Mycorrhizal Association and its Benefits to *Allanblackia parviflora* Tree Seedlings in the Nursery

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Abstract

Allanblackia species are high value multipurpose indigenous fruit trees whose seeds contain edible oil that has become a foreign exchange earner for rural-based enterprises. Wild harvesting could not sustain the supply to industry and therefore domestication was focused on developing propagation techniques, selecting and collecting elite planting materials. Little emphasis was placed on the soil nutrient requirements where preliminary results showed seedlings grown in rhizosphere soil of wild trees had good growth performance. A study was undertaken to examine microbial-*Allanblackia parviflora* plant interactions and determine their benefits to nursery seedlings. Roots of wildlings and rhizosphere soil from *A. parviflora* trees were collected from three forest reserves and the roots assessed for mycorrhizal colonization. *Allanblackia parviflora* seedlings were raised in different potting media with different ratios and their height and diameter determined. Soil treatments were also analyzed for nutrient and chemical contents. Vesicles, arbuscular structures, hyphal coils and intercellular hyphae were found on root tips of wildlings collected from rhizosphere soil of *Allanblackia* (AB) trees and seedlings grown in soil treatments containing AB soil. Root colonization of *A. parviflora* was largely in the form of extensive cell-to-cell growth of hyphal coils characteristic of Paris-type morphology. Addition of Agricultural field soil (Ferric Acrisol, Afs) or Humus (H)+Afs to AB improved height of seedlings. Seedlings grown in AB soil alone increased best in height with age followed by those grown in combination of 75% AB soil and 25% Afs. Available P was highest in Afs (220.84 mgP/kg) and low in AB soil (6.54 mgP/kg) while combination of H + Afs to AB increased K level to 341.34 mgK/kg. The improvement in growth must be due to both vesicular-arbuscular mycorrhizal fungi and soil chemical content of AB soil.

Introduction

Sub-Saharan Africa has a number of undomesticated indigenous fruit trees that have the potential of enhancing livelihood of smallholder farmers, and at the same time with high potential demand but very poorly organized value chains.

One recent exception to this is from the *Allanblackia* tree of the family Clusiaceae, a native African tree whose seeds contain oil found useful in food manufacturing. Traditionally, *Allanblackia* fruits are collected from wild stands and high-value edible oil extracted from the seeds. The fat content has for long been used by local communities for food, medicine and animal feed (Meshack, 2004). The oil has received attention from industry as a result of its use in the production of spreads and soap (EFSA, 2007). In 2004, it was realized that wild harvesting alone cannot sustain supply to industry, therefore Unilever, the World Agroforestry Centre (ICRAF) and their national partners promoted the domestication of *Allanblackia*. A socio-economic study by Ofori *et al*. (2006) showed that farmers were

willing to adopt *Allanblackia* for integration into agroforestry systems provided there is ready market, attractive prices, early bearing varieties and methods for propagation are known. Rural communities could therefore improve their livelihoods not only from the benefits from the tree itself, but also through private-public-partnerships (Jamnadass *et al.,* 2010).

Domestication work has since focused on developing propagation techniques, selecting and collecting elite planting materials, and initiating central and community nursery activities. However, little emphasis has been placed on the soil nutrient requirements which will be important for growth and domestication of *Allanblackia* in order to realize the full harvesting potential.

Preliminary results on management of *Allanblackia* at the nursery showed that seedlings grown in rhizosphere soil of trees performed better than those of non-rhizosphere seedlings, suggesting a mutualistic plant-microbial association (Owusu-Yeboah *et al*., 2008). Research on *Allanblackia stuhlmannii* in Tanzania (Ström, 2013) indicates that the species forms an association with vesicular arbuscula-mycorrhiza (VAM). This symbiotic relationship plays a major role in function, maintenance and evolution of biodiversity and ecosystem stability, composition and productivity (Smith and Read, 2008; van der Heijden *et al*., 2008). To understand the biology and appropriate cultivation practices of *Allanblackia*, studies were undertaken to find out whether or not the trees form mycorrhizal association and establish their benefits to nursery seedlings so as to facilitate the domestication process.

Methodology

Sampling of wildlings and soil from Allanblackia *trees in the field*

Collection of wildlings and soil (0-10cm) from around *Allanblackia* trees was done in New Edubiase in Ashanti, Benso in the Western and Dikoto in the Central Regions of Ghana. Ten seedlings and rhizosphere soils were collected from around each of five AB older trees. The soils were pooled and stored in plastic bags at 4 ^{o}C prior to processing. Roots from rhizosphere soils were extracted by washing them several times using tap water. Fine roots of wildlings and those extracted from soil rhizosphere were divided into two and one half stored in 50% ethanol prior to assessment of mycorrhizal colonization.

Soil samples (0–10 cm) were collected randomly from four spots in a Ferric Acrisol in which cassava was intercropped with plantain around the Forestry Research Institute of Ghana (FORIG) nursery. Organic residue used was a mixture of leaves and pod wastes of *Tetrapleura tetraptera* (Family Fabaceae) that was left to decompose around the nursery for six months. The mixtures were turned and mixed thoroughly every two weeks.

Determination of growth of AB seedlings in different potting media

Germinated seeds of AB were potted in poly bags of size 25 cm × 25 cm, one germinated seed per bag of eight soil treatments as follows: (i) Rhizosphere soil collected from the AB roots (AB), (ii) 50% soil from AB roots, Humus and Agricultural field soil (50% AB + 25% H+ 25% Afs), (iii) Soil from 50% AB roots and 50% Agricultural field soil (50% AB + 50% Afs),

(iv) Soil from decomposed *Tetrapleura tetraptera* chopped pods (Tts), (v) Humus soil (H) , (vi) 50% Humus soil and 50% Ferric Acrisol (50% H + 50% Afs), (vii) Ferric Acrisol (Afs), (viii) Fifty percent soil from decomposed *Tetrapleura tetraptera* chopped pods and 50% Ferric Acrisol (Tts + Afs)

There were five replicates per treatment. Thus the total number of seedlings used in the treatments was forty. The germinating seeds were kept in the shade house and watered daily with distilled water. The temperature in the shade net varied from 25 °C to 35 °C on very hot days. Seedling growth (height and diameter) was measured monthly for six months. The height was measured using a tape while the diameter was measured using a caliper. Seedlings were harvested after the sixth month, with the bulk soil separated from the roots. Five seedlings per treatment were sampled for biomass. One half of samples (shoots and roots) were oven-dried and weighed while fresh roots of the other half were stored in 50% ethanol prior to assessment for mycorrhizal colonization.

Assessment of growth of Allanblackia seedlings in different soil ratios

Allanblackia parviflora soil was collected from under mature AB trees. AB soil and Agricultural field soil were then mixed in different ratios of 1:0, 1:0.3, 1:1, 1:3, 1:7, 1:15, and 0:1 respectively as follows:
i. 100% rhizosphere soil of AB roots. (ii) 75% rhizosphere soil of AB roots and 25% Afs. (iii) 50% rhizosphere soil of AB roots and 50% Afs. (iv) 25% rhizosphere soil of AB roots and 75% Afs. (v) 12.5% rhizosphere soil of AB roots and 87.5% Afs
vi. 6.25% rhizosphere soil of AB roots and 93.75% Afs. (vii) 100% Afs.

Soils were put into black polythene bags of size 25 cm × 25 cm. Three replicates of each soil and control were prepared and arranged in a systematic manner. Newly germinated AB seeds of approximately equal height were placed in these poly bags with one germinated seed per bag. The number of roots on each seedling was counted and the length of each measured. The height and diameter of seedlings were measured monthly.

Determination of AM root colonization

Five replicate samples each of young wildlings and seedlings raised in AB soil were carefully excavated. Fine roots of mature plants were collected in three replicates by careful digging along a major root from the stem base to the root tips. A minimum of ten fine root tips of seedlings from the eight soil treatments and those from wildlings were excised and prepared (cleared with KOH stained with acidic Trypan blue and stored in acidic glycerol) Koske & Tessier (1983). Samples of 30 stained 5 mm length root fragments from three replicates of each sample were examined microscopically to determine arbuscular mycorrhizal fungal colonization and presence of arbuscular mycorrhizal structures (eg. vesicles and arbuscules). Rhizosphere soil from mature plants was collected at 10 cm depths, mixed, air-dried and analyzed for chemical properties.

Soil analysis

Soil *p*H was measured in 1:1 soil to water ratio (McLean, 1982). The Walkley Black procedure was used to determine soil organic carbon (Schumacher, 2002). Total nitrogen (N) was determined using the Kjedahl digestion and distillation procedure. The

cation exchange capacity (CEC) at *p*H 7 was determined by the NH_4OAc method. Calcium (Ca) and Magnesium (Mg) were determined by atomic absorption spectrophotometry while Potassium (K) and Sodium (Na) were determined by flame photometry. The effective cation exchange capacity (ECEC) was determined as the sum of exchangeable cations and exchangeable acidity. Exchangeable acidity (H^+ and Al^{3+}) was extracted with 1 M KCl and determined by titration with NaOH before and after addition of NaF (Sims, 1996). Available Phosphorus was determined using Bray 1. extraction solution (Iatrou, 2014). Heavy metals were determined in 0.5 M EDTA soil extract on atomic absorption spectrophoto-meter (Model VGL Buck scientific).

Results

Determination of AM root colonization

Microscopic examination of the stained roots revealed vesicles, arbuscular structures, hyphal coils and intercellular hyphae from root tips of wildlings collected from around AB trees and seedlings grown in all treatments containing AB soil. Mycorrhizal structures occurred randomly

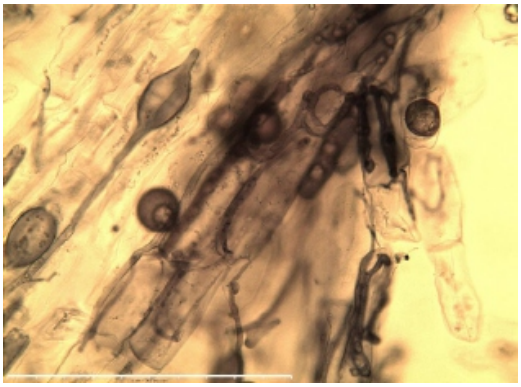

Plate1a. Mycorrhizal structures in root of wild seedling

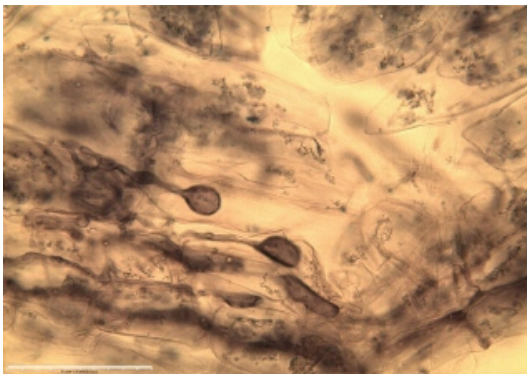

Plate1b. Mycorrhizal structures in root of seedlings in AB soil

in the root tips (Plates 1a, b). Root colonization in *Allanblackia* was largely in the form of extensive cell-to-cell growth of hyphal coils characteristic of Paris-type morphology (Plates 1a, b). The number of vesicles, arbuscular structures, hyphal coils and intercellular hyphae were lower in roots of seedlings grown in treatments containing AB soil. The association observed was the vesicular arbuscular type.

Assessment of Allanblackia seedling growth in different nursery potting media

Results obtained on height and diameter measurements are shown in Figs.1 and 2. There were variations in height among the different treatments. Seedling height was highest with those in AB soil. Addition of Afs or H + Af soils to AB soil improved growth in height of seedlings. Height of seedlings in H or H +Af soils was low (8 to 9 cm); H and H+Afs soils did not improve growth in height of Tts (Fig. 1). There were significant differences in height of seedlings between combinations of Tts + Afs, AB soil only, Afs only, AB + Afs and H + Af + AB soils.

Mean diameter of seedlings grown in Af soil was 0.055 cm while those grown in H

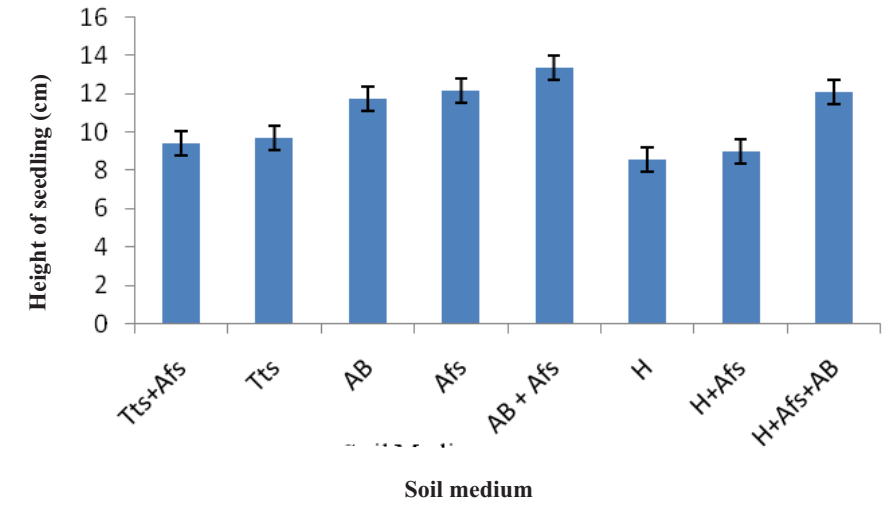

Fig 1. Height of AB seedlings grown in different soil media

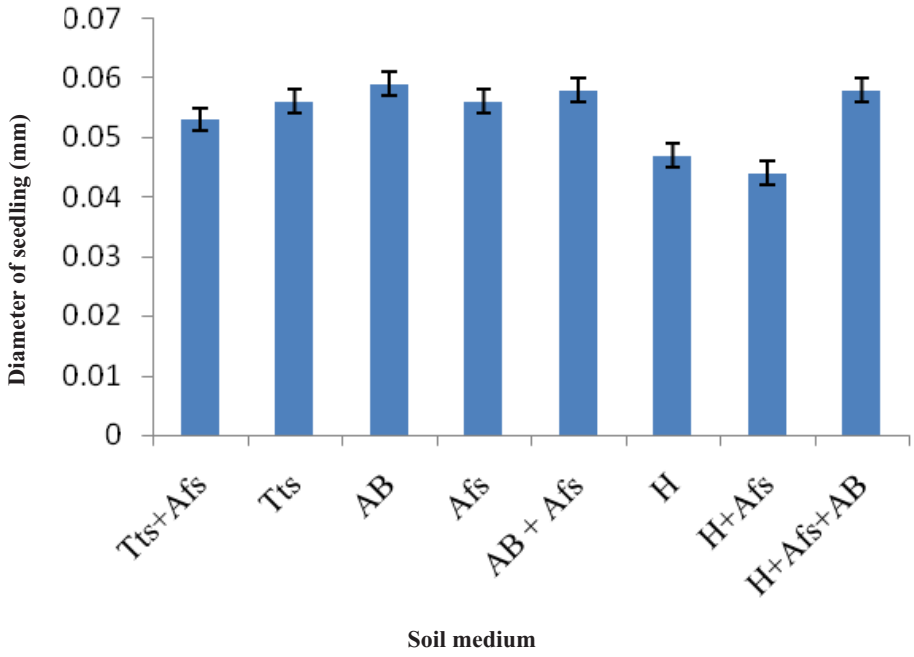

Fig. 2. Diameter of AB seedlings grown in different soil media

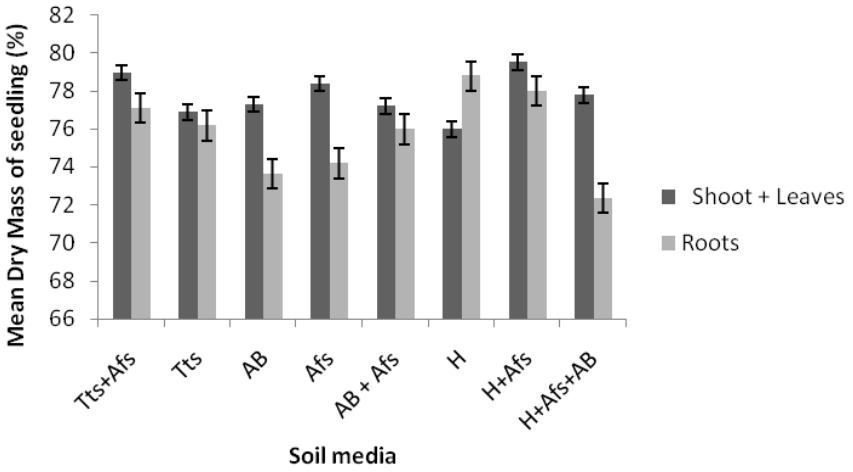

Fig. 3. Dry mass of seedlings grown in different soil media

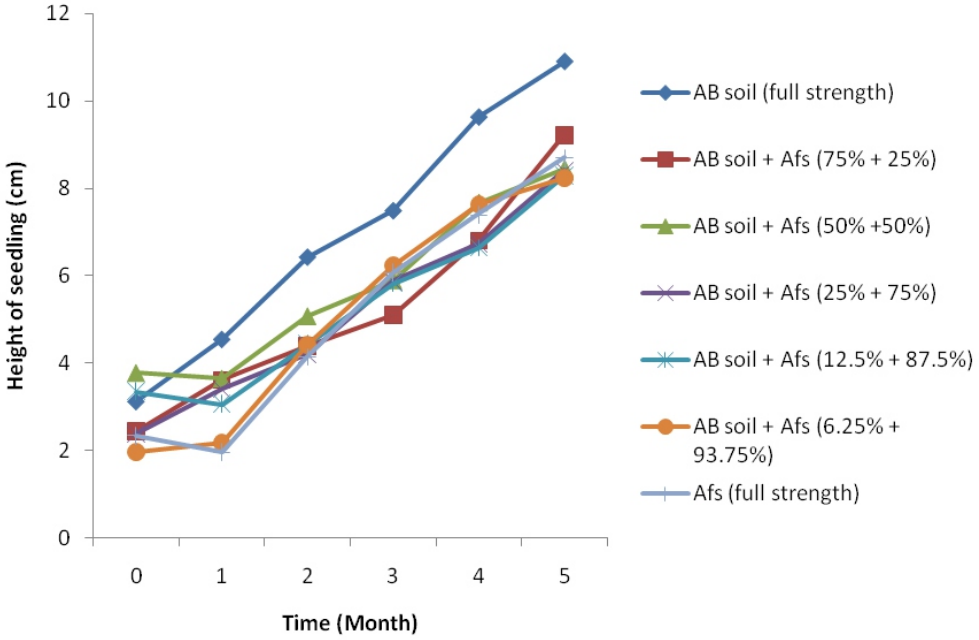

Fig. 4. Height of seedlings in different concentrations of AB soil media

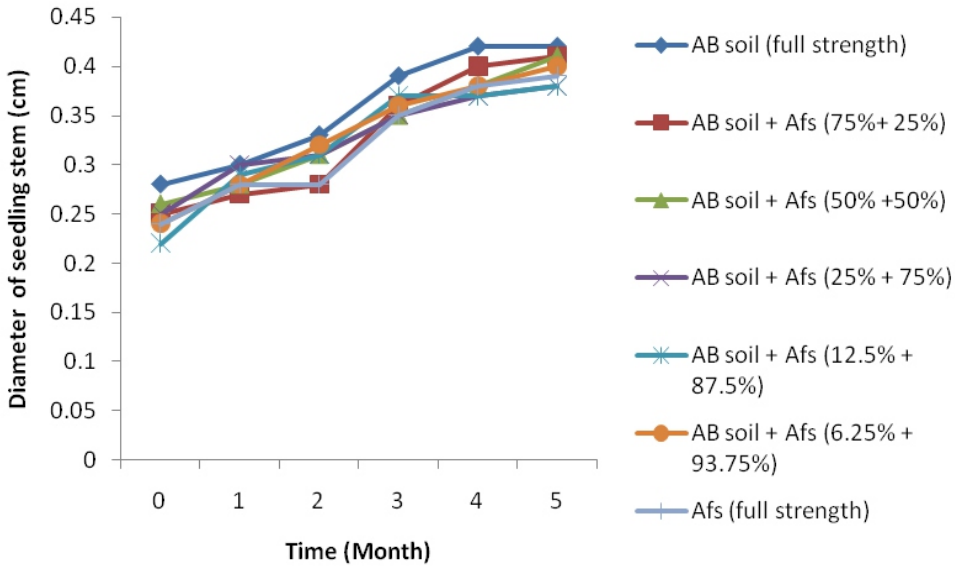

Fig. 5. Seedling diameter in different concentrations of AB soil media

TABLE 1
Chemical properties of soil mixtures with AB soil

Chemical component	*Soil Treatment*								
	AB	*AB+H+Afs*	*AB+Afs*	*Tts*	*H*	*H+Afs*	*Afs*	*Tts + Afs*	*L S D (0.05)*
*p*H (1:1H$_2$0)	7.39^{a}	6.74^{b}	7.06^{a}	7.01^{a}	7.36^{a}	5.00^{e}	6.85^{b}	5.68^{d}	0.349
