlings of *P. soyauxii* grown under full light was positively and highly correlated with fractional mycorrhizal colonization, conversely to *L. alata.*

Differences between tree species in dependency on and responsiveness to AM fungi has been attributed to various characteristics. It was proposed that early successional species are generally less dependent on arbuscular mycorrhizas than late successional and climax forest tree species [2]. *L. alata* is considered a pioneer tree that may have expanded in the "Forêt littorale" during the 18th and 19th centuries following forest clearings and man-induced fallowing; conversely, *P. soyauxii* is a climax tree species of Atlantic Biafrean moist forests of south Cameroon [33]. Thus, the higher growth response of *P. soyauxii* seedlings than that of *L. alata* seedlings is consistent with the successional hypothesis on mycorrhizas. Seedlings of small-seeded pioneer species also were more dependent on AM inocula for initial survival and growth [18]. However, mycorrhizal dependency of pioneer, shade tolerant and light-demanding Brazilian woody species varied from highly to very highly mycorrhizal dependent [3].

Root features have also been implicated as a determinant of mycorrhizal dependency and responsiveness [34]. Incidence of root mass may be more important than other root features such as root diameter, root density, root hair incidence, and root hair length [35]. Species with a lower root production are predicted to be highly mycorrhizal dependent. In this study, six months-old seedlings of *P. soyauxii* and *L. alata* did not markedly differ in carbon allocation to roots and likewise lacked root hairs. After soil inoculation, root-to-shoot ratio of *P. soyauxii* seedlings significantly decreased in all land use types but fallow and re-vegetated landings, conversely to *L. alata* seedlings, with the exception of soils from skid trails. Decrease in carbon allocation to roots of *P. soyauxii* seedlings also suggests the efficiency of *Pterocarpus* soil inoculum. Both groups of seedlings also markedly differ in growth rate after addition of quality inoculum. Seedlings of *P.*

soyauxii grew very rapidly following tree-specific inoculation while those of *L. alata* did not [7].

It was suggested that non-mycotrophic and facultatively mycotrophic species have lighter seeds than obligately mycorrhizal species [2]. Our data do not conform to this hypothesis, as light-seeded seedlings of *P. soyauxii* were more responsive to tree-specific addition than heavy-seeded seedlings of *L. alata.* The high phosphorus requirements of a nodulating legume might make such species more mycotrophic, independent of seed size. However, in an earlier study with seedlings of three timber species [4], it was observed that the non-nodulating legume *Distemonanthus benthamianus* (Caesalpiniaceae) with heavy seeds had a larger mycorrhizal inoculation effect than *Terminalia superba* (Combretaceae), thus, confirming the nutrient effect stirring up mycorrhizal demands. By contrast, seedlings of the large-seeded caesalp tree species *E. falcate* seemed less responsive to mycorrhizas [36].

Seedlings of both timber species differed in specific mycorrhizal inoculation effect. In general, MIE was large and positive for *P. soyauxii* seedlings. For *L. alata* seedlings, MIE was low and negative in soils from fallow and re-vegetated landings. Fallow soils showed high mycorrhizal inoculum potential across various sites in South Cameroon [7]. However, both seedlings varied in growth in soils from agricultural practices. Poor growth of *P. soyauxii* contrasted with relatively good growth of *L. alata* seedlings in these soils, suggesting possible difference in light demand. If indeed *L. alata* is an early light-demanding species, then, it can readily establish on former agricultural land, conversely to *P. soyauxii.*

Both seedlings grew poorly on soils from skid trails and bare landing, to different extent, though, owing to the negative impacts of heavy machinery on physical and chemical soil surface properties in addition to reduction in mycorrhizal propagules (Table 1). A substantial increase in bulk density and soil compaction was also recorded along skid trails and landings, which might be partially responsible for the reduced performance of seedlings on soils of forestry practices. Earlier authors also reported negative effects of logging practices [37]. We regularly observed seedlings of both species on bare landings, but they all died within a few years. However, in our experiment, *P. soyauxii* seedlings grew very well in soils from re-vegetated landings. These logged-over sites are rapidly invaded by the early successional, facultative mycorrhizal tree, *Mussanga cecropioides* [22], and generally show a progressive build up in surface soil horizon and organic matter. It is also possible that contaminating debris from various logging operations could enrich such re-vegetated landings by mixing of different soils horizons.

