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Diversity of arbuscular mycorrhizal fungi communities in contrasted plantain field soils in Côte d'Ivoire as revealed by Illumina Miseq

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Abstract

The arbuscular mycorrhizal fungi (AMF) communities of plantain rhizosphere soils were investigated by Illumina MiSeq technology. We analyzed the possible correlation between soil characteristics, AMF abundance and community composition in plantain field soils within three different agro-ecological zones. We used principal component analysis to test the relative contribution of each agro-ecological zone in explaining AMF community composition variation in field soils. Pearson correlations were used to identify the soil properties that significantly explained AMF community compositions within the three zones. The results showed that despite the fact that the three zones exhibited contrasted soils, AMF communities within the three zones were dominated by Glomeraceae, with Rhyzophagus as the main genus (72.75% of AMF identified genera). Soil types determined the distribution of AMF communities in plantain field soils, and this effect was attributed to total phosphorus, organic matter, Ca2+, Mg2+ and Na+.

Keywords: Glomeromycota, diversity, Illumina Miseq, Plantain, Rhyzophagus.

Résumé

Les communautés des champignons mycorhiziens à arbuscules (CMA) des sols de la rhizosphère du bananier plantain ont été étudiées par la technologie Illumina MiSeq. Nous avons analysé la corrélation possible entre les caractéristiques du sol, l'abondance et la composition des communautés CMA dans les sols de bananiers plantains dans trois zones agro-écologiques différentes. Nous avons utilisé une analyse en composantes principales pour tester la contribution relative de chaque zone agro-écologique à l'explication de la variation de des communautés de CMA. Les corrélations de Pearson ont été utilisées pour identifier les propriétés du sol qui expliqueraient de manière significative la composition des communautés de CMA dans les trois zones. Les résultats ont montré que, malgré le fait que les trois zones présentaient des sols contrastés, les communauté des CMA dans les sols des champs de plantains étaient dominée par la famille des Glomeraceae, Rhyzophagus étant le genre principal (72,75% des genres identifiés). Le type de sol a déterminé la distribution des communautés de CMA dans les sols de bananier plantain, et cet effet a été attribué au phosphore total, à la matière organique, au Ca2+, au Mg 2+ et au Na+.

Mots clés : Glomeromycota, diversité, Illumina Miseq, Plantain, *Rhyzophagus*.

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Introduction

Of many microbes in rhizospheres, Arbuscular Mycorrhizal Fungi (AMF) that belong to the phylum Glomeromycota (Schüßler et al., 2001), constitute a multifunctional partner in the mutualistic interaction they develop with most land plants. Indeed, AMF provide the mycorrhizal plant with water and essential nutrients such as phosphorus and nitrogen (He et al., 2003; Smith and Read, 2008). In addition AMF improve plant tolerance to both biotic and abiotic stresses (Augé, 2001, 2004; Ortas et al., 2001; Plenchette et al., 2005; Al-karaki, 2006; Pozo and Azcón-Aguilar, 2007; Porcel et al., 2011; Augé et al., 2015). This has led to the development of mycorrhizal inoculants as biofertilizers for a sustainable agriculture. Mycorrhizal inoculation has been applied for decades to promote better plant growth for various crop plants notably in Sabsaharian Africa (Séry et al. 2016, Kouadio et al. 2017) where access to chemical fertilizers remain expensive (Plenchette et al., 2005). In Côte d'Ivoire, plantain with 1.6 million tons over the last decade (FAO 2016) is an important food crop. However, the production system of this culture is essentially based on low-performing and mostly marginal farming techniques without fertilizer (Traoré et al., 2009). The positive impact of AMF inoculation on banana productivity has been demonstrated (Jaizme-Vega et al., 2002; Elsen et al., 2003; Hol and Cook, 2005; Jaizme-Vega and Rodriguez Romero 2004). Moreover, mycorrhizal inoculation was shown to decrease the incidence of nematodes such as Radopholus similis (Koffi et al., 2013) and Pratylenchus goodeyi (Elsen et al., 2003) and fungal pathogens such as Cylindrocladium spathiphylli (Declerck et al., 2002), Fusarium oxysporum var cubense (Jaizme-Vega et al., 1997) and more recently M. fijiensis (Oye Anda et al., 2015) in banana. However, AMF inoculation for plantain productivity should require prior knowledge of endogenous AMF communities in order to provide a better selection of AMF inocula for sustainable agricultural practices (Séry et al 2018). Nowadays, high-throughput sequencing technology such as the Illumina MiSeq is fundamental to investigating fungal communities (Balint et al., 2014; Mao et al., 2017, Séry et al 2018). Metabarcoding through DNA identification and high-throughput DNA sequencing provide an unprecedented insight into the composition of unknown communities (Bik et al., 2012; Shi et al., 2016). The aim of this work was to identify the AMF communities associated to plantain fields in 3 agro-ecological zones of Côte d'Ivoire. The main objective was to understand the ecology of AMF communities in plantain field soils using the Illumina sequencing approach.

We wanted, through this molecular tool to (i) determine the composition and structure of arbuscular mycorrhizal fungi in the plantain rhizosphere soils and (ii) analyze the impact of soil physico-chemical characteristics on the composition and structure of AMFs species communities.

Materiels and Methods

Soil sampling

We collected soil samples from plantain fields in three different agroecological zones (Bouaflé, Azaguié and Abengourou) in Côte d'Ivoire.

