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Evaluation of mycorrhizal efficiency of soils under cocoa farming by measuring the impact of inoculation on maize growth

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ABSTRACT

The decline in soil fertility in Côte d'Ivoire is one of the main causes of the low productivity of cocoa farms. This study was aimed at assessing the mycorrhizal efficiency of soils under cocoa cultivation for a possible production of inoculum based on endogenous mycorrhizae. The soils were collected from cocoa fields in four localities (Dagbabouo, Kibouo, Toroguhue and Zépreguhue) in the agroecological zone of Daloa, a cocoa production zone in Côte d'Ivoire. The chemical fertility of soils, the mycorrhizogenic infectious potential (MIP) and its impact on the growth of maize (*Zea mays*, L.) var GRMP18 were evaluated by performing bioassays. The soils of the four localities are poor in organic matter (OM) with a low cation exchange capacity. Zepreguhue soils are distinguished from others by their high levels of Mg^{2+}, Na^{+} and assimilable phosphorus. In Zepreguhue, the highest MIP, the highest phosphorus concentration and the best agronomic performance on maize (*Z. mays* L.) were observed. Zepreguhue soils have the most successful mycorrhizal efficiency. These results constitute background data allowing to implement mycorrhization technology for sustainable cocoa and other crop cultivation in Côte d'Ivoire.

INTRODUCTION

Côte d'Ivoire has occupied, from the end of the 1970s until now, the rank of the world's highest producer of cocoa. Despite this performance, farm productivity levels remain low. The observed yields vary on average between 300 to 500 kg/ha against 2000 to 2500 kg/ha as predicted by research (Assiri et al., 2009; KPMG, 2012). The main causes of this low yield are the depletion of forest reserves, but also include the decline in soil fertility and the emergence of new diseases (Kouakou et al., 2011). Management of soil nutrients is critical to the overall health of the cocoa tree (Snoeck and Dubos, 2018). In cocoa farms in Côte d'Ivoire, the drop in yield due to soil impoverishment can reach 30% (Koko et al., 2009).

The use of NPK fertilizer had a positive impact on the growth and development of young cocoa plants (Lambert et al., 2020). However, continual use of inorganic fertilizers presents a number of concerns including decrease in soil organic matter (OM), deterioration of soil structure, soil hardening and soil acidification which are identified as the most serious (Kopittke et al., 2019). In order to reduce these constraints, various research programs aiming at the development of alternative systems of sustainable, intensive and competitive cocoa cultivation, with a limited environmental impact, have been undertaken. These include the improvement and selection of high-yielding and resistant varieties, the development of cocoa regeneration and rehabilitation techniques, cultivation techniques

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Table 1. Geographic coordinates of sampling points.

Cocoa fields	Sampling points	Geographic coordinates		
		Altitude	North	West
Kibouo	kib1	255.5 m	06° 49.540	006° 30.535
	kib2	249.9 m	06° 49.474	006° 30.516
	kib3	243.5 m	06° 49.595	006° 30.486
	kib4	236.6 m	06° 49.541	006° 30,444
Toroguhue	tor1	261.7 m	06° 56.491	006° 27.581
	tor2	251.5 m	06° 56.514	006° 27.561
	tor3	256.9 m	06° 56,531	006° 27.534
	tor4	256.3m	06° 56.532	006° 27.534
Dagbabouo	dag1	257.8m	06° 44.382	006° 27.886
	dag2	252.5m	06° 44.344	006° 27.899
	dag3	254m	06° 44.405	006° 27.908
	dag4	249.9m	06° 44.372	006° 27.939
Zepreguhue	zep1	253.8m	06° 54.019	006° 21.841
	zep2	257.2m	06° 53.980	006° 21829
	zep3	259.9m	06° 54.012	006° 21.782
	zep4	265.9m	06° 54.061	006° 21.793

kib: Sampling point in Kibouo's cocoa fields; **tor:** Sampling point in Toroguhue's cocoa fields; **dag:** Sampling point in Dagbabouo's cocoa fields; **zep:** Sampling point in Zepreguhue's cocoa fields.

combining cocoa and forest species and fertility management in cocoa trees (Dumont et al., 2014; Kebe et al., 2016). However, the results from these research programs have been below expectations.

Another little popularized approach in Côte d'Ivoire consists of using the microbiological potential of the soil, in particular arbuscular mycorrhizal fungi (AMF). These soil fungi form symbiosis with the roots of most land plants to create mixed organs called mycorrhizae. These mycorrhizae improve water and mineral absorption, and the resistance of plants to biotic and abiotic stresses (Smith and Read, 2008). Today around the world, several inocula of AMF have been developed to ensure the sustainable cultivation of a large number of plants in agriculture. However, the integration of this technology in agriculture in general and particularly in Ivorian cocoa farming requires inocula designed from endogenous strains adapted to the ecological conditions of crops in Côte d'Ivoire. Exotic strains sometimes present difficulties in adapting to local ecological conditions (Faye et al., 2013). Their integration into cropping systems is often unsuccessful (Kouadio et al., 2017). AMF inocula development can be accomplished either on an individual strain basis or a consortium of strains naturally found in soil (Torres-Ariasa et al., 2017). In this case, it is imperative to determine the mycorrhizal efficiency of soils before considering using the AMFs they contain as a source of inoculum. In this work, the mycorrhizal efficiency of soils under cocoa cultivation was determined through soil parameters, their ability to induce mycorrhizal structures in maize roots (*Zea mays*, L., 1753) and to promote maize growth.

