

Plantain mycorrhization with native consortium of arbuscular mycorrhizal fungi (AMF) induce solubilisation of metals (Fe^{2+} and Al^{3+}) in soil from Azaguié (south-east of Côte d'Ivoire)

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Abstract

New agroecological practices propose to manage soil fertility using soil microorganisms such as arbuscular mycorrhizal fungi (AMF). However, few studies have been conducted on the impact of plantain mycorrhization and metal (Fe^{2+} and Al^{3+}) solubilisation in soil. This study evaluates the effectiveness of native AMF on plantain growth and metal leaching from soil. Trap plants (maize, sorghum and cowpea) were grown to produce various inoculums. Then, plantains were grown under controlled conditions, with six treatments (control, plantain without inoculum, plantain with maize root inoculum, vigna root inoculum, sorghum root inoculum and mixed root inoculum) replicated five times. Growth parameters were measured, and the rate of plantain root colonization was evaluated by determining the frequency or intensity of infection. The contents of metals in leached solutions were analyzed using ICP-OES. Results indicated the rate of plantain roots colonization by fungi was not significantly different between the different treatments. Plantain biomass remained very low whatever the treatment. However, plant inoculated with vigna roots inoculum had the highest biomass while plant inoculated with sorghum root inoculum showed the lowest biomass. Leached solutions from soils with inoculated plants had on average a pH value of one pH unit lower than leached solutions from soils without inoculum. In addition, plantain mycorrhization can promote the leaching of Fe^{2+} from the soil by acidification, whereas the difference between treatments was not significant for the concentration of Al^{3+} in solutions. These results suggest that the association of plantain with microorganisms remains a way for agroecological banana production in Côte d'Ivoire.

Keywords: *Musa* AAB, ferralsol, native fungi, metal leaching

1 Introduction

Global agricultural needs are increasing with rapid population growth while land resources are becoming increasingly limited to meet these needs (FAO, 2017). Increasing agricultural yields on small production areas is therefore a challenge for agricultural producers. Thus, in Côte d'Ivoire, farmers generally use large quantities of chemical fertilisers to improve and restore soil fertility (Roose *et al.*, 2015). However, the intensive use of these chemical fertilisers accentuates soil degradation including acidification and a reduction in the functioning of the fauna or flora activities

as well as soil capacity to sustain agricultural production (Siavoshi *et al.*, 2011). To reduce the massive use of synthetic fertilisers, new agricultural practices suggest the efficient management of soil microorganisms such as arbuscular mycorrhizal fungi (AMF).

Mycorrhizal fungi form a symbiotic association (mycorrhiza) with the roots of more than 90 % of terrestrial plants (Strullu-Derrien & Strullu, 2007) and play a major role in the spatiotemporal evolution of terrestrial plant ecosystems (Kisa *et al.*, 2007). In addition to their direct impact on plant growth via mainly improved mineral nutrition of the host plant, it has also been shown that the structure of the plant canopy as well as its development were intimately

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linked to the establishment of mycorrhizal symbiosis (van der Heijden *et al.*, 2004; Sanon *et al.*, 2010). These mycorrhizal fungi play an important role in the biogeochemical cycling of phosphorus and its bioavailability to plants. Due to the phosphorus deficiency found in many tropical soils, interest in these microorganisms has increased significantly in recent decades (Richardson *et al.*, 2011). Different mechanisms are used by mycorrhizal fungi to increase the availability and acquisition of phosphorus by plants through the solubilisation of minerals. The main mechanism of inorganic phosphorus solubilisation is the acidification of the soil solution (Plassard *et al.*, 2011). Indeed, the ability of fungi to solubilize minerals is mainly due to their ability to acidify the soil solution as well as to the release of organic ions into solution (Khan *et al.*, 2007). Acidification is due to the release of protons at the outer walls in exchange for the removal of cations by the ATPase (Rodríguez *et al.*, 2008). The acidification of the environment causes an imbalance in the soils and a change in the solubility of some minerals, leading to their dissolution, including iron and Al^{3+} oxides present in abundance in soils (Kraemer *et al.*, 2006). At a low pH (~4.3) trivalent aluminium (Al^{3+}) is in soil the most abundant form and this soluble Al^{3+} is toxic for plant growth. The main effect of Al^{3+} toxicity being inhibition of root growth (Rout *et al.*, 2001; Kopittke *et al.*, 2016). Similarly, in some conditions, Fe^{2+} toxicity results from an excess Fe^{2+} (Audebert & Fofana, 2009). Thus, wouldn't the introduction of mycorrhizal fungi in tropical agrosystems be a source of toxicity for plants through the solubilisation of aluminium and iron?