In conclusion, our results demonstrate that seedlings of timber species do not always form effective mycorrhizal associations with all kinds of arbuscular mycorrhizal fungal communities, confirming the hypothesis that the quality of the mycorrhizal propagules in soils determines seedling fitness. For sustainable timber management and conservation, there is a need for the assessment of inoculum quality for the effective and rapid mycorhization of seedlings of important timber species. This aspect of practical mycorrhization should be considered for successful rejuvenation of forests.

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REFERENCES

1. Janos, D.P. 1980. Vesicular-arbuscular mycorrhizae affect lowland tropical rain forest plant growth. *Ecology* 61: 151-162.
2. Janos, D.P. 1996. Mycorrhizas, succession and rehabilitation of deforested lands in the humid tropics. in: J.C. Frankland, N. Magan & G.M. Gadd (eds), Fungi and environmental change, p. 129-161. Cambridge University Press, Cambridge.
3. Siqueira J.O & Saggin-Junior OJ 2001. Dependency on arbuscular mycorrhizal fungi and responsiveness of some Brazilian woody species. *Mycorrhiza* 9 (6): 245-255.

4. Onguene N.A, Kuyper Th.W. 2005. Growth responses ofthree native timber species to soils with different arbuscular mycorrhizal inoculum potentials in South Cameroon. Indigenous inoculum and effect of addition of grass inoculum. For. *Ecol. Manag*. 210: 283-290
5. Michelsen, A 1992. Mycorrhiza and root nodulation in tree seedlings from five nurseries in Ethiopia and Somalia. For. *Ecot. Manag.* 48: 335-344.
6. Cuenca G., Z. De Andrade., G. Escalante 1998. Arbuscular mycorrhizae in the rehabilitation of fragile degraded tropical lands. *Biol. Fertil. Soils* 26: 107-111.
7. Onguene A.N. 2000. Diversity and dynamics ofmycorrhizal associations in tropical rain forests with different disturbance regimes in south Cameroon. Ph.D thesis, Wageningen University, Tropenbos Documents Series 3, 167p
8. Sieverding, E. 1991. Vesicular-arbuscular mycorrhiza management in tropical agrosystems. Eschborn, Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ) GmbH.
9. Alexander, I., N. Ahmad & L.S. See 1992. The role of mycorrhizas in the regeneration of some Malaysian forest trees. Phil. Trans. *R Soc. Lond*., ser. B 335: 379-388.
10. Carpenter F.L., Mayorga S.P., Quintero E.G., Schroeder M. 2001. Land-use and erosion of a Costa Rican ultisol affect soil chemistry, mycorrhizal fungi and early regeneration. *For. Ecol. Manag.* 144: 1-17
11. Musoko, M., F.T. Last & P.A Masan 1994. Populations of spores of vesicular-arbuscular mycorrhizal fungi in undisturbed soils of secondary semi-deciduous moist tropical forest in Cameroon. *For. Ecot. Manag*. 63: 359-377.
12. Mikola P. 1973. Applications of mycorrhizal symbiosis in forestry practice. in: C. G. Marks & T.T. Kozlowski (eds), Ectomycorrhizae - their ecology and physiology, p. 383-411. New York, Academic Press.
13. Guissou T, Bâ AM., Oualba J-M., Guinko S., Duponnois R 1998. Responses of *Parkia biglobosa* (Jacq.) Benth, *Tamarindus indica* L. and *Ziziphus mauritiana* Lam. To arbuscular mycorrhizal fungi in a phosphorus-deficient sandy soils. *Biol. Fertil. Soils*. 26: 194-198.
14. Gaur A, Adholeya A 2002. Arbuscu1ar-mycorrhizal inoculation of five tropical fodder crops and inoculum production in marginal soil amended with organic matter. *Biol. Fertil. Soils*. 35: 214-218
15. De Grandcourt A, Epron D., Montpioo P., Louisanna E., Béreau M, Garbaye J, Guehl lM. 2004. Contrasting responses to mycorrhizal inoculation and phosphorus availability in seedlings of two tropical rain forest tree species. *New Phytol*. 16: 865-875
16. Bever, J.D. 2002. Host-specificity of AM fungal population growth rates can generate feedback on plant growth. *Plant and Soil*. 244: 281-290
17. Muthukumar T, Udaiyan K 2000. The role of seed reserves in arbuscular mycorrhizal formation and growth of Leuceana leucocephala (Lam.) de Wit and Zea mays L. *Mycorrhiza* 9(6): 323-330
18. Kiers E. T., Lovelock C.E., Krueger E.L., Herre E.A 2000. Differential effects of tropical arbuscular mycorrhizal fungal inocula on root colonization and tree seedling growth: implications for tropical forest diversity. *Ecot. Lett.* 3: 106-113
19. Schmitt C., Brou G., Kuyper Th., Onguene N.A 2001. Mycorrhizal selectivity of three timber species in the tropical rain forest in south Cameroon. *Mitt. Deutsch. Bodenk. Ges*. 96: 371-372
20. Lovelock C.E., Andersen K., Morton R 2003. Arbuscular mycorrhizal communities in tropical forests are affected by host tree species and environment *Oecologia*. 135: 268-279
21. Van Gemerden, B.S. & G.W. Hazeu 1999. Landscape ecological survey (1:100 000) of the Bipindi - Akom il - Lolodorf region, Southwest Cameroon. Tropenbos Cameroon Documents 1: 1-164.
22. Onguene N.A, Kuyper Th.W. 2001. Mycorrhizal associations in the rain forest of south Cameroon. *For. Ecol. Manag*. 140: 277-287
23. Ngonkeu M. E. L., 2003. Biodiversité et potentiel des mycorhizes à arbuscules de certaines zones agro-écologiques du Cameroun. Thèse de Doctorat 3e cycle. Université de Yaoundé I Cameroun 258p.
24. Ngonkeu M. E.L., 2009. Tolérance de certaines variétés de maïs aux sols à toxicité aluminique et manganique du Cameroun et