MATERIAL AND METHODS

Soil sampling

Soil samples were collected from cocoa fields in agroecological zone of Daloa. This area is an important site of cocoa production in Côte d'Ivoire (Aguilar et al., 2003; Anon, 2006). Samples were collected in September during the harvesting period from four fields which are Dagbabouo, Kibouo, Toroguhue and Zepreguhue (Table 1). At each field, three soil samples of about 1 kg were collected at a depth of 20 cm in the rhizosphere of cocoa plants according to the diagram proposed by Huang and Cares (2003). The soils were brought up to the laboratory. Each replicate was air-dried.

Soil chemical analyses

The soil samples characteristics were determined by routine analysis methods. Air-dried soils were used to determine chemical characteristics. pH values of soils were determined according to Pansu and Gautheyrou (2006). Organic carbon was evaluated using the method of Walkley and Black (1934) and nitrogen in soil by the Kjeldahl method (Bremner, 1960). OM was calculated as:

OM (%) = organic C × 1.724 (Schaffer, 1975). Three classes of OM content of soil fertility namely poor OM content (1–2%), moderate OM content (2–4%) and rich OM content (> 4%) were defined (El Boukhari et al., 2016). The cationic exchange capacity (CEC) and total P were determined using the method of Duchaufour (1977), Pansu and Gautheyrou (2006), respectively. The CEC was measured on a KCl suspension after mechanical stirring of 5 g of soil sample. For total phosphorus, it was determined after total wet digestion of 5 g ground soil with reagent composed of 60% perchloric acid, nitric acid (density=1.4) and distilled water. Available P was determined from Olsen (1952). Three phosphorus content classes of soil fertility were defined (Calvet and Villemin, 1986): very low P content (< 30 ppm), low P content soil (30–50 ppm), and moderately poor content (50–100 ppm). The exchangeable cations (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}) were measured in a suspension of KCl after mechanical stirring of 5 g of soil sample.

Maize inoculation

An amount of 100 g of each field soils were used as inoculum and 3000 g of sterilized compost (autoclaved at 110°C, 2 kg/cm^2, 3 h; characteristics: pH = 7.1; OM = 2.81%; total nitrogen = 0.15%; available phosphorus = 55 mg/kg) was used as substrate. Thus inoculated pot cultures were established using 3000 g of sterile substrate and 100 g of each sampled soil. Control pot made using 3000g + 100 g of sterile substrate.

Maize (*Z. mays* L., 1753) var GRMP18 seeds were surface sterilized with sodium hypochlorite (10% v/v) for 10 min, thoroughly rinsed with sterilized water then were pre-germinated. After germination, seedlings were selected for uniform size and then transplanted into pot culture (5 L). 35 pots per each field sample were established and 35 non-inoculation pots were also established as control. The pots were placed in greenhouse. Cultures were grown for 60 days. Each pot contained one maize plant. Maize growth parameters were recorded and soil core samples (50 g) were taken from each pot for AMF spore extraction.

Assessment of mycorrhizal development

Evaluation of arbuscular mycorrhizal fungal (AMF) spore production by field soil

AMF spores were isolated every two weeks until the sixtieth day from field soil samples (50 g) or pot culture (50 g). Spores were extracted using wet-sieving and decanting (Gerdemann and Nicolson, 1963) with sieves of different sizes (45, 90, 125 and 500 µm) and the modified sucrose density gradient centrifugation method (Walker et al., 1982). AMF spores were counted using binocular magnifying glass (EUROMEX Holland STO 11738). Spore density was expressed as the number of spores per gram of dry soil.

Assessment of maize root colonization

Fine maize roots of three plants per treatments were sampled every two weeks until the sixtieth day after planting. Roots were rinsed and cut into 1 cm fragments. These roots fragments were cleared by boiling in 10% (w/v) KOH and stained with 0.05% (v/v) trypan blue in lactoglycerol according to Phillips and Haymann (1970) method. Ten pieces of roots per plant were placed in glycerol (50%) between slide and coverslip (Kormanik and McGraw, 1982) and observed under an optical microscope. The colonized roots were observed and evaluated according to Trouvelot et al. (1986).

Assessment of Inoculation effect on maize growth in greenhouse

Plant growth parameters (height, collar diameter, foliar surface area and biomass) were assessed every 15 days for 8 weeks. Three plants per treatment were chosen for the measurements. Plant height was estimated from the base of the stem to the crossing point of the last two leaves. Collar diameter was tacked at the base of the stem, while the leaf area is calculated according to the formula $S = L \times l \times 0.75$ where L= maximum leaf length and l = maximum leaf width (Bonhomme and Varlet-Grancher, 1978). Dry biomass was also determined by the destructive method. Three plants per treatment were uprooted from the ground with their roots. Whole plants were weighed after ovendrying at 50°C for 5 days (that is, until the weight measured no changes).