In Côte d'Ivoire, mycorrhizal fungi are not widely used in the field under natural conditions due to the low reproducibility of the effect of inoculation on the plant host (Duponnois *et al.*, 2013). This is mainly due to the lack of compatibility of the inoculant with the edaphic characteristics of the local soil. This incompatibility results in the disappearance of the introduced strain in the soil after a few years of application (Hart *et al.*, 2017; O'Callaghan *et al.*, 2022). It is therefore advantageous to select native strains adapted to the soil conditions where the crop will be planted (Meddich *et al.*, 2015). The main objective of this study is therefore to evaluate the effect of the growth of inoculated plantain plantlets with native consortium of mycorrhizal fungi on the solubilisation of iron and aluminium.

2 Materials and methods

2.1 Study area

Soil used in this experiment was sampled at the research station of the Institut des Nouvelles Techniques Agricoles

(INTA) located near Azaguïé (south-east of Côte d'Ivoire). Azaguïé is located at 5 km North from Abidjan, between latitudes $5^{\circ}38'$ and $6^{\circ}15'$ N and longitudes $3^{\circ}57'$ and $4^{\circ}90'$ W (Fig. 1). In the research station, soils are classified as ferralsol, developed on granite as bedrock. These soils are sandy-clay, acidic soil with high aluminium and iron content, desaturated with a high content of coarse elements, and vegetation in dense forest. (Kouame *et al.*, 2014).

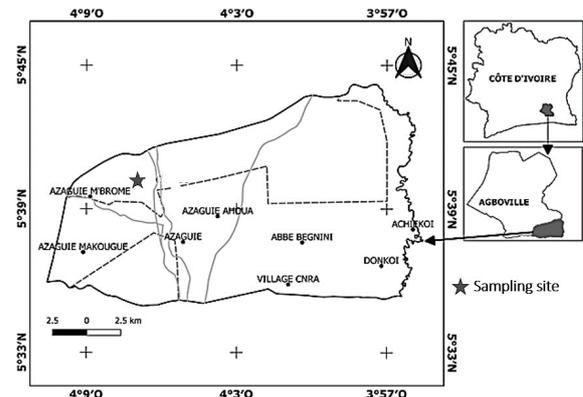


Fig. 1: Sampling soil and study location

2.2 Biological material

In this study, three species with an agronomic interest were used as fungi trap-plants to multiply indigenous soil mycorrhizal fungi. These species were maize (*Zea mays*L.), sorghum (*Sorghum vulgare*L.) and cowpea (*Vigna unguiculata* L.). In addition, the test plant used in this experiment consists of plantain plantlet cultivars (Musa AAB, cv Corne 1), mainly grown in Côte d'Ivoire.

2.3 Inoculum production

In order to trap mycorrhizal fungi, forty elementary soil samples were taken on the whole site using a systematic 10 m x 10 m square grid sampling method. These samples were, following equivalent mass, mixed to constitute one composite sample. In addition, rhizosphere soils of a poaceae (*Panicum*) and two leguminous plants (*Pueraria phaseoloides*, *Acacia mangium*) were also sampled around the research station. Fine root fragments of these plants were collected because these species are known for their strong mycorrhizal dependence.

In the laboratory, bulk soil, rhizosphere soil and root fragments were mixed, homogenized and used as a growing medium for the trap-plants grown in a device as described in Fig. 2A. This set-up consisted of a 5 cm thick layer of mycorrhized soil-root mixture sandwiched between two layers of sand (5 and 10 cm thick), pre-treated with concentrated nitric

acid (69 %, EMSURE®) for 24 hours and rinsed thoroughly with distilled water. Maize, sorghum and vigna seeds ster-

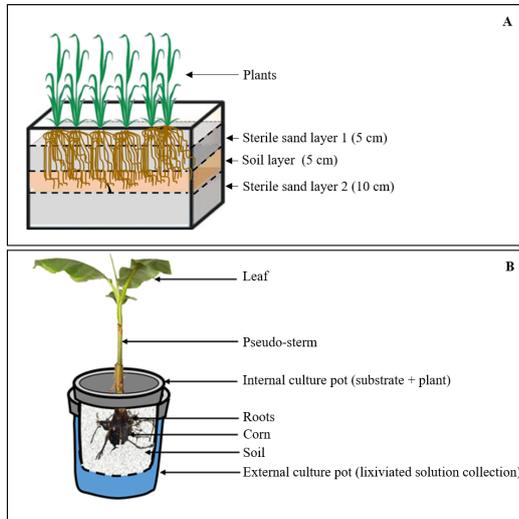


Fig. 2: Experimental plant growth device for (A) trap plant and AMF multiplication, (B) plantain plantlet growth under controlled conditions in inner pot (2500 cm^3) inserted in outer pot (3000 cm^3).

ilized with 2.4 % calcium hypochlorite were pre-germinated on agar medium in Petri dishes. The pre-germinated seeds were then transferred to the cultivation device with a density of 250 seedlings per 0.5 m^2 , with 5 lines containing 50 plants spaced by 8 cm between lines and 1.8 cm between each plant per line. The plants were watered daily with distilled water. At 60 days after transplanting, maize and orghum plant were cut 1 cm above the substrate layer surface and watering was stopped in order to induce water stress. Ten days after weaning, all the seedlings were then removed and the soil samples transferred to the laboratory for extraction and spore enumeration.

Fungi spores were isolated from soil according to the wet sieving method using a series of nested sieves (1000, 500, 150, 80 and $45 \mu \text{ m}$) (Brundrett *et al.*, 1996). Soil samples from the rhizosphere of trap-plant and bulk soil samples (100–200 g) from the experimental device were mixed into a 1 L beaker of water before pouring through the series of sieves. Then, the contents of each sieve were washed thoroughly to remove traces of sugar solution, backwashed into a Petri dish and observed using a stereomicroscope.

In addition to spore extraction, root infestation by fungi was evaluated by microscope observation of root mycorrhization structures. Collected roots of each host plant were stained according to the staining method based on the use of ink and vinegar (Vierheilig *et al.*, 1998). After the checking of root mycorrhization, the remaining roots were harvested and cut into fragments of about 1 cm, gently dried at room

temperature and mixed with the rhizosphere soil for its use as inoculum of indigenous fungi consortium.

2.4 Experimental plantain growth

Plantain plantlets were disinfected in a hypochlorite solution (2 %), abundantly rinsed and soaked in distilled water for 48 hours. Plantlets were grown in a device as described in Fig. 2B. The inner pot ($v = 2500 \text{ cm}^3$) for growing plantlets contained a sterilized mixture of sand and sieved soil from the research station (1/3, v/v). Thirty (30) g of inoculum were applied around the plantlet bases. Crop trial was conducted in a completely randomized design with 5 replications of 6 treatments:

- T0: Control without plantain and without inoculum;
- T1: plantain without inoculum;
- T2: plantain inoculated with maize roots inoculum;
- T3: plantain inoculated with vigna roots inoculum;
- T4: plantain inoculated with sorghum root inoculum;
- T5: plantain inoculated with mixed roots inoculum.

The experiment was conducted for 12 weeks under a shelter at the Centre National Floristique of the University Félix Houphouët-Boigny (Abidjan, Côte d'Ivoire). Throughout the experiment, plantain was watered twice a week with distilled water at a rate of 350 ml per watering event. Measurements of growth parameters (height, pseudo-trunk diameter and number of leaves) were carried out every week from 28 days after transplanting. Leached soil solutions were also collected weekly in the outer pot. At the end of the experiment, plantlets root infestation by fungi was assessed according to the methods previously described in the above sections.

2.5 Sample treatment and analysis

Plant biomass (pseudo-trunk and leaves) was determined after drying in an oven at $70 \text{ }^\circ\text{C}$ for 5 days. Dry biomass was finely ground to $50 \mu \text{ m}$. Fifty mg of the ground sample was mineralised in a mixture of 2 ml of hydrogen peroxide (H_2O_2) and 4 ml of nitric acid (HNO_3 65 %) for 8 hours at room temperature. The mixture was then heated on a plate at $80 \text{ }^\circ\text{C}$ for 24 hours. After cooling, all solutions from plant mineralization were filtered and diluted to 15 ml with double-distilled water. The percolation solutions were collected and filtered through Whatman filter paper® and separated in two fractions. The first one was used to determine the pH and the second one was stored at $-4 \text{ }^\circ\text{C}$ until analysis of soluble Fe^{2+} and Al^{3+} . Concentrations of Fe^{2+} and Al^{3+} in biomass and leached solutions were measured by inductively

coupled plasma optical emission spectrometry (ICP-OES). Blanks consisted of double-distilled water only and standards with known Fe^{2+} and Al^{3+} contents were used in the analysis sequence.

2.6 Statistical analysis

The analysis of variance of the soil and biomass analysis data as well as the plantlet growth data was carried out using SAS.9 (SAS Institute Inc., Cary, NC, USA). The data were analysed for their mean and standard deviation. Comparisons between means were made using the Newman-Keuls test at 5 % probability.

3 Results

3.1 Characterisation of mycorrhization of trap plants

Spore distribution per class size under trap plants is shown in Table 1. Four (4) spore sizes were identified: $\geq 500 \mu\text{m}$, $500\text{--}150 \mu\text{m}$, $150\text{--}80 \mu\text{m}$ and $80\text{--}45 \mu\text{m}$. The distribution of spores according to diameters showed that spores between $45 \mu\text{m}$ and $80 \mu\text{m}$ represented more than 50 % of the total spores. Then followed by the spores in the $150\text{--}80 \mu\text{m}$ class which constituted about a quarter of the total number of spores collected under the trap plants. Total spores

Table 1: Spores number per class size and total spores under trap plants.

SS (μm)	Spores per 100 g of soil		
	Vigna	Maize	Sorghum
500	96.00 \pm 10.53 ^c	180.00 \pm 19.31 ^d	89.00 \pm 17.57 ^c
150	126.67 \pm 12.01 ^c	340.33 \pm 14.64 ^c	148.67 \pm 11.24 ^c
80	308.67 \pm 15.31 ^b	711.67 \pm 11.01 ^b	597.33 \pm 60.05 ^b
45	663.67 \pm 26.41 ^a	1467.67 \pm 33.53 ^a	1413.0 \pm 93.82 ^a
TS	1195.00 \pm 64.26 ^a	2699.67 \pm 78.49 ^{***}	2248.00 \pm 182.68 ^{**}

Note: SS: sieve size; TS: total spores; Means followed by the same letter within a column are not significantly different and for total spores, the values followed by the same symbol do not indicate a significant difference ($p > 0.05$).

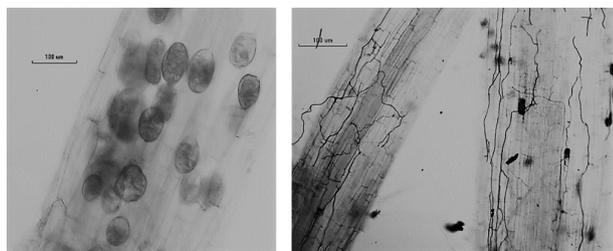


Fig. 3: The different structures of arbuscular mycorrhizae (hyphae; vesicles) on the roots of plants inoculated with mycorrhiza ($G. \times 400$).

were significantly ($p < 0.05$) higher for the grasses than for the legume (Table 1), about 125 % and 88 % for maize and sorghum respectively. The average number of spores counted is higher under the maize which is statistically higher compare to the number of spores under the sorghum. Microscopic observations of the roots of these trap plants showed mycorrhizal structures such as vesicles, hyphae and arbuscular (Fig. 3). The distribution of mycorrhization structures indicated that arbuscular and mycelia are well observed in vigna and sorghum, whereas these structures were rare in maize roots. In contrast, vesicles were more abundant in maize roots than in vigna and sorghum. These vesicles represented about 80–85 % of the fungal structures observed in maize roots.

The rate of root infection, defined by the frequency and intensity of mycorrhization, also showed differences between plants (Fig. 4). The mycorrhization intensity of vigna is about 35 % and 45 % higher than that of sorghum and maize, respectively. For root mycorrhization frequency, sorghum showed a lower mycorrhization frequency (78.33 \pm 2.89 %) compared to maize (87.88 \pm 21 %) and vigna (87.44 \pm 6.54 %), with no significant difference ($p > 0.05$).

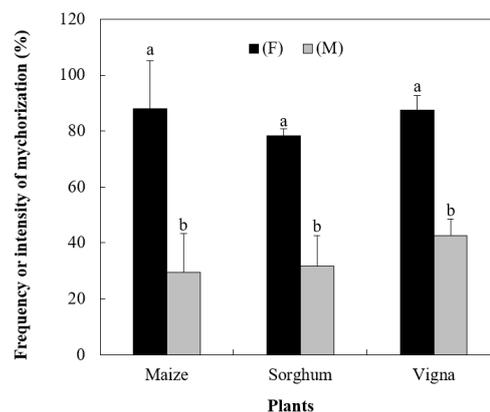


Fig. 4: Mean mycorrhization parameters of host plants: frequency (F %); intensity (M %). Note: Means followed by the same letter are not significantly different ($p > 0.05$).

3.2 Plantain mycorrhization and growth

Root infection rate is presented in Table 2. The data obtained showed relatively low root colonization rates. The mean values of the colonization rates are not significantly different for either the frequency or the intensity of infection. However, the trends indicated that higher root infection rate was observed with T3 treatment (17.12 %) while the lowest root infection was highlighted with T2 treatment (9.11 %). The mycorrhization intensity of the roots of inoculated ba-

Table 2: Root mycorrhization rate of plantain plantlet inoculated with indigenous soil consortium of arbuscular mycorrhiza fungi (AMF).

Treatment	Frequency (%)	Intensity (%)
T1	0.00 ^c	0.00 ^b
T2	9.11 ^b	0.10 ^a
T3	17.12 ^a	0.17 ^a
T4	14.39 ^a	0.14 ^a
T5	14.39 ^a	0.14 ^a

Note: Means followed by the same letter within a column are not significantly different ($p > 0.05$). T1: plantain without inoculum; T2: plantain inoculated with maize roots inoculum; T3: plantain inoculated with vigna roots inoculum; T4: plantain inoculated with sorghum root inoculum; T5: plantain inoculated with mixed roots inoculum.

nana plants remained very low for all treatments and did not exceed 1 % after 3 months of inoculation.

Table 3: Average growth parameters of plantain inoculated or not with native strains of AMF after eight weeks of cultivation.

Tr.	Pseudo-trunk		Leaf area cm ²	Number of leaves -	Biomass dry weight g per pot
	height cm	circumf. cm			
T1	8.00 ^d	0.62 ^c	26.40 ^a	1.75 ^c	0.75 ^c
T2	11.75 ^c	0.90 ^b	40.73 ^a	3.25 ^b	1.25 ^b
T3	14.30 ^a	1.20 ^a	64.00 ^a	4.75 ^a	1.75 ^a
T4	8.00 ^d	0.65 ^c	27.90 ^a	2.00 ^c	0.70 ^c
T5	12.90 ^b	1.00 ^b	51.20 ^a	4.25 ^a	1.45 ^{ab}

Note: Means followed by the same letter within a column are not significantly different ($p > 0.05$). Tr.: treatments. See table 2 for the explanation of the treatments.

Table 3 reports the results of growth parameters of plantain plantlets. Inoculated plants were significantly taller with about 46.87 %, 78.75 % and 61.25 % for T2, T3 and T5, respectively, as compared to the non-inoculated control T1. No significant difference was observed between plants from T4 and the non-inoculated control plant (T1). The pseudo-trunk data indicated a similarly trend. The number of leaves was higher within inoculated plants than non-inoculated ones. An increase of 86 %, 171 %, 14 % and 143 % in the number of leaves was recorded for plants from T2, T3, T4 and T5 treatments, respectively, compared to non-inoculated control plant (T1). However, the total leaf area of the plants did not vary significantly between the different treatments although the inoculated plants had a higher leaf area than the non-inoculated control plants. The biomass of the plants remained very low whatever the treatment. However, plants from treatment T3 had the highest biomass while those from treatment T4 showed the lowest biomass, similar to the biomass of the control plants.

3.3 Solubilisation of Fe²⁺ and Al³⁺ in soils

The leached solutions from the substrates with inoculated plants had very low pH values, significantly lower on average by 1.23 ± 0.10 pH units compared to the control solutions (Table 4). The concentrations of Fe²⁺ leached by the percolating solutions were significantly ($p < 0.001$) higher with the inoculated plants within the measurement uncertainty, while there was no difference in the concentration of aluminium. Iron contents in plant shoots, whether inoculated or not, appeared to be marginal in contrast to aluminium. The pH is negatively correlated with leached iron and Al³⁺ as well as with the Al³⁺ content in the biomass. Furthermore, leached cations are strongly and positively correlated with the content of these cations in the plants (Table 5).

4 Discussion

4.1 Mycorrhizal inoculum potential in field soils

Mycorrhizal potential or inoculum potential are terms used in many studies as an indicator of propagule density and mycorrhizal activity in the soil. It allows the quality and infectivity of soil inoculum to be evaluated and is used as a biological indicator. This potential reflects therefore the richness of the soil in propagules (spores, mycelia, mycorrhizal root fragments) able to generate host plant infection. Soil samples taken in the research station of Azaguié revealed the presence of AMF propagules. Their overall small size suggests that they belong to the genus *Glomus* (Danesh *et al.*, 2016). Cardoso & Kuyper, (2006) and Haougui *et al.*, (2013) have reported that this genus is the most abundant AMF propagule in tropical forest soils. The presence of AMF mainly dominated by the genus *Glomus* in soils of the Azaguié region has been demonstrated in previous studies too (Zeze *et al.*, 2007; Bivoko *et al.*, 2013). In terms of sporulation density, grasses (maize and sorghum) appear to be more efficient host plants than legumes (vigna). The fibrous roots of monocotyledons may have higher sporulation due to the stress that occurs after irrigation ceases. According to Chave *et al.* (2017), the water stress induced by weaning applied to the grasses would probably have caused massive production of reproductive spores in the rhizosphere of the plants, as water stress makes the plants more susceptible to AMF propagules. It is generally known that maize is a well-adapted host for AMF that can stimulate the presence of high numbers of infectious propagules in the soil (Wang *et al.*, 2008). Maize roots were richer in vesicles than the other two plants when comparing mycorrhizal structures. Similar results were observed by Thunot *et al.* (2004) and vesicle richness of maize roots can be partly explained by the high

Table 4: Solution acidity, concentration of leached Fe^{2+} and Al^{3+} ($\mu g ml^{-1}$), and shoot Fe^{2+} and Al^{3+} content ($\mu g g^{-1}$).

Treatments	pH	Leached ($\mu g ml^{-1}$)		Shoot ($\mu g g^{-1}$)	
		Fe^{2+}	Al^{3+}	Fe^{2+}	Al^{3+}
T0	5.63±0.07 ^a	1.60±0.30 ^c	0.54±0.01 ^a	n.d.	n.d.
T1	4.89±0.47 ^{ab}	1.95±0.15 ^{bc}	0.57±0.022 ^a	0.03±0.00 ^a	0.05±0.00 ^d
T2	4.40±0.20 ^b	2.95±0.15 ^{ab}	0.60±0.03 ^a	0.04±0.01 ^a	0.26±0.02 ^a
T3	4.35±0.10 ^b	3.55±0.35 ^a	0.63±0.03 ^a	0.08±0.02 ^a	0.19±0.01 ^c
T4	4.30±0.10 ^b	3.10±0.40 ^{ab}	0.63±0.04 ^a	0.04±0.01 ^a	0.28±0.01 ^a
T5	4.55±0.04 ^b	3.80±0.10 ^a	0.59±0.04 ^a	0.07±0.01 ^a	0.22±0.02 ^b

Note: Means followed by the same letter within a column are not significantly different ($p > 0.05$).

T0: Control without plantain and inoculum; T1: plantain without inoculum; T2: plantain inoculated with maize roots inoculum; T3: plantain inoculated with vigna roots inoculum; T4: plantain inoculated with sorghum root inoculum; T5: plantain inoculated with mixed roots inoculum. n.d.: Not determined.

Table 5: Relation between Fe^{2+} and Al^{3+} in leachate solution, biomass and pH.