Located in the center of Côte d'Ivoire, the Bouaflé area was characterized by an average temperature of 30° C with annual amplitude of 5° C. It was characterized by two rainy seasons and two dry seasons. The soils were for the most part ferralitic soils moderately denatured. The vegetation was that of a zone of contact between the forest and the savannah, with clear forest and gallery forest along the rivers and areas of wooded or shrub savannah.

The agro-ecological zone of Azaguié was located in the southeast of Côte d'Ivoire in forest zone with ferralitic soils. The climate of Azaguié was a tropical humid with two rainy seasons and two dry seasons. The average annual precipitation was 1700 mm (Mollard, 1993). The Azaguié soil belongs to the category of ferralitic soils. It was characterized by its great depth, reddish color, satisfactory permeability and the presence of gravel. The parent rock was a birrimian schist, of the arkosic type or sometimes a variegated shale rich in silica. The average content of organic matter was is of the order of 1.7 to 2.5%. The pH was acidic (4.1 to 6.2).

The Abengourou agro-ecological zone was located in the east of Côte d'Ivoire with a subequatorial climate, constituted by dense rainforest. It included two rainy seasons that alternate with two dry seasons. Rainfall varied between 1200 mm and 1800 mm and the average annual temperature varies between 25° and 28° C. Soils were generally ferralitic (Perraud, 1971; Yoro et al., 1995).
Four fields were sampled for each agro-ecological zone, (Table 1). In each field, three samples (1 kg each) were collected at a depth of 20 cm in the plantain plant rhizosphere according to the method of Huang and Cares (2004). Two major varieties of plantain (French and Faux Corne) were grown in the fields at three surveyed areas.
In Abengourou, the fields surveyed were traditional monoculture farms on which fallow land were before banana cultivation. In the Azaguié area, they were also traditional farms, with crop associations with cocoa or cola seedlings. In Bouaflé, the fields surveyed were traditional farms of cultivation associations (banana-young plants and cocoa) for the fields.

Soil analyses

Dried soils from plantain fields were used to determine chemical and physical characteristics. The contents of nitrogen N, carbon C, organic matter OM (OM= C*1.724; International method NF ISO 14235), total phosphorus (ppm), available phosphorus (ppm), cation exchange capacity CEC (cmol.kg-1), Ca2+ (cmol.kg-1), K+ (cmol.kg-1), Na+ (cmol.kg-1) were analysed. The pH of the soil was determined according to Pansu and Gautheyrou (2003a). Organic carbon was evaluated using the method of Walkley and Black (1934) and nitrogen in soil by the Kjeldahl method (Kjeldahl, 1983). The total phosphorus was determined after total wet digestion by attacking 5g ground soil with reagent composed of 60% perchloric acid, nitric acid (density=1.4) and distilled water. For soil's cationic exchange capacity (CEC) and total P, they were determined using the method of Duchaufour (1977), Pansu and Gautheyrou (2003b), respectively. The CEC was measured on a KCl suspension after mechanical stirring of 5g of soil sample. The available P was determined from Olsen (1952) and the exchangeable bases (Ca2+, Mg2+, K+, and Na+) were measured on a suspension of KCl after mechanical stirring of 5 g of soil sample.

Molecular analysis

Total genomic DNA was extracted from 1g of dry soil using the Fast Prep method according to Plassart et al. (2012). In a first step, 2µl of 1/10 diluted initial total genomic DNA was amplified using the universal eukaryotic primers LR1 and NDL22 (van Tuinen et al., 1998). The PCR reaction was performed in a final volume of 20 µl in a mixture containing 250 µM dNTP, 100 µM each primer and 1U of Taq polymerase (MP Biomedical). The amplification reaction was carried out in a thermocycler using the following program: denaturation for 5 min at 95 ° C, followed by 30 cycles of 94 ° C (1 min), 58 ° C (30 sec), 72°C (45 sec), 72 ° C for 5 min and 25°C for 1 sec. PCR product was used as template for a second PCR. The glomeromycetes specific primers FLR3-T (5'-TTG AAA GGG AAA CGA TTG AAG 3 ') and FLR4 (Gollotte et al., 2004) coupled to a half Illumina adapter, were used for the second PCR. The same PCR mixture was used as described above. The amplification reaction was carried out in a thermocycler using the following program: denaturation for 5 min at 95 ° C, followed by 30 cycles of 94 ° C (1 min), 58 ° C (30 sec), 72 ° C (45 sec), 72 ° C for 5 min and 25 ° C for 1 sec. The PCR amplification products were separated in a 1.2% agarose gel and visualized under ultraviolet light after staining with ethidium bromide.
Bioinformatic analyses were performed to the adapted pipeline defined by Balint et al. (2014) as used by Séry et al. (2108). This pipeline comprises a set of procedures designed to (1) ensure the quality of the reads. Raw read pairs were filtered for an average read quality, (2) assemble in pairs the data from the NGS (reads) with PANDAseq software (Masella et al., 2012), (3) remove primer artefacts, (4) reorient reads to 5'-3 'using grep-type commands to separate reads containing the forward and reverse primers, (5) demultiplexing where we retained only those reads that contained a perfectly matching primer + label combination on both ends. We used a script that relied on fqgrep (https://github.com/indraniel/fqgrep). Files were then pooled and primers and labels removed using a command from the Fastx Toolkit to trim labels and primers. Extraction of the informative zone LSU and the identification of the AMF's OTUs were done using VSEARCH software (Balint et al., 2014). The quality of the sequences is ensured by the assembly of the reads and the suppression of the reads of low quality, the suppression of the chimeras and the clustering. The matching of the reads Forwards and Reverse sequenced was important for completely reconstructing the LSU region and subsequently performing solid demultiplexing. This highly variable LSU region was subject to blasting (Nilsson et al., 2010). The reads were grouped in OTUs using sequences similar to 97% (clustering) before starting phylogenetic studies and ecological diversities. Operational taxonomic unit (OTU) delimitation and taxa assignment were done using the Maarjam database (Opik et al., 2010) and NCBI database as reference. The number of clones for each AMF's OTU in each sample was used to calculate the rarefaction curves (Goods coverage plot) with Explicet Version 2 software. A Venn diagram on the distribution of OTUs between agro-ecological zones was made with the Venny 2.1.0 software (http://bioinfogp.cnb.csic.es/tools/venny/).

Analysis of arbuscular mycorrhizal fungi diversity

The observed OTUs-(S) and the Chao1 index were

Table 1: Fields geographic coordinates

Zone	Fields	points	Geographic coordinates		
			North	*West*	*Altitude (m)*
Abengourou	Aniansuié 1	Ab 1/1	06°41.556'	003°41.698'	147 SE
		Ab 1/2	06°41.551'	003°41.671'	148 SO
		Ab 1/3	06°41.521'	003°41.737'	161 N
	Aniansuié 2	Ab 2/1	06°40.350'	003°38.837'	170 N
		Ab 2/2	06°40.344'	003°38.852'	165 SO
		Ab 2/3	06°40.347'	003°38.834'	166 N
	Dramanekro 1	Ab 3/1	06°42.676'	003°36.960'	152 N
		Ab 3/2	06°42.674'	003°36.934'	147 N
		Ab 3/3	06°42.654'	003°37.021'	161
	Dramanekro 2	Ab 4/1	06°42.913'	003°37.220'	177
		Ab 4/2	06°42.903'	003°37.200'	176 N
		Ab 4/3	06°42.892'	003°37.184'	176
Azaguié	Ahoua	Az 1/1	05°40.352'	004°02.385'	47 NO
		Az 1/2	05°40.410'	004°02.397'	48 N
		Az 1/3	05°40.324'	004°02.386'	47
	Blida 1	Az 2/1	05°37.221'	004°03.354'	77
		Az 2/2	05°37.231'	004°03.394'	74
		Az 2/3	05°37.260'	004°03.415'	70
	Blida 2	Az 3/1	05°39.169'	004°07.317'	52 N
		Az 3/2	05°39.147'	004°07.325'	52 N
		Az 3/3	05°39.150'	004°07.287'	53 N
	Mbromé	Az 4/1	05°31.947'	004°03.712'	74
		Az 4/2	05°31.970'	004°03.712'	70
		Az 4/3	05°31.944'	004°03.692'	63
Daloa	Garango 1	Bo 1/1	06°57.581'	005°50.737'	195
		Bo 1/2	06°57.588'	005°50.773'	194
		Bo 1/3	06°57.585'	005°50.809'	195
	Garango 2	Bo 2/1	06°55.746'	005°48.553'	213
		Bo 2/2	06°55.711'	005°48.545'	216
		Bo 2/3	06°55.691'	005°48.533'	217
	Garango 3	Bo 3/1	06°55.814'	005°48.121'	231
		Bo 3/2	06°55.879'	005°48.128'	234
		Bo 3/3	06°55.791'	005°48.147'	234
	Koudougou	Bo 4/1	06°55.411'	005°40.752'	168
		Bo 4/2	06°55.445'	005°40.807'	162
		Bo 4/3	06°55.486'	005°40.820'	162

calculated by analysis of the rarefaction curves using Explicet Version 2 software to asymptotically estimate the richness of the AMFs of each site. The alpha diversity: the Simpson reciprocal index (1/-Σpi2) and the Shannon-Wiener index (H'= - Σpi.lnpi) (pi relative abundance of the ith species) was calculated to estimate the diversity of AMFs by the metagenomic approach. The community evenness was assessed by the Piélou evenness index (J'= H' / lnS). The number of sequences assigned to each OTU was considered as an estimator of the abundance of molecular species. Principal Component Analyses (PCA) of the AMF community composition and soils parameters in the plantain rhizosphere soils in the three agro-ecological zones were done with XSLSTAT 2015 and Statistica software Version 7.1. It permitted to assess the effect of agro-ecological zones on soil properties, on the diversity of mycorrhizal fungal communities on the other. A (Pearson (n)) correlation matrix between diversity and soil physico-chemical parameters was calculated using Statistica software Version 7.1

Results

Soil characteristics of the three agro-ecological zones

The Abengourou and Bouaflé soils were basic (respectively pH 6.69 and 6.76) in contrast to Azaguié soils (Table 2). Abengourou soils were rich in organic matter (OM: 3.54, C: 2.05 and N: 0.23) compared with Azaguié (OM: 2.64, C: 2.64, N: 0.14) and Bouaflé (OM: 2.6, C: 1.51, N: 0.18).The levels of total and assimilable phosphorus were statistically identical for the three agroecological zones. There was a good decomposition of organic matter with a C/N ratio of (9 to 11) in the soils of the three zones (Abengourou, Azaguié and Bouaflé). CEC values (13.94 cmol.kg-1) were high in Abengourou indicating good nutrient storage capacity, such as Ca2+, Mg2+ and K+. In fact, Abengourou soils contained a large quantity of cations (respectively 3.3, 1.38 and 0.346 cmol.kg-1) meaning that the Abengourou soils were more fertile than the Azaguié soils; more acid (pH water: 5.94) and poor in cations (Ca2+: 1.3, Mg2+: 0.73, K+: 0.18) soils from Bouaflé that had basic pHs grouped both with those of the Abengourou and Azaguié zones (Figure 1).

AMF diversity and species richness within the plantain field soils.

The rarefaction curves of the OTUs for each study area reached the asymptotes in all cases (Fig. 2). A total of 766 200 sequences were obtained from these three agro-ecological zones, respectively 173 900 for Abengourou, 270 302 for Azaguié and 321 998 for Bouaflé (Table 3). A total of 110 OTUs were obtained from the plantain field soils (Fig. 3).

Abengourou, Azaguié and Bouaflé’s area had 32 OTUs in common (Fig.2). We noticed also specific OTUs in each agroecological zone respectively 3 OTUs in Abengourou, 5 OTUs in Bouaké and 6 OTUs in Azaguié. Azaguié zone had the most OTUs specifics number contrary to Bouaflé and Abengourou. Abengourou and Bouaflé zone have the most OTUs in common (13) Twelve genera of AMF were identified in the banana rhizosphere in Côte d'Ivoire. The different genera were: Acaulospora, Ambispora, Archeospora, Claroideoglomus, Funneliformis, Gigaspora, Glomus, Paradentiscutata, Paraglomus, Racocetra, Rhizophagus and Septoglomus. We noticed seven, height and eleven genera respectively in Abengourou, Azaguié and Bouaflé. Species belonging to the genera Rhizophagus were abundant in Abengourou (73.07%), Azaguié (76.53%) and Bouaflé (69.4%). The genera Acaulospora was also abundant in Abengourou (8.07%) and Azaguié (1.6%). The dominant genera in the banana rhizosphere in the three regions were the genera Rhyzophagus (72.75%); Archeospora (2.82%); Acaulospora (2.8%); Septoglomus (2.2%) and Glomus (2.084%) with a high number of OTUs respectively (29, 12, 10, 8 and 5 for Glomus). Respectively heighteen, sixteen and twenty species were identified in Abengourou, Azaguié and Bouaflé. Abengourou area contained more species. A total of twenty five species of AMF were associated with the banana rhizosphere (Table 4). Some of these species were very abundant (20-30%)

Table 2: Physico-chemical characteristics of the plantain field soils within the three agro-ecological zones

Zones	pH_{water}	O.M (%)	C%	N%	P. total (ppm)	P. ass. (ppm)
Abengourou	$6.69^a±0.74$	$3.54^a±1.16$	$2.05^a±0.67$	$0.23^a±0.06$	$480.09^a±456.2$	$31.62^a±23.6$
Azaguié	$5.94^b±0.53$	$2.64^b±0.65$	$1.53^b±0.38$	$0.14^b±0.03$	$257.0^a±132.7$	$35.3^a±15.8$
Bouaflé	$6.76^a±0.48$	$2.6^b±0.63$	$1.51^b±0.37$	$0.18^b±0.06$	$395.72^a±237.4$	$25.18^a±13.6$
Zones	**C/N**	**CEC**	**Ca^{2+} (cmol.kg-1)**	**Mg^{2+} (cmol.kg-1)**	**K^+ (cmol.kg-1)**	**Na^+ (cmol.kg-1)**
Abengourou	$9.2^a±2.9$	$13.94^a±5.1$	$3.3^a±1.5$	$1.38^a±0.46$	$0.346^a±0.15$	$0.287^a±0.13$
Azaguié	$11.2^a±2.25$	$9.3^b±2.3$	$1.3^b±0.38$	$0.73^b±0.2$	$0.18^b±0.05$	$0.18^a±0.2$
Bouaflé	$9.2^a±2.46$	$12.49^{ab}±6.1$	$2.37^a±1.37$	$0.98^b±0.52$	$0.21^b±0.1$	$0.27^a±0.18$

N.B: N, nitrate; P, phosphorus; P. ass, assimilable phosphorus; P. total, total phosphorus; C, carbon; CEC, cation exchange capacity; Ca^{2+}, Calcium ; Mg^{2+}, Magnesium ; K^+, Potassium ; Na^+, Sodium.
Means with different letters were significantly different at the 5% level. LSD test

and very present in all agroecological zones of plantain. Five species (1-10%) present in the plantain rhizosphere regardless of the area were: Rhizophagus intraradices, Archeospora sp, Septoglomus viscosum, Glomus sp and Acaulospora kentinensis. All the zones soils had some mycorrhizal fungi specific to the sampling area. Two mycorrhizal fungi isolated only in their collection area were identified in Abengourou, one in Azaguié and four mycorrhizal fungi specific to Bouaflé. The Chao1, Evenness (Pielou), Shannon and Simpson reciprocal indices were calculated for each site in order to evaluate rare species and give an estimate of the specific richness (Table 5). These indices were significantly different between zones. The Chao indices showed that Bouaflé zone contained the rarest species compared to the other two areas. Shannon diversity index H measure the biodiversity incorporates both OTUs richness and OUT eveness into a single value. Bouaflé area counted greater OTUs (5 OTUs, 321998 sequences) and more uniform distribution of OTUs followed by Azaguié (6 OTUs, 270302 sequences) and Abengourou (3 OTUs, 173 900 sequences). Based on the α-diversity index AMF communities from Bouaflé were more diverse than those from Azaguié and Abengourou.