diversité moléculaire et fonctionnelle des mycorhizes à arbuscules. Thèse de Doctorat Ph.D Université de Yaoundé I Cameroun, 224p.
25. Hetrick, RAD., G.T. Wilson, D.G. Kitt & AP. Schwab 1988. Effects of mycorrhizal contribution to growth of big bluestem grass in non-sterile soil. *Soil Biol. Biochem*. 20: 501-507.
26. SAS Institute Inc. 2004. SAS/STAT users' guide, V.9.13, SAS Campus Drive, NC, USA
27. Smith, S.E. & D.I. Read 1997. Mycorrhizal symbiosis, 2nd ed. London, Academic Press.
28. Klironomos lN., Hart M.M. 2002. Colonization of roots of arbuscular mycorrhizal fungi using different sources of inoculum. *Mycorrhiza*. 12: 181-184
29. Moore M., Opil M., Sen R., Zobel M. 2004. Native arbuscular mycorrhizal fungal communities differentially influence the seedling performance of rare and common *Pulsatilla* species. *Funct Ecot*. 18: 554-562
30. Michelsen, A 1993. Growth improvement of Ethiopian acacias by addition of vesicular arbuscular mycorrhizal fungi or roots of native plants to non-sterile nursery soit. *For. Ecot. Manag.* 59: 193-206.
31. Gehring C.A 2003. Growth responses to arbuscular mycorrhizas by rain forest seedlings vary with light intensity and tree species. *Plant Ecology*. 167: 127-159.
32. Béreau M, Barigah T.S., Louisanna E, Garbaye J. 2000. Effects of endomycorrhizal development and light regimes on the growth of *Dicotynia guianensis* AmshotT seedlings. *Ann. For. Sci*. 57: 725 -733
33. Letouzey R. 1968. Etude phytogéographique du Cameroun. P.Lechevalier, 511p, Paris
34. Baylis, G.T.S. 1975. The magnolioid mycorrhiza and mycotrophy in root systems derived from it. In: F.E. Sanders, B. Mosse & P.B. Tinker (eds), Endomycorrhizas, p. 373-389. London, Academic Press.
35. Manjunath, A & M. Habte 1991. Categories ofvesicular-arbuscular mycorrhizal dependency of host species. *Mycorrhiza* 1: 3-12.
36. Baratolo C. 2001. Tradeoffs between neotropical tree seedling traits performance in contrasting environments. PhD thesis. Ann. Arbor, MI, USA: University of Michigan
37. Nadian H., Smith S.E., Alston AM., Murray R.S. 1996. The effect of soil compation on growth and P uptake by *Trifolium subterraneum:* interactions with mycorrhizal colonization. *Plan Soit*. 182: 39-49.
38. Porter, W.M. 1979. The 'Most Probable Number' method for enumerating infective propagules of vesicular arbuscular mycorrhizal fungi in soil. *Aust. J. Soil Sci*. 17: 515-519.