	Leached Fe^{2+}	Leached Al^{3+}	Shoot Fe^{2+}	Shoot Al^{3+}	pH
Leached Fe^{2+}	1.00	0.56	0.77	0.78	-0.69
Leached Al^{3+}		1.00	0.21	0.64	-0.91
Shoot Fe^{2+}			1.00	0.20	-0.18
Shoot Al^{3+}				1.00	-0.87
pH					1.00

spore density of this host plant (Dabire *et al.*, 2007). Under these conditions, the difference in sporulation for grasses may be due either to the appearance of new spore types at a threshold not detectable before trapping or to a selection of these spores by the grasses. Bouamri *et al.* (2006) argued that the use of various host plants during the trapping of mycorrhizal fungi in the same soil may reveal the presence of different species. However, it is not obvious to test this hypothesis in this study because the number of spores and species of fungi initially present in the soil was unknown. Nevertheless, Bivoko *et al.* (2013) demonstrated that inoculation of vigna with soil from cassava fields in Azaguié increased spore densities. However, in our study vigna had the lowest spore density. Despite this low sporulation density of vigna compared to grasses, the root infection results showed that vigna trap roots induced a higher mycorrhization intensity of banana roots than grasses (sorghum and maize). Vigna is a legume capable of forming a symbiotic relationship with atmospheric nitrogen-fixing bacteria (Rhizobium) that can enhance the infectivity to mycorrhizal fungi. To this end, Megueni *et al.* (2011) highlighted the importance of this double rhizobium-AMF inoculation in improving the production of two vigna varieties. This symbiosis could create a synergy between rhizobia and mycorrhizae favouring the root infection (Haro *et al.*, 2012). The high mycorrhization intensity and low sporulation of vigna suggest that not only

spores can infect again the plants. Since there are also mycorrhizal roots present in the soil and root mycelia (Trepanier, 1998; Fogain 2001) or other factors that may intervene as in the case of legumes. In this case, the result obtained reflects the complexity of spore abundance in the establishment of root colonization. Therefore, it can be hypothesised, as suggested by Olah *et al.* (2005), that a combined effect of Myc and Nod factors stimulates mycorrhizal symbiosis and new root formation in vigna.

4.2 Plantain mycorrhization and growth

Several works have shown the ability of plantain plants to establish symbiosis with AMF (Fogain *et al.*, 2001; Tsané *et al.*, 2005; Emara *et al.*, 2018). The results of this study indicate that infection rates were quite variable depending on the treatment. Furthermore, the average mycorrhization frequency of banana plants was less than 15 % and the mycorrhization intensity did not reach 1 % for all treatments. These percentages are much lower than the data observed in the literature. Emara *et al.* (2018) showed that the infection of banana roots could reach a rate of 80 %. The low colonization rates obtained in this study could be due to several parameters. The first parameter would be the clayey texture of the soil, which could have inhibited a good root development of banana plants (Olivares *et al.*, 2020). The second explanation would be the variability of the substrate in this

study and literature (Tsané *et al.*, 2005; Frey & Ellis, 1997; Siqueira *et al.*, 1982). Plant growing substrate can significantly affect the formation and function of AMF. Tsané *et al.* (2005) demonstrated that species of mycorrhizae can affect the same plant species differently following the substrate qualities. These authors showed that for the same banana variety, good root colonization was obtained on less fertile soils. Finally, the last explanation could lie in the experimental period, which was not sufficient for good root development to penetrate the substrate and come into contact with the introduced inoculum (Emara *et al.*, 2018).

Despite these low infection rates, inoculation increased the growth of inoculated plantain plantlets compared to the control. A beneficial effect attributed to mycorrhization is a better plant growth (Hamel & Plenchette, 2007; Fortin *et al.*, 2008; Thioye, 2017) because of acidification of the mycosphere which favours mineral weathering (Lepleux, 2012). In this study, mineral weathering induced by acidification resulted in the drainage of Fe^{2+} and Al^{3+} in the leachate (Hinsinger *et al.*, 2003). These contents are significantly higher within the inoculation treatments. It can be assumed that there is also a solubilisation of minerals releasing nutrients (Ca, Mg, K, P...) essential for plant growth. It is also recognised that inoculation improves mineral nutrition (Declerk *et al.*, 1995; Jaizme-Vega & Azcon, 1995) and phosphate nutrition in particular (Smith *et al.*, 2003). This mineral nutrition is coupled with an improvement of the water nutrition of the plants (Heijden *et al.*, 2004; Tanwar *et al.*, 2013). This mineral and water nutrition then results in a significant effect on the number of leaves emitted and the average height of the plants (Fogain *et al.*, 2001; Tsané *et al.*, 2005; Emara *et al.*, 2018).

In this study, the inoculum prepared from vigna roots was most efficient in mycorrhizal plantain compared to maize and sorghum roots. This could be attributed to the different structures (spores, hyphae, arbuscules) colonizing the roots which play different functions in the colonization, nutrition and growth of the inoculated plants. The arbuscules are the site of active exchange between the plant and the fungus (Harrison, 2012). The efficiency of the vigna inoculum can therefore be related to the high presence of arbuscules found in the roots. Also, the nodules in the vigna roots would indicate that this AM fungus-nitrogen fixing bacteria combination allowed for better nutrient accumulation in the substrate. This improved the phosphorus and nitrogen nutrition of the inoculated plants and favoured plant growth (Artursson *et al.*, 2006).

4.3 Plantain mycorrhization and solubilisation of Fe^{2+} and Al^{3+} in soil

The symbiosis of plants with fungi results in acidification of the mycorrhizosphere (Bago & Azcón-Aguilar, 1997). Thus, in tropical soils, the risk of this association is a potential solubilisation of Fe^{2+} and Al^{3+} in soils, followed by their transfer to plants. Aluminium toxicity is known to be a major factor limiting the growth of plants grown in acid soils and has been demonstrated in banana in the tropics (Rufyikiri, 2000). The presence of Al^{3+} caused a reduction in water and nutrient uptake and plant growth. In the present study, percolation solutions were used to measure acidity and leaching of free Fe^{2+} and Al^{3+} in plantain plantlets. The results showed higher acidity in the percolation solutions of the inoculated plants compared to the non-inoculated controls and compared to the original soil pH. The decrease in pH would be due to the production or excretion of protons by both the mycelia of AM fungi (Villegas & Fortin, 2001) and the roots of plantain plantlets, which increased leaching of Fe^{2+} and Al^{3+} . Overall, inoculation of plantain plantlets favoured the solubilisation and leaching of Fe^{2+} more than Al^{3+} . This could be explained by a high Fe^{2+} content in the soil compared to Al. Nevertheless, there is a greater transfer of Al^{3+} into the shoots than Fe^{2+} . These results are in agreement with the work of Redon (2009) who argued that AM fungi are also able to modify their environment to take up and transport elements to the plant. Also, Rufyikiri (2000) argued that inoculated plantain plants accumulate a good amount of aluminium in their roots but also in their leaves. In this study, plant inoculated with vigna inoculum displayed the lowest Al^{3+} content in shoot. This association decreased Al^{3+} transfer to the plant by improving nutrient absorption, through Al accumulation in the fungal mycelia as indicated by Moyer-Henry *et al.* (2005), and active organic acid production, especially oxalic acid (Eldhuset *et al.*, 2007). These results suggest that the association of plantain plantations with legumes remains a way to explore agroecological banana production.

5 Conclusion

The aim of this study was to evaluate the efficiency of native AMF consortium in plantain mycorrhization and growth. This study demonstrated that grasses are the best trap plants for soil mycorrhizal fungi. The inoculum produced from indigenous soil consortium of fungi strains had little effect on the root colonization of plantain plantlets and their growth. Nevertheless, the experiment showed that plantain mycorrhization can influence the solubilisation and promote the

leaching of metals (especially for Fe^{2+} , and to a lesser extent for Al^{3+}) from the soil. In this way, the bioavailability of nutrients (i.e., phosphorus) in acidic soil could increase, as soluble inorganic phosphorus is bound by these metals. However, analyses of phosphorus and nutrients such as Ca, K, Mg, N, Zn will have to be carried out in the banana-soil agrosystems to better define the interactions between banana growth and dynamics of these elements. In addition, further investigations need to be conducted to constrain fungi and/or bacteria strains in Azaguié soils to find the efficient strain for plantain in the context of agroecological banana production.

Conflict of interest declaration

The authors have no conflicts of interest to declare.

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