Original Research Article

Pathogenic fungi communities in contrasted yam field soils are dominated by the genus *Fusarium*in Centre Côte d'Ivoire

Abstract

Below-ground pathogenic fungi of yam, a globally important food crop are shown to be responsible of field and storage diseases and as such are important threats to yam health. However, yam soil pathogenic fungi community composition and soil determinants remain unclear. Here, we explored the effects of soil physicochemical characteristics on soil fungal phytopathogens in contrasted yam fields.Illumina miseq was used to characterize pathogenic fungi communities in yam field soils. In this work, we hypothesise that pathogenic community composition could be linked to specific physicochemical properties within yam contrasted soils. Principal Coordinate Analysis (PCoA) and variance partitioning were used to identify the soil properties that significantly influence pathogenic fungi community compositions within the three sites in a yam production zone. The results have shown that despite the fact that the three sites exhibited contrasted soils grouped in two types according to physicochemical parameters, four ubiquitous genera including *Fusarium*, *Penicillium*, *Aspergillu*s and *Trichoderma* were identified within pathogenic fungi communities in yam field soils, with Fusarium as the most dominant core genus.Soil type determined the distribution of pathogenic fungi communities in yam field soils, and this effect was attributed to soil properties related to yam soil cation exchange capacity (CEC) and silt content as well as three micronutrients including Na, N and total phosphorus.

Keywords: Yam, Soil pathogenic fungi, Community

Introduction

Healthy, sustainable and inclusive food systems are critical to achieve the world's development goals. However of lots of problems, crop production and food security worldwide are threatened by various soil-borne phytopathogens (Doran et al. 2000, Abawi et al. 2000, Janvier et al.2007, Chakraborty et al. 2010, Chaparro et al. 2012). The production of Yam (*Dioscorea spp*.) which is a tuber plant cultivated in West Africa, the Pacific and the Caribbean, and more occasionally in East Africa and tropical America (Quénéhervé, 1998) is not left behind. West Africa (Nigeria, Côte d'Ivoire, Ghana, Benin, Togo) is widely regarded as "the yam belt" (Orkwor et al. 1998,

Ntui et al. 2021) since it represents more than 92% of yam world production (FAO, 2013). Yam represents a strategic culture for food security and the fight against poverty in West Africa. It constitutes the main source of income for many small producers, particularly women who are involved at all levels of the sector. There is a strong demand for yam in urban areas which, combined with its high market value, explains the continued growth in its production (Diby, 2005). In Côte d'Ivoire, yam is the leading food crop in terms of production with 5.54 million tons (FAO, 2013). It is mainly cultivated in four production basins. In the Center and Center-West, production is mainly intended for self-consumption; in the North and North-East on the other hand, most of the production is intended for marketing (Doumbia et al., 2006). Although cultivated areas are increasing, yields have seen a continuous decline in recent years, going from 8.8 to 6.3 t/ha/year, an overall drop of 25% (FAO, 2013). Furthermore, the potential yield of yam has been estimated between 27 and 51 t/ha/year of fresh tubers (Diby et al., 2009). The drop in yields as well as the significant gap between potential and actual yields can be explained by both the traditional production systems and yam diseases. Indeed, within these traditional systems which are extensive, the yam culture is placed ahead of a crop rotation after clearing primary vegetation or long-term fallow. Under these conditions, the duration of natural fallows is greatly reduced and consequently impacts the soil fertility in yam fields.The second problem is yam diseases including field diseases and storage diseases due to nematods, fungi and viruses (Amusa et a. 2003, Dongzhen et al. 2020, Yacouba et al. 2021, Diouf et al. 2022). Among yam diseases, wilt, rots and damping-off diseases caused by pathogenic fungi are the most common (Dongzhen F et al 2020, Amusa et al. 2003). In Côte d'Ivoire, in a recent study, fungi belonging to genera *Colletotrichum, Fusarium, Pestalotiopsis, Pestalotia, Botryodiplodia, Aspergillus, Mucor, Curvularia,* and*Phytophtora*responsible of yam leaf diseases were isolated of which*Colletotrichum*was the most abundant (Soulemane et al. 2023). Yam pathogenic fungi can infect both the above-ground and below-ground parts of yam starting at the early stage of yam development, with infected tissues continuously chanagecolour, resulting in vascular wilt, and eventually rot and plant death. During storage, any damage to the tuber provide an entry point for fungal pathogens allowing these diseases to continue their spread even after harvesting, during storage of yams (Morse 2020). It means that the knowledge of the above-ground pathogenic fungi communities is importance for a best management of yam disease both in field and during storage. The occurence and causes of fungal pathogens in the soil-plant systems have been