Soil factors that shape AMF community compositions in the plantain fields

The Principal-Component Analysis (PCA) showed that AMF community compositions in the plantain were shaped by some soil factors (Figure 4, Figure 5). As can be seen, the Abengourou and Azaguié zones (Ab3, Ab4, Az3, Az1 and Az2) contributed positively the most to the F1 axis, while Bouaflé (Bo4, Bo1 and Bo3) contributed the most only to the F2 axis. CEC, N, C/N, Mg2+, Ca2+, C, O and AMF communities contributed the most to the F1 axis. Meanwhile K+, Paraglomus, Septoglomus,

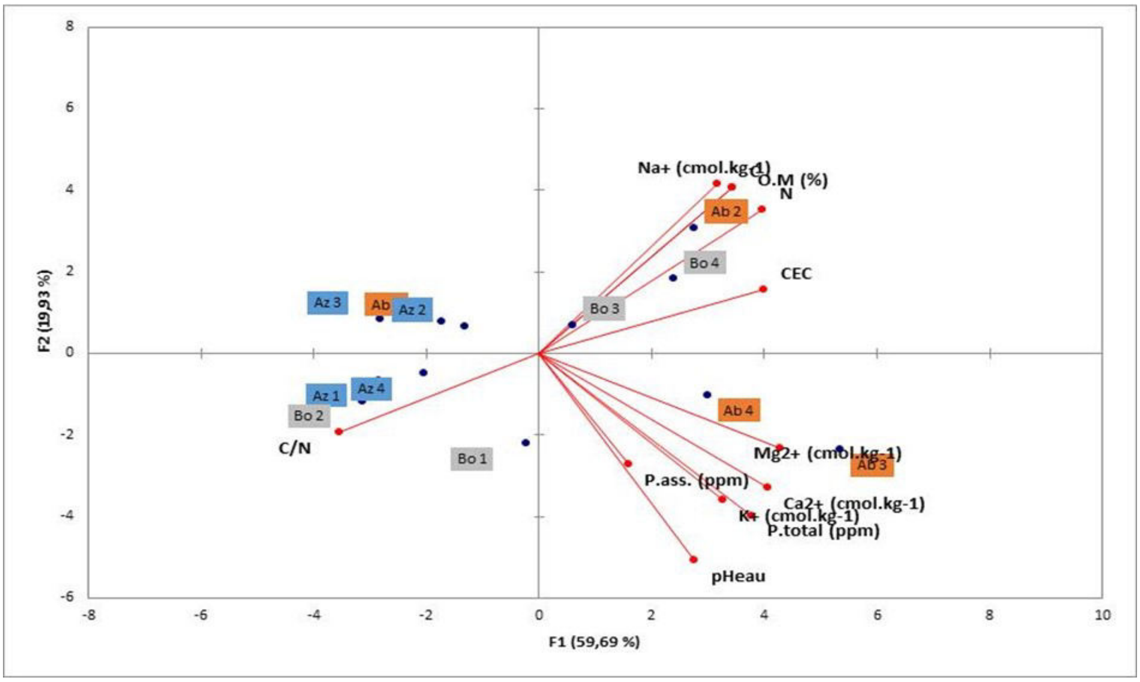

Figure 1. : Principal-Component Analysis (PCA) of plantain field soil parameters in the three agro-ecological zones. The amounts of variation explained by the PCA axes were as follow: F1: 59.69 %; F2:19.93%. The model explained 79.62 % of the whole variance

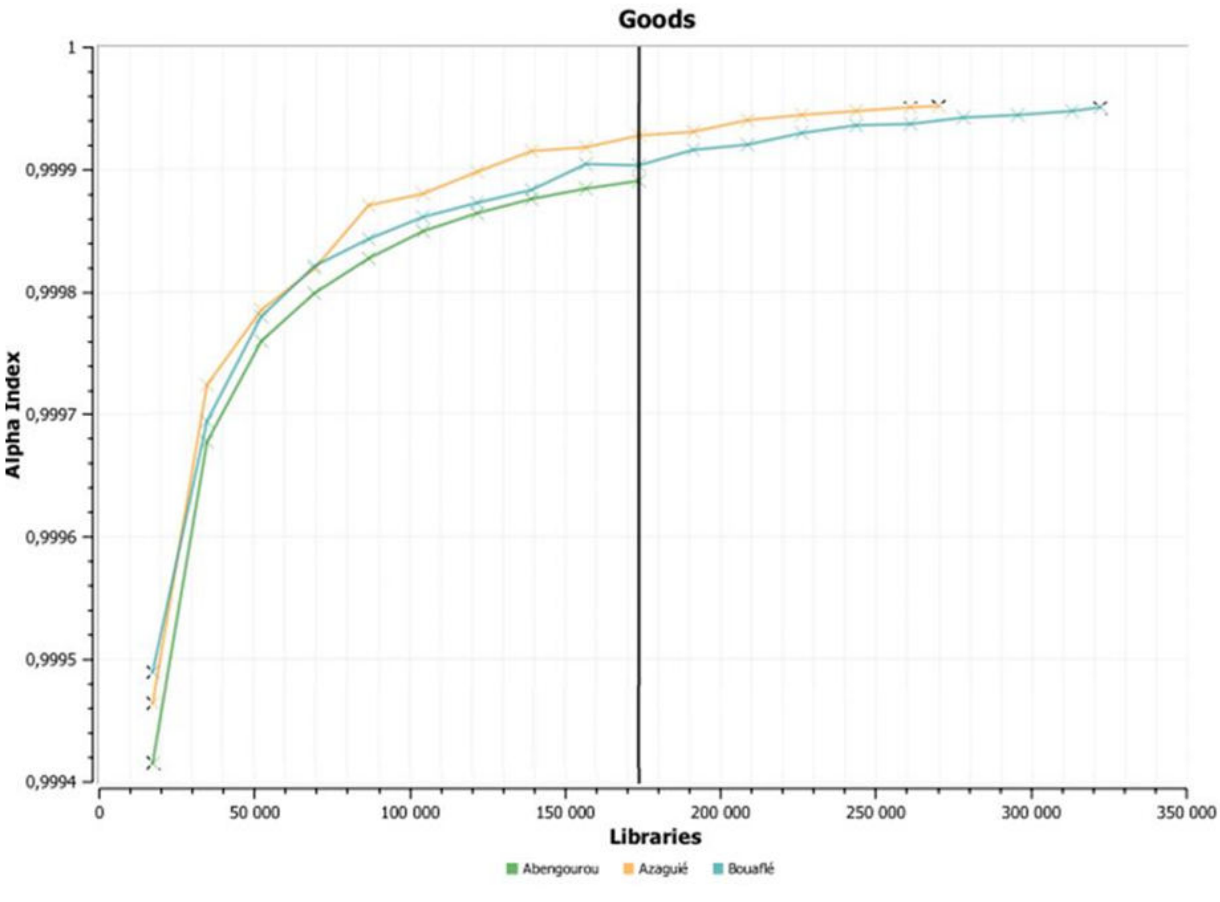

Figure 2. Good's coverage plot of the OTUs distribution within the three agro-ecological zones of plantain fields

Table 3 Distribution and abundance (%) of AMF genera in plantain field soils within the three agro-ecological zones

Genre	Abengourou (%)	Azaguié (%)	Bouaflé (%)	Total	OTUs number per genera. (OTUs differ from each other by 3%)
Acaulospora	8.7	1.6	0.64	2.8	10
Ambispora	x	0,00037	0,0003	0,00026	1
Archeospora	x	8,003	x	2,823	1
Claroideoglomus	0,0086	0,007	0,2245	0,098	12
Funneliformis	2,37	x	0,0062	0,54	1
Gigaspora	x	x	0,00031	0,00013	1
Glomus	0,067	0,00223	4,921	2,084	5
Paradentiscutata	0,012	x	0,474	0,202	1
Paraglomus	x	x	0,00124	0,0005	2
Racocetra	x	0,00074	0,00031	0,0004	1
Rhizophagus	73,07	76,53	69,403	72,75	29
Septoglomus	3,1	0,0085	3,55	2,2	8
Unkown	12,67	13,875	20,77	16,5	38

Rhyzophagus, Glomus contributed most to the F2 axis. Moreover, there were significant correlations between AMF diversity and soil total P, C/N, OM, C, Ca2+, Mg2+ and Na+ (Table 6).

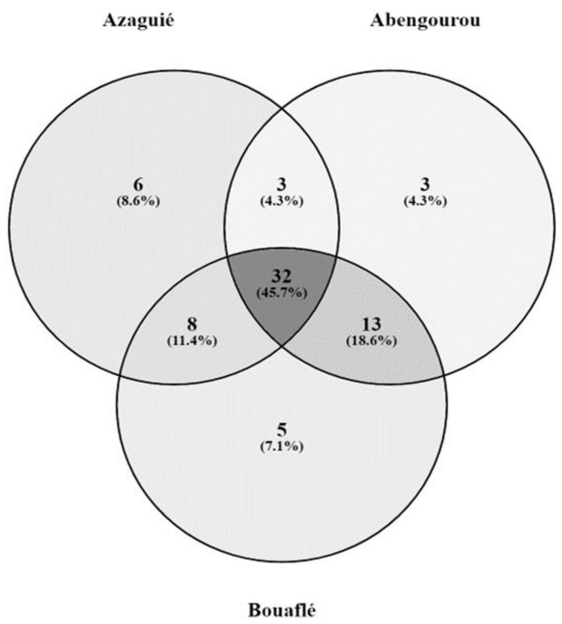

Figure. 3. Venn diagram of the OTU distribution within the three agro-ecological zones of plantain fields

Table 4 Distribution of AMF species in plantain field soils within the three agro-ecological zones in Côte d'Ivoire.

Species	Abengourou	Azaguié	Bouaflé
Acaulospora cavernata	+	+	+
Acaulospora kentinensis	++	+	+
Acaulospora laevis	++	+	+
Acaulospora scrobiculata	+	-	+
Acaulospora sp	+	+	+
Acaulospora WUM18	+	+	+
Ambispora appendicula	-	+	+
Archeospora sp	-	++	-
Claroideoglomus	+	+	+
Claroideoglomus claroi-deum	+	-	-
Claroideoglomus sp	+	+	-
Funneliformis mosseae	++	-	-
Gigaspora margarita	-	-	+
Glomus marcocarpum	+	-	-
Glomus sp	+	+	++
Paradentiscutata bahiana	+	-	+
Paraglomus laccatum	-	-	+
Paraglomus sp	-	-	+
Racocetra fulgida	-	+	+
Rhizophagus intraradices	++	++	++
Rhizophagus sp	++++	++++	++++
Septoglomus sp	+	+	-
Septoglomus constrictum	-	-	+
Septoglomus jasnowskae	+	+	-
Septoglomus viscosum	++	+	++
Unknown	++	++	+++

- : Absent 0%; +: Present <1%; ++: Abundant [1-10% [; +++: Very abundant [20-30% [; ++++: Dominant [40-60% [; +++++: Highly dominant [70-100%]

Table 5: AMF α-diversity within the three agro-ecological zones in Côte d'Ivoire

Zones	Chao 1	Shannon	Reciprocal Simpson	Pielou
Abengourou	49.67^{b}±5,1	2.5^{c}±0,0	3.436^{c}±0.002	0.709^{c}±0
Azaguié	50.15^{b}±2,07	2.58^{b}±0,0	3.53^{b}±0.005	0.716^{b}±0
Bouaflé	60.8^{a}±4.36	2.75^{a}±0,0	3.59^{a}±0.0025	0.722^{a}±0

Means with different letters are significantly different at the 5% level. LSD test.

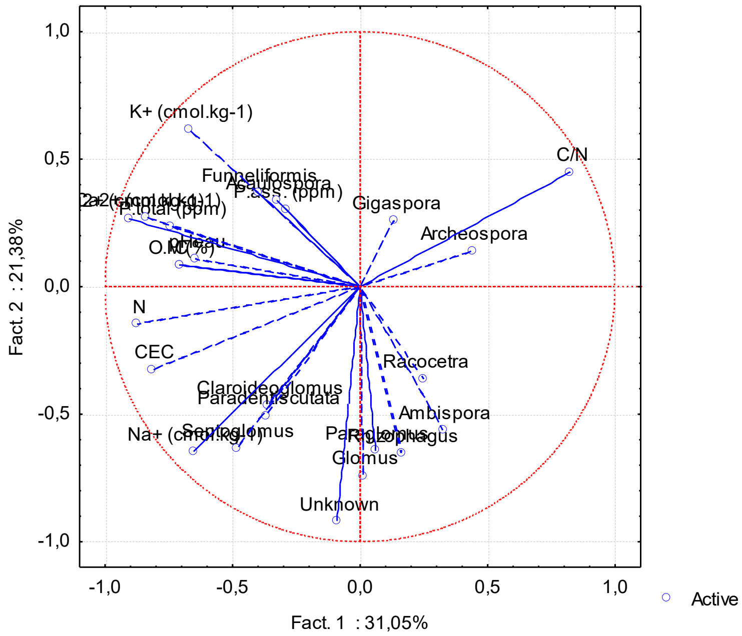

Figure 4. Principal-Component Analysis (PCA) of the AMF community composition and plantain field soil parameters in the three agro-ecological zones. Projection of variable. The amounts of variation explained by the PCA axes were as follow: F1, 21, 38%; F2, 31, 05%. The model explained 52.43% of the whole variance

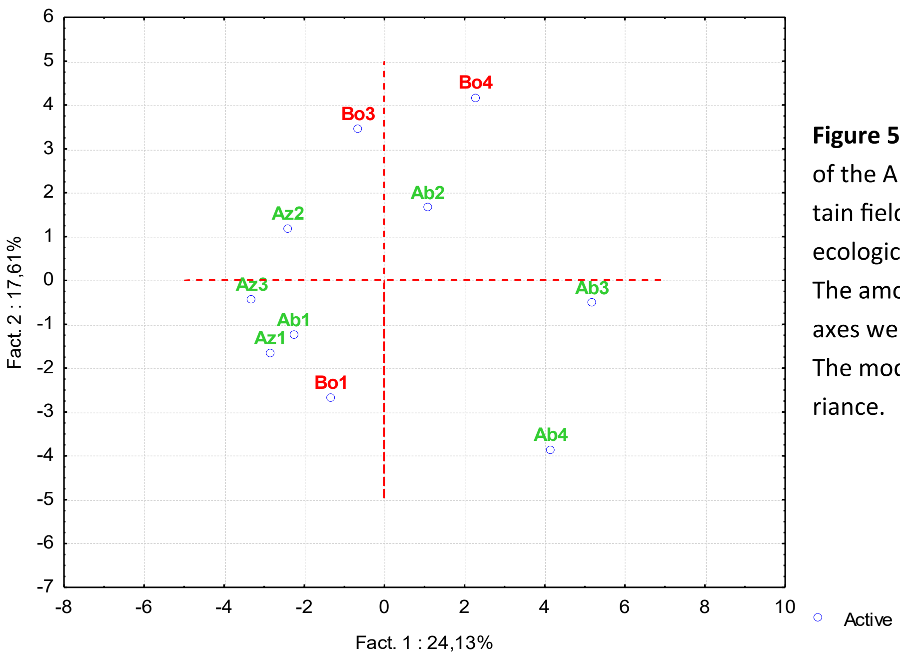

Figure 5. Principal-Component Analysis (PCA) of the AMF community composition and plantain field soil parameters in the three agro-ecological zones. Projections of observations. The amounts of variation explained by the PCA axes were as follow: F1, 17.67%; F2, 24.13%. The model explained 41.8% of the whole variance.

Discussion

The AMF communities of the banana rhizosphere soils were identified in this work for the first time with the Illumina Miseq technology. The rarefaction curves of the OTUs for each study area showed that the sequencing effort was sufficient to cover all the diversity present in each agro-ecological zone. Overall, a total of 110 OTUs described the diversity of communities associated with the banana rhizosphere soils. The most Glomeraceae identified in the banana rhizosphere included the genera Rhyzophagus (72.75%); Archeospora (2.82%); Acaulospora (2.8%); Septoglomus (2.2%) and Glomus (2.084%). The predominance of Glomeraceae corresponded with the known wide distribution of that family in Côte d'Ivoire (Kouadio et al., 2017; Séry et al. 2018) and elsewhere (Öpik et al., 2010, Garcés-Ruiz et al. 2017). The most represented genus in the three zones within the banana field soils was Rhizophagus with Azaguié (76.53 %%), Abengourou (73.07%) and Bouaflé (69.4%).