Statistical analyses

All from greenhouse experiments were subjected to statistical analyses by performing either one or two-way analysis of variance (ANOVA) using SPSS. The significance of the treatment effects was determined using Duncan test with P = 0.05.

RESULTS

Cocoa field soil physicochemical characteristics

Chemical characteristics of cocoa field in Daloa are given in Table 2. The soils of Dagbabouo (pH = 6) and Kibouo (pH = 5.8) were acidic while the soils of Toroguhue (pH =

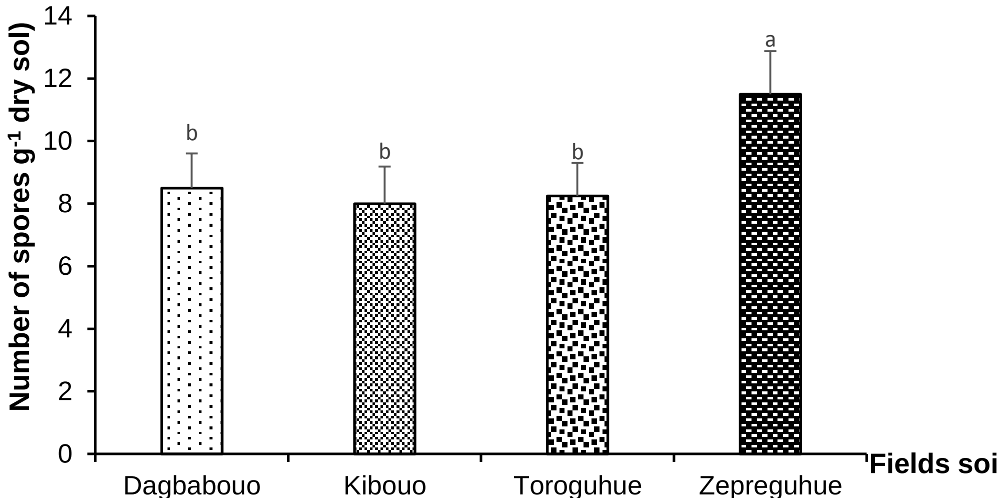

Figure 1. Density of spores in different sampling soils. All the values are means of the three replications (N = 3). Error bar indicate standard deviation. Means with different letters were significantly different at 5% level.

Table 1. Chemical characteristics of cocoa field soils.

Chemical properties	Dagbabouo	Kibouo	Toroguhue	Zepreguhue
pH	6.0^{b}	5.8^{b}	6.9^{a}	6.8^{a}
C (%)	1.38^{b}	2.03^{a}	1.85^{ab}	1.49^{ab}
N (%)	0.12^{b}	0.16^{a}	0.15^{ab}	0.12^{b}
C/N	11.5^{a}	12.69^{a}	12.33^{a}	12.42^{a}
OM (%)	2.37^{b}	3.49^{a}	3.19^{a}	2.57^{ab}
P ass. (ppm)	67^{b}	72^{ab}	71^{ab}	75^{a}
CEC (cmol*kg^{-1})	6.6^{a}	5.6^{a}	8.6^{a}	7.4^{a}
Ca^{2+} (cmol*kg^{-1})	1.74^{bc}	1.30^{c}	3.45^{a}	2.27^{b}
Mg^{2+} (cmol*kg^{-1})	0.85^{a}	0.83^{a}	1.013^{a}	1.02^{a}
K^{+} (cmol*kg^{-1})	0.27^{a}	0.25^{a}	0.27^{a}	0.21^{a}
Na^{+} (cmol*kg^{-1})	0.08^{b}	0.06^{b}	0.07^{b}	0,68^{a}

N, Nitrate; **OM,** organic matter; **P ass,** assimilable phosphorus; **C,** carbon; **CEC**, cation exchange capacity. Means with different letters are significantly different at the 5 % level.

6.9) and Zepreguhue (ph = 6.8) were neutral. The nitrogen proportion of the soils of Dagbabouo and Zepreguhue were average while those of Kibouo and Toroguhue soils were higher. The C/N ratios of the four soils were high with values between 11.5 and 12.69. All soils were moderately rich in OM. The lowest (2.37%) and highest (3.49%) OM concentrations were observed in Dagbabouo and Kibouo soils respectively. All four soils were moderately poor in available phosphorus. However, Zepreguhue soils had the highest available phosphorus content (75 ppm), while the locality of Dagbabou had the lowest available phosphorus content (67 ppm).

The CEC of the soils of the four fields were low with values less than 10 cmol/kg. However, cation content varied depending on the locality. A very high concentration of Na^{+} ions was observed in the Zepreguhue soils (0.68 cmol/kg). The soils of Toroguhue had the highest content of calcium (3.45 cmol/kg) with the lowest calcium concentrations observed Kibouo (1.30 cmol/kg^{-1}). There was no significant difference between the magnesium and potassium contents of all soils.

Mycorrhizal potential of each soil

The spore densities of arbuscular mycorrhizal fungi from soils sampled in cocoa fields ranged from 8 to 11.5 spores g^{-1} dry sol (Figure 1). Zepreguhue soils had the highest density while the densities of spores in Dagbabouo, Kibouo and Toroguhue were the same.

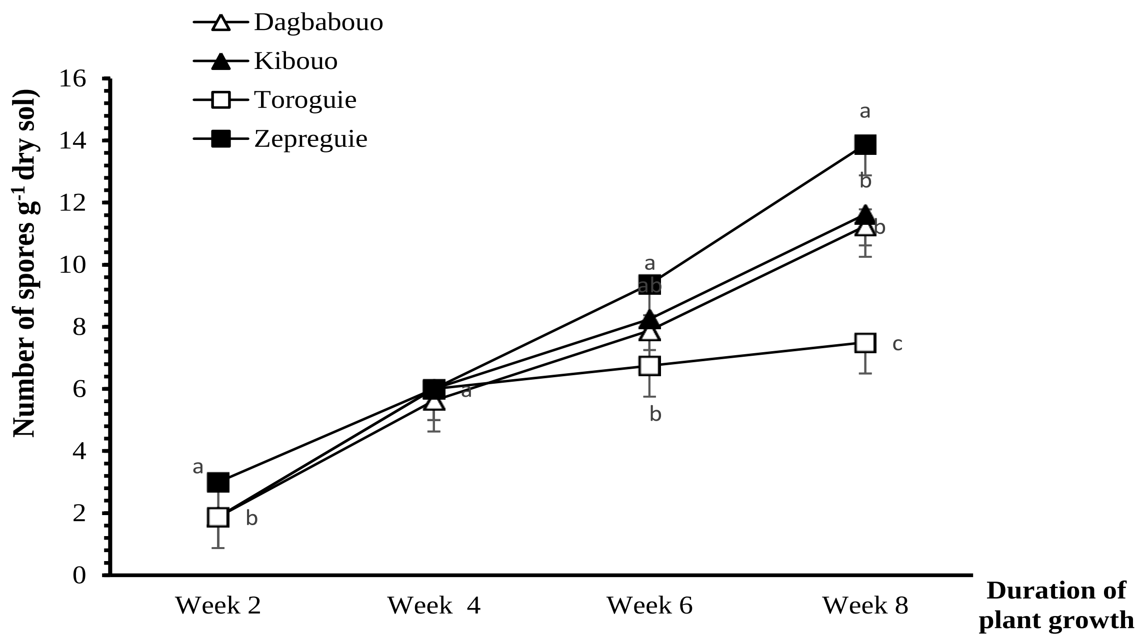

Figure 2. AMF spore density in maize-grown soil over time for 4 field soils. Error bar indicate standard deviation. Means with different letters were significantly different at 5% level.

Changes in AMF spore soil density during maize growth in pot culture

AMF spore densities from all four soils increased from week 2 to week 8 with value ranging from 2 to 14 spores g^{-1} dry soil (Figure 2). The soils of Zepreguhue contained the highest densities, especially from the 6th week to the 8th week. Whereas, the lowest densities were obtained from Toroguhue from the 6th week to the 8th week. Zepreguhue zone stands out with higher spore densities.

Evolution of mycorrhizal colonization in maize roots

The mycorrhization colonization of the four cocoa soils obtained on the roots of maize (*Z. mays* L.) increases from the second (2nd) week to the sixth (6th) week with no changes in colonization between weeks 6 and 8 (Figure 3). The mycorrhization colonization of Zepreguhue soils was higher than those of other fields, especially from week 4 through week 8. No significant difference was noted between the MIs of Dagbabouo, Kibouo and Toroguhue.

Impact of cocoa field soil potential on maize growth

Generally, plant heights (Figure 4A) increased continuously for the first six weeks with no change in plant height between weeks 6 and 8. Also, there was no significant difference in height from week 2 to week 4. However, from the sixth to the eighth week, the heights of Zepreguhue plants (31.67 to 32.8 cm) were higher than those of the controls (24.13 to 25.29 cm). During the same period, no significant difference was noted between the heights of the plants on pots with Zepreguhue, Toroguhue and Dagbabouo soils used as inoculum.

The collar diameter of plants cultivated in Zepreguhue soil increased in diameter from week 2 to week 6 and then declined from week 6 to 8 (Figure 4B). While those of the plants (controls, Dagbabouo, Kibouo and Toroguhue) increased from the second to the fourth week and regressed from the sixth week. The collar diameter of Zepreguhue plants were the largest, especially from the sixth week (4.8 cm) to the eighth week (4.26 cm).

All leaf areas (Figure 4C) increased rapidly during the first four weeks. In the fourth week, the leaf areas of plants inoculated with soils from Zepreguhue and Dagbabouo were higher than those of control plants and plants Kibouo and Toroguhue soils. From the fourth week to the sixth week, the leaf areas of the maize plants on soil of Dagbabouo, Kibouo and control plants, regressed, while those on the soil of Toroguhue and Zepreguhue grew until the sixth week, then regressed from the eighth week. Also, it is noted that from the sixth week to the eighth week, the maize plants on Zepreguhue soils, recorded the highest values of leaf areas in comparison with all the other plants. The dry biomass of plants in Zepreguhue soils increased from week 2 to week 6 with values between 0.88 and 4.44 g, dry biomass is unlikely to decrease over time (Figure 4D). Changes in dry biomass of plants in Toroguhue soil

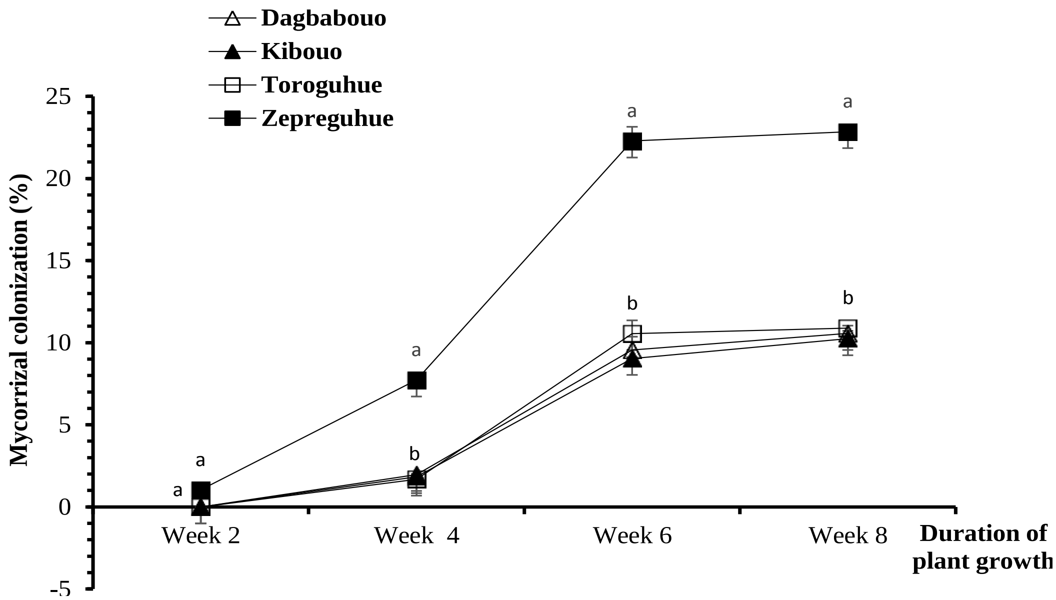

Figure 3. Changes over time in maize root colonization by AMF from 4 field. Error bar indicate standard deviation. Means with different letters were significantly different at 5% level.

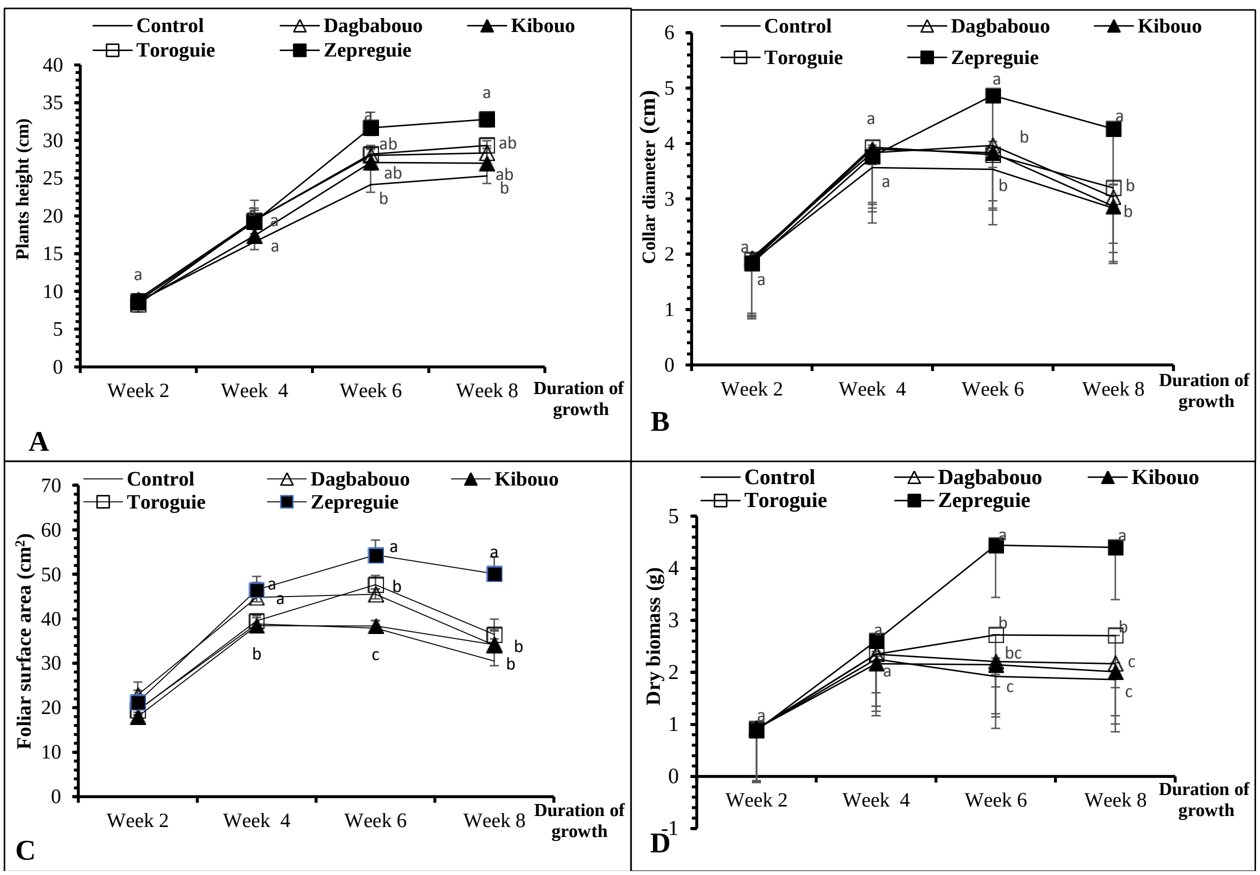

Figure 4. Change in maize growth parameters over time in pot-grown plants in 4 field soils. **A,** Plants height; **B,** Plants collar diameter; **C,** Plants surface foliar; **D,** dry biomass. Error bar indicate standard deviation. Means with different letters were significantly different at 5%.

Soil chemical properties

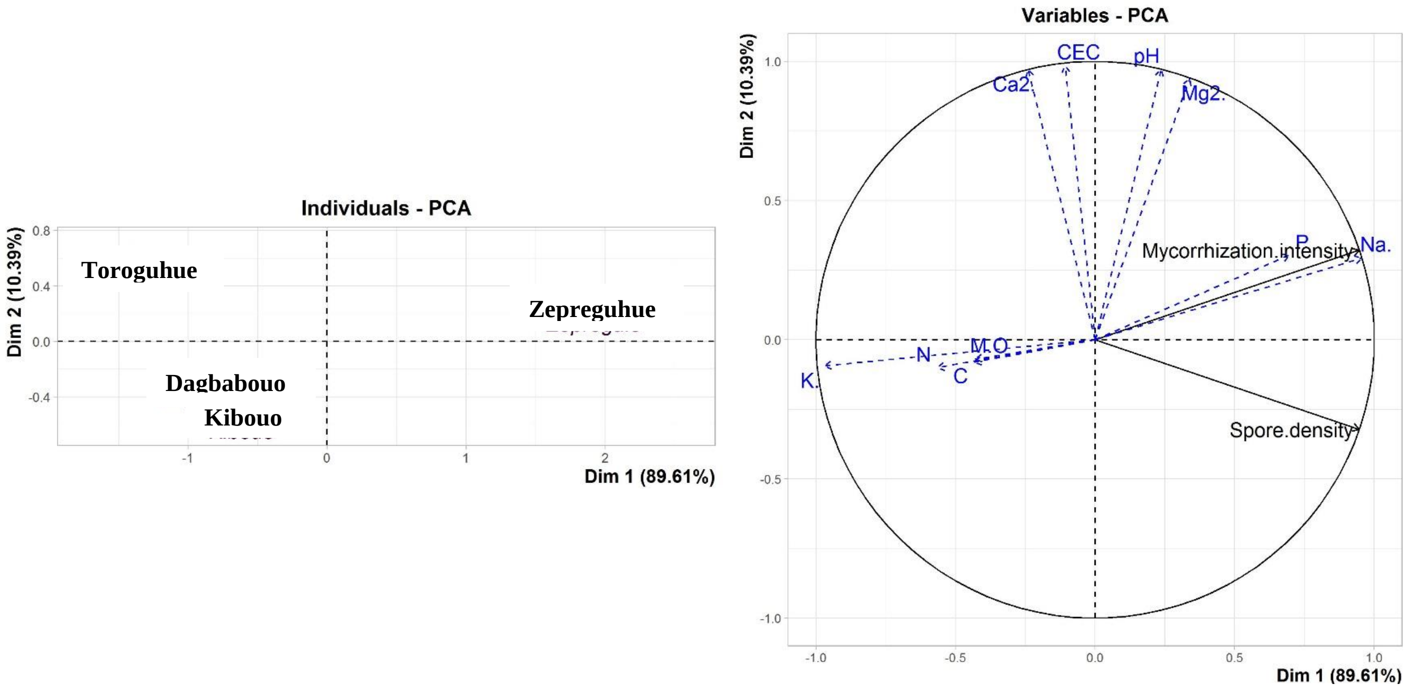

Figure 5. Multiple component analyses (PCA) between mycorrhization potential and soil chemical properties.

were not significant between the fourth and the eighth week (2.35 to 2.70 g). In Dagbabouo, Kibouo soils, and the control dry biomass is unlikely to decrease over time. Maize plants in soils in the locality of Zepreguhue followed by those in Toroguhue recorded the highest values of dry biomass.

Mycorrhizal efficiency assessment through correlation analyses

Correlation between chemical properties of soil and mycorrhizal potential of soils

Correlations were performed in order to demonstrate the interactions between the chemical properties of soils and the mycorrhization parameters of soils under cocoa cultivation. The graph (Figure 5) revealed that the localities of Dagbabouo and Kibouo have similar chemical parameters measured in the soils while Toroguhue and Zepreguhue had unique and different characteristics. Mycorrhizal colonization, spore density (DS) show positive correlations with chemical parameters such as the content of available phosphorus (Pass), sodium (Na^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), the CEC as well as the pH while chemical parameters such as potassium content (K^+), proportion of nitrogen (N), organic carbon (C) as well as OM proportion have negative correlation with mycorrhizal colonization, and density of spores. The soil of Zepreguhue had both the highest level of assimilable phosphorus and mycorrhization of the 4 field soils.

Impact of mycorrhizal potential on maize vegetative growth

Figure 6 and Pearson-R correlations (Table 3) show the interaction between maize growth parameters and the mycorrhization parameters of the 4 field soils. Dagbabouo, Kibouo and Toroguhue soils were similar in maize growth performance. Maize plants inoculated with these soils would show similarities in agronomic growth, while the plants inoculated with Zepreguhue soils showed unique and different characteristics. The mycorrhization parameters were positively correlated with the various agronomic growth parameters (Height / MI: R = 0.74); Biomass / IM: R = 0.88; Height / DS: R = 0.78; Biomass / DS: R = 0.75).

DISCUSSION

In this study, the objective was to assess the mycorrhizal efficiency of soils under cocoa cultivation with the aim of

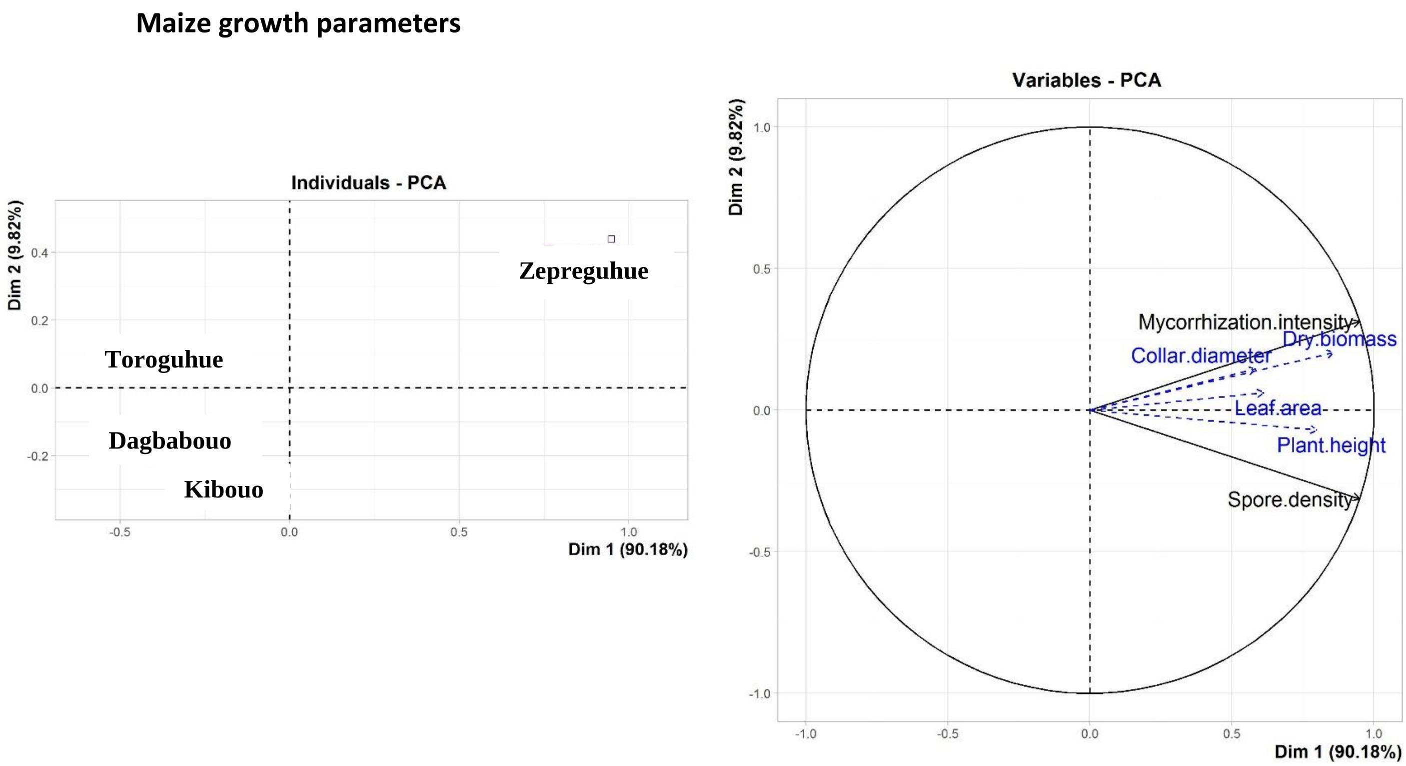

Figure 6. Multiple component analyses (PCA) between mycorrhization potential and maize growth parameters.

Table 3. Pearson correlation coefficient (R).

Growth parameters	R Mycorrhizal colonisation	P-value
leaf areas	0.6014864	1.02e-06
plant heights	0.7382777	2.18e-12
Plants collar diameter	0.6004496	2.85e-05
Dry biomass	0.8797747	5.59e-14

possibly enhancing the endogenous mycorrhizal potential as a source of inoculum easy to produce by farmers for the sustainable cultivation of cocoa in Ivory Coast.

After eight weeks of maize cultivation, the spore densities of AMF in the pots with Zepreguhue soils as inoculum were the highest of the 4 field soils tested, only the soils of Zepreguhue resulted in mycorrhizal colonization of more than 25%, showing a good infectivity of mycorrhizal propagules (Cuesta, 2013). The development of AMF has been linked to various biotic and abiotic factors in soil including chemical element content (Voko-Bi et al., 2013). The soils of both Dagbabouo and Kibouo are acidic with average nitrogen proportions, a high C/N ratio reflecting reduced biological activity with difficult mineralization (Valé, 2006). The soils of Toroguhue and Zepreguhue have neutral pH, with average proportions of nitrogen, a high C/N ratio as in the soils of Kibouo and Dagbabouo. Biological activity is expected to be reduced in Toroguhue and Zepreguhue.

Chemical analysis showed that all of the sampled soils had a deficit in OM and low mineral fertility. Indeed, the cation exchange capacities of soils and the levels of available phosphorus were low. The monoculture of cocoa on these soils for several years would result in a reduction in biological activity, leading to unavailability of minerals for assimilation by the plant. This reduction in organic activity could also be explained by the continued use of chemical inputs in the cocoa fields. Indeed, organic and chemical inputs are increasingly used by cocoa producers to compensate soil fertility deficits (Ruf, 2015). The biological activity of soil microorganisms is necessary for the proper decomposition of OM followed by the mobilization of mineral elements that can be assimilated by the plant. Thus, an unavailability of assimilable minerals would reflect low activity of soil microorganisms. This low biological activity could be particularly related to that of AMFs whose action is important in the mobilization of assimilable minerals (Duponnois et al., 2011). However, Zepreguhue soils stood out by their elevated magnesium content, significantly higher sodium concentrations and above all their higher assimilable phosphorus contents. In Zepreguhue soils, the activity of microorganisms, in this case that of AMFs, is expected to be higher than the other 3 field soils. Indeed, several authors have demonstrated that the presence of AMFs is positively correlated with high levels of minerals in soil, specifically with assimilable phosphorus (Jakobsen et al., 2002). This conclusion was confirmed with the evaluation of the mycorrhizal potential of each soil. Indeed, it was in the locality of Zepreguhue that the high mycorrhizal intensity and the highest spore densities were observed. In the cacao fields in Dagbabouo and Kibouo, the application of fertilizers is common. The soils in these fields have elevated levels of potassium, nitrogen, and OM, yet these chemical parameters have negative correlations with the mycorrhizal colonization. It has been shown that high OM content and NPK amendments are generally unfavorable to the development of AMFs (Dai et al., 2014; Garbaye, 2013); this would explain why mycorrhization in these two areas is low.

The growth of maize with each of the soils used as inoculum gave variable agronomic performances according to the locality and mycorrhizal potential of each soil. The best maize growth was obtained with Zepreguhue soils. Regardless of the agronomic parameter considered, the soils of the locality of Zepreguhue with the strong mycorrhizogenic potential gave the best plant growth. Mycorrhization is correlated with improved vegetative growth in plants (Sery et al., 2016). Other authors have shown that the increase in the biomass of mycorrhizal plants is associated with the activity of AMFs improving the uptake of limiting elements particularly phosphate (Lambers et al., 2008). Improving phosphate nutrition by mycorrhization could also induce resistance to biotic and abiotic stresses (Duponnois et al., 2011). In the current study, the mycorrhizal potential, was positively correlated with the concentrations of phosphorus, sodium and the growth parameters of maize. The transfer of phosphorus by AMFs has been demonstrated by several authors (Harrison et al., 2002; Javot et al., 2007). The association between mycorrhization, the mobilization of mineral elements and the agronomic performance of maize could make it possible to define the mycorrhizal efficiency of a soil as the capacity of this soil, taken as inoculum, to induce mycorrhizal structures in plants roots and to promote plant growth based on the established symbiosis. These benefits are improved mineral and water absorption, protection against pathogens, improved growth and yield (Smith and Read, 2008). Cocoa soils in Daloa zone taken as inoculum do not have the same capacity to induce mycorrhizal structures or to promote the growth of maize. Zepreguhue soils presented the highest mycorrhizal potential, the highest phosphorus concentration as well as the best agronomic performance on maize (*Z. mays*). Thus, Zepreguhue soils have the highest mycorrhizal efficiency. The enhancement of the mycorrhizal potential of the soils in Zepreguhue could lead to the production of effective inocula for the sustainable cultivation of cocoa in Côte d'Ivoire.

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