investigated for important crop plants including maize, wheat and rice and provided important information on the mechanisms by which soil fungi interact with the host (Nguyen et al.2016, Möller et al. 2017,Qiu et al. 2019). Apart from a recent work (Du et al. 2022), information on the prevalence and diversity of the phytomycobiome in agricultural soils notably in yam field soils remain scarse. Consequently, deciphering the community composition of soil fungal pathogens and their determinants in yam fields is crucial to protect crop yam from field and storage diseases due to fungal pathogens.The aim of this work was to use Illumina sequencing approach in order to (i) deeply determine the composition and structure of soil borne fungi pathogens in the yam rhizosphere soils and (ii) analyze the impact of soil physico-chemical characteristics on the composition and structure of these soil borne fungus population.

Material and Methods

Soil sampling

Soil samples were collected from yam fields in three different fields (Seman, Logbakro and Zambakro) in Yamoussoukro Côte d'Ivoire. This region is an important yam production zone in Côte d'Ivoire (Digbeu et al 2009). The Yamoussokro production zone is located in the center with a subequatorial climate marked by a bimodal rainfall regime with four seasons. Average rainfall ranges from 1100mm to 1600mm and the average annual temperatures vary from 24.6°C to 27.9 °C. The vegetation is a mosaic of Guinean savannah and dense moist semi-deciduous forest type with Aubrevilleakerstingii (N'Guessan, 1990). Three fields were sampled. In each field three samples were collected at a depth of 20cm in the yam plant rhizosphere. For each sample, 12 primary samples collected from around the stem in the tuber/root region according to the diagram proposed by Huang and Cares (2004) were collected and mixed up to 1kg.

Soil physico-chemical analyses

Soil analyses included 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 ca pacity CEC (cmolkg-1), Ca2+ (cmolkg-1), K+ (cmol.kg-1), Na+ (cmolkg-1), and texture (Clay, silt, and sand). Three organic matter content classes of soil fertility have been

defined (Schaffer, 1975): poor OM content (1–2%), moderate OM content (2–4%), rich OM content (>4%). Dried soils were used to determine chemical and physical characteristics. 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, 1883). The soil's cationic exchange capacity (CEC) and total P 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. For total phosphorus, it 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. The available P was determined from Olsen (1954) and the soil particle size by hydrometric methods and sieves.

DNA extraction, PCR amplification and bioinformatic processing

Total soil DNA was extracted using the MoBioPowerSoil DNA isolation kit (MoBIO, Laboratories, Inc. Carlsbad, CA) following the manufacturer's instructions. The internal transcribed spacer (ITS) region was amplified using fungal-specific primers (White et al., 1990) ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA 3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). DNA was amplified using the HotStarTaq Plus Master Kit (Qiagen, Valencia, CA). Amplicon products from different samples were mixed in equal concentrations and purified using AgencourtAmpure beads (Agencourt Bioscience Corporation, USA). Paired-end 2 × 250 bp sequencing was performed on an Illumina MiSeq instrument (Illumina Inc., San Diego, CA, USA). Amplification and sequencing were performed by the Molecular Research LP next-generation sequencing service (http://www.mrdnalab. com). Quality processing of ITS rRNA gene sequences was performed in Qiime (v.1.9.1) following a pipeline found on the Qiime website. The FASTA and QUAL files were converted to FASTQ files using the convert_fastaqual_fastq.py command. Then the mapping file was validated using the validate_mapping_file.py command. Barcodes were extracted and libraries were split. Chimeric sequences were removed through the filter_fasta.py command. Potential soil phytopathogens genera were retrieved from PhytoPath (https://phytopath.org) and PHI base r5.0 (Urban et al, 2022) and matched with the taxonomy obtained from the RDP classifier v2.13 (Wang et al., 2008).