Carbon (%)	1.95^{c}	2.19^{a}	2.19^{a}	2.29^{a}	1.31^{b}	2.48^{a}	1.95^{c}	1.31^{b}	0.504
Total N (%)	0.14^{d}	0.16^{b}	0.15^{c}	0.16^{b}	0.10^{e}	0.18^{a}	0.15^{c}	0.09^{f}	0.001
Organic matter (%)	3.36^{c}	3.78^{b}	3.78^{b}	3.95^{b}	2.26^{e}	4.28^{a}	3.36^{c}	2.26^{e}	0.224
Exch. Ca (cmol$_c$/kg)	22.96^{c}	13.08^{h}	14.42^{g}	14.69^{f}	14.15^{g}	4.01^{i}	6.94^{i}	11.75^{g}	0.686
Exch. Mg (cmol$_c$/kg)	4.27^{c}	3.47^{d}	2.94^{e}	2.14^{g}	3.20^{d}	2.67^{f}	3.74^{c}	3.20^{d}	1.399
Exch. K (cmol$_c$/kg)	0.45^{c}	0.18^{g}	0.30^{e}	1.08^{a}	0.34^{d}	0.23^{f}	0.28^{e}	0.32^{d}	0.002
CEC (cmol$_c$/kg)	27.99^{a}	17.01^{h}	17.92^{g}	18.83^{f}	17.96^{g}	7.61^{i}	11.28^{h}	15.94^{e}	5.547
Available P mg/kg soil^{-1}	6.54^{g}	145.10^{b}	70.16c	100.46^{d}	108.43^{c}	210.48^{a}	220.84^{a}	66.17^{f}	12.595
Available K mg/kg soil^{-1}	192.68a	341.34^{b}	267.37c	322.94d	232.01^{e}	294.07^{f}	340.26^{b}	228.76^{g}	3.148

soil was 0.048 cm. Seedlings in H+Af soils had decreased diameter but when AB soil was added, it increased the diameter. There were no significant differences among diameters of seedlings grown in combinations of Tt + Af soils, Tt soil only, H + Af soils, H + Af + AB soils. There was also no significant difference between diameters of seedlings grown in Tt soil and that of Af + AB soils; as well as that of AB soil only and AB + Af soils (Fig. 2).

There were variations among the dry mass of the seedlings grown in the different soil media (Fig. 3). In terms of shoot, soil treatments with AB soil added gave the highest mass. This was followed by those from soil treatments with Af soil added. Soils without either top soil or AB soil added gave the lowest dry mass. However, with the root dry mass Af soil, AB soil + Af soil gave higher mass than AB soil alone as well as those from the other treatments. Generally there were significant differences among treatments.

Assessment of growth of Allanblackia seedlings in different soil proportions

Seedling height increased with age mostly in AB soil followed by those in the combination of 75% AB soil and 25% Afs, 50% AB soil and 50% Afs in decreasing order (Fig. 4). Combinations of AB and Afs at concentrations below 25% AB soil had no effect on seedling height (Fig. 4). There was no marked difference in the diameter of tree seedlings raised in the different soil proportions with AB soil (Fig. 5).

Soil analysis

Soil *p*H ranged from 5.00 to 7.39. Addition of Afs to Humus (H) decreased *p*H by 1.85 units (6.85 to 5.00); *p*H of H and Afs were 7.36 and 6.85, respectively. Addition of Afs decreased *p*H of AB by 0.33 while addition of H+ Afs to AB soil further decreased the *p*H to 6.74. There were no significant differences among *p*H of soil treatments AB, AB + Afs, Tts and H at $P \leq 5\%$.

Addition of H + Afs + AB or Afs alone resulted in increase of soil carbon in AB which originally was 1.95%. The carbon content of H+ Afs, Tts, AB+ Afs, AB+H+ Afs were significantly higher than those of either H alone (1.31%), Afs alone (1.95%), AB or Tts + Afs. *Tetrapleura tetraptera* soil (Tts) alone had 2.29% carbon content but addition of Afs lowered the carbon content to 1.3%.

Interestingly, low nitrogen content of Tts + Afs (0.09%) was accounted for by the low organic carbon content (1.31%) as well as that of H (carbon, 1.31% and nitrogen, 0.10%). The high total N of H+ Afs is accounted for by the high organic carbon content, an indication of high fertility in H+Afs. Addition of H+Afs to AB increased the nitrogen and carbon contents. The nitrogen contents of the soil treatments were significantly different at $P \leq 5\%$.

The organic matter content of AB and Af soils were not significantly different but different from those of all the other soil treatments.

Available P was highest in Afs (220.84 mg/kg P) followed by that in H+Afs (210.48 mg/kg P) and lowest in AB soil (6.54 mg/kg P). Addition of H+ Afs increased the available phosphorus in AB soil to 145.10 mg/kg P. There were significant differences between all the treatments except those between Tts and H, and H+Afs and Afs alone at $P \leq 5\%$.

Similarly, available K in Afs when added to AB increased K level to 267.37 mg/kg K (60%) and H + Afs to AB increased K level to 341.34 mg/kg K. Addition of Afs to Tts rather decreased available P and K levels from 100.46 to 66.17 mg/kg P and 322.94 to 228.76 mg/kg P, respectively which was not expected. There were significant differences between all the treatments except those between AB+H+ Afs and Afs at $P \leq 5\%$.

Addition of H+ Afs or Afs alone to AB decreased Ca, Mg, K and Na levels in the soil. Thus, this decreased the pH of soil, not providing available soil nutrients for plant growth. There were significant differences between treatments in exchangeable cations of K while Magnesium cations in treatments Tts, H+ Afs differed significantly from those of the other soil treatments.

Discussion

Vesicles and arbuscular structures with hyphal coils were observed from root tips of wildlings of AB and seedlings raised in *Allanblackia parviflora* soil in the nursery as observed in *A. stuhlmannii* by Ström (2013) where a large number of potential AM symbionts (Glomerales) were found, both on young and old roots. Majority of tropical trees are considered to form arbuscular mycorrhiza (De Carvalho *et al*., 2010). This morphological type of arbuscular mycorrhizal association might be defined as the Paris-type which is characterized by the absence of intercellular hyphae, but replaced by extensive intracellular hyphal coils referred to as arbusculate coils as described by Smith and Smith (1996, 1997) to be found in plants growing in natural ecosystems.

The frequency of vesicles, arbuscular structures, hyphal coils were lowered in roots of seedlings grown in treatments containing AB soil, as compared to seedlings from the wild probably because the density or population of mycorrhizal spores in the soil has reduced as a result of soil disturbances (Jasper *et al*., 1987; Bellgard, 1993).

Seedlings grown in AB soil alone gave the highest height which explains the fact from other experiments that mycorrhizal fungi can overcome nutrient limitation to plant growth by enhancing nutrient acquisition, especially Phosphorus (Marschner & Dell, 1994; Clark & Zeto, 2000).

Seedlings grown in AB soil combined with other soil media showed higher increase in height than the others in which no AB soil was added (Fig. 1). This confirms that plants hosting AMF in their roots show enhanced growth and mineral uptake, and a high tolerance of biotic and abiotic stress, compared with non-mycorrhizal plants (Smith and Read 2008). However use of Af soil, humus soil and their combinations could promote almost the same height growth of seedlings as AB soil and combinations. This might be because these treatments have enough phosphorus and other minerals that could enhance growth of the plant just as AB soil and their combinations. Therefore, *A. parviflora* seedlings could be raised in combinations of AB and agricultural field soils in the nursery.

Seedling height increased with time in full strength AB soil while with the mixing of agricultural field soil with AB soil in different ratios, height growth of seedlings was slow. This might be as a result of decrease in density of mycorrhizal spores in the mixtures. Experiments have shown that any form of soil disturbance such as tillage, fallowing agricultural soils (Thompson, 1987), crop rotation, top soil stripping (Jasper *et al*., 1987; Bellgard, 1993) markedly reduce

populations of mycorrhizal fungi.

Except for Af soil, Phosphorus levels were observed to be extremely low in AB soil (6.54 mg/kg) followed by those in 50% AB + 50% Af soil (70.16 mg/kg) and very high in Af soil (220.84 mg/kg). This indicates that available P increases as ratios of AB soil in potting mixtures are augmented. The result confirms that a vast number of tropical soils and sand dune ecosystems (Ahulu *et al*., 2005) are sufficiently low in available phosphate. Besides, the extremely low level of P in AB soil indicates that the hyphae of vesicular-arbscular mycorrhizal fungi growing in depleted soil can absorb phosphate rapidly, and thus increase the inflow of Phosphorus to the host plant. Abbot and Robson (1979) observed that levels of soil Phosphorus greater than that required for plant growth eliminated the development of arbuscles of vesicular-arbuscular types of mycorrhizae. In general, growth of the seedlings and Phosphorus uptake from insoluble sources are enhanced by mycorrhizal colonization (Alexander, 1989).

Mycorrhiza colonization in roots of plants growing in AB soil alone decreased as quantity of AB soil decreased from 75 to 25% AB soils. Vesicles were also observed in roots growing in full strength AB soil and 75% AB soil but only arbuscles appeared in 50% and 25% AB soils. An increase in the level of soil phosphate results in reduction in chlamydospore production by the fungus (Menge *et al*., 1978). Amijee *et al*. (1989) observed that when the soil level of bicarbonate Phosphorus exceeded 140 mg kg^{-1} (140 mg/kg) the rate of colonization was found to decrease. Schubert and Hayman (1986) also found that the development of mycorrhizal relationships were greatest when soil Phosphorus levels were at 50 mg/kg. It is well established that colonization by mycorrhizal fungi is significantly reduced at high soil Phosphorus levels (Amijee *et al*., 1989, Koide and Li, 1990).

Conclusion

Vesicles, arbuscular structures, hyphal coils and intercellular hyphae occur in the root tips of wildlings and nursery grown seedlings. The frequency of vesicles, arbuscular structures, hyphal coils and intercellular hyphae reduced in roots of seedlings grown in soil treatment containing AB soil. AB soil supported the highest seedling height growth. Application of 25% and above AB soil (soil collected under matured AB tree) to agricultural field soil in raising AB seedlings had an effect on growth (height and diameter). The effect on growth is due to both vesicular-arbuscular mycorrhizal fungi and soil chemical composition of AB soil. *Allanblackia parviflora* seedlings could be raised in AB soil amended with agricultural field soil in the nursery.

Acknowledgement

We wish to thank ICRAF for supporting the study by providing funding and technical assistance. We are also grateful to Ms. Emmanuella Lawson-Lekete and Messrs Philemon Gyamfi, Francis Owusu Nuamah and Michael Opoku for their help in the laboratory and nursery.

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