Roughly one fourth of the 110 OTUs identified from the banana rhizosphere soils were found in the three zones. These OTUs were related to the genera Rhizophagus, Septoglomus and Acaulospora as well as Archeospora and Glomus for which no OTU was identified at the species level. This clearly showed that there was a diverse core of AMF taxa already pre-established in a large part in the banana field soils. The AMF communities within the banana field soils can be considered highly diverse as compared to the diversity found when morphological description used (Jefwa et al, 2012). In this study the Illumina Miseq technology allowed the identification of 25 species distributed in 12 genera within the three agroecological zones. Indeed, the use of the Illumina Miseq approach gave a wide coverage of the Glomeromycota phylum and species (Krüger et al., 2012, Redecker et al., 2013). However, previous studies revealed that banana and plantain fields were dominated by the genera Acaulospora (Jaizme-Vega & Azcón, 1995; Fotso et al., 2016).

The dominance of an AMF genus may not be linked to the presence of a particular crop species or variety in an agro-ecological zone (Jefwa et al., 2012). Indeed similar agro-ecological zones (Abengourou and Azaguié) exhibited the dominance of the genus Rhizophagus in cassava rhyzosphere fields (Séry et al, 2018) while PCA analyses of the banana field soils did not allow a clear cut deliniation of each agroecological zone. Moreover, the diversity indexes (Simpson, Shannon and Pielou) were significantly different between zones confirming differences among AMF communities from one zone to another. Indeed the Bouaflé AMF communities were more diverse compared to Azaguié and Abengourou based on the α-diversity index. The Bouaflé area had higher OTU number (5 OTUs, 321998 sequences) and uniform OTU distribution than Azaguié (6 OTUs, 270302 sequences) and Abengourou (3 OTUs, 173 900 sequences). Bouaflé plantain rhizospheres distinguish also from other zone by great proportion of glomus genus (4,921%). Meanwhile, Abengourou area contains a great proportion of Acauslospora genus (8.7%) compared to Bouaflé (0.64%) and Azaguié (1.6%). Several studies in banana and plantain fields have revealed that the species Acaulospora and some species described as Glomus were also abundant in plantain rizosphere (Jaizme-Vega & Azcón, 1995; Fotso et al., 2016). Azaguié zone is the only area where, we found AMFs species belong to Archeospora (8%). As shown in previous studies, soil physicochemical characteristics were a major factor influencing AMF community (Séry et al, 2018, Alguacil et al., 2016, Jansa et al., 2014, Koorem et al., 2014, Santos-Gonzalez et al., 2011). Indeed in this study soil total P, C/N, organic matter (O.M), C, Ca^{2+}, Mg^{2+} and Na^{+} significantly influenced AMF diversity at both genera and species level in the banana field soils.

Table 6: Correlation matrix (Pearson (n)) between AMF species, genera and soil physico-chemical parameters

Genera/ Species	pH water	O.M (%)	C	N	C/N	Total. P (ppm)	Ass. P (ppm)	CEC	Ca^{2+} (cmol.kg-1)	Mg^{2+} (cmol.kg-1)	K^{+} (cmol.kg-1)	Na^{+} (cmol.kg-1)
Septoglomus	.3696 p=.293	.1269 p=.727	.1269 p=.727	.4111 p=,238	***-.7419*** ***p=.014***	.1287 p=.723	-.2827 p=.429	.3818 p=.276	.2565 p=.474	.2498 p=.486	-.0075 p=.984	***.6618*** ***p=.037***
Claroideoglomus claroideum	.4986 p=.142	.1638 p=.651	.1638 p=.651	.2250 p=.532	-.2329 p=.517	***.8156*** ***p=.004***	.6287 p=.052	***.6363*** ***p=.048***	***.7052*** ***p=.023***	***.7361*** ***p=.015***	.5474 p=.101	.1401 p=.699
Glomus marcocarpum	.4986 p=.142	.1638 p=.651	.1638 p=.651	.2250 p=.532	-2329 p=.517	***.8156*** ***p=.004***	.6287 p=.052	***.6363*** ***p=.048***	***.7052*** ***p=.023***	***.7361*** ***p=.015***	.5474 p=.101	.1401 p=.699
Rhizophagus intraradices	***-.7185*** ***p=.019***	.5305 p=,115	.5305 p=,115	.3463 p=,327	.0237 p=,948	-,3658 p=.299	.1356 p=.709	.1101 p=.762	-.4475 p=.195	-.3314 p=.350	-.3425 p=.333	.2287 p=.525
Septoglomus jasnowskae	-.3455 p=.328	***.7146*** ***p=,020***	***.7146*** ***p=,020***	.6311 p=,050	-.2926 p=.412	-.0909 p=.803	-.1469 p=.685	.2636 p=,462	-.0115 p=.975	.0514 p=.888	.1245 p=.732	.2847 p=.425
Septoglomus viscosum	.3881 p=.268	.0772 p=.832	.0772 p=.832	.3635 p=.302	***-.7128*** ***p=.021***	.1332 p=.714	-.2694 p=.452	.3592 p=.308	.2541 p=.479	.2433 p=.498	-.0156 p=.966	.6341 p=.049

The values in bold are different from 0 to a significance level alpha = 0.05

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