Results

The yam field soils are contrasted according to physicochemical characteristics

pH values of the yam fields ranged from 5.23 to 7.55 with Logbakro soils having lower pHs.While the soils in Logbakro were loamy, the ones in Zambakro and Seman were sandy clay. The CEC was lower in Logbakro than in Zambakro and Seman where the values were greater than 9 cmol/kg. Chemical analyses revealed that there was a clearcut demarcation between the Logbakro fields on one hand and on the other fields from Zambakro and Seman (Figure 1).Logbakro soils C, N and Mg2+ contents which characterized the fields at this site were statistically different (p value 0.05)from the values in Zambakro and Seman. Zambakro and Seman soils contained assimilable P, Na, Ca2+, K, N and total P that were significantly different (p value) from the ones in Logbakro.

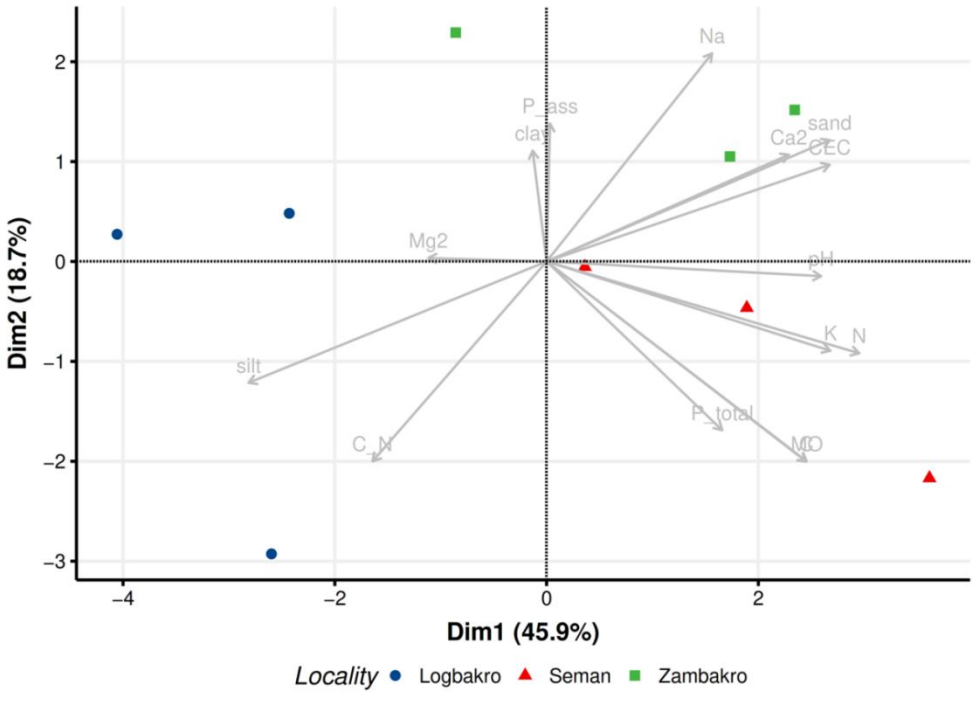

Figure 1: Principal component analysis of yam field according to soil physicochemical characteristics. Sampled sites are colored

Phytomycobiome diversity variation between the contrasted yam field soils

After quality filtering, a total of612.768sequences were obtained from the three yam fields, resulted into 2319 ASVs (Amplicon SequenceVariant) of which 170 ASVs (7.33 %) were from the phytomycobiome. The phytomycobiome of the Zambakro field was grouped in 12genera (Table 1) and dominated by *Fusarium*(1.90%),*Aspergillus*(0.70%) and Penicillium(0.10%). The Ceman field was grouped in 12 genera and dominated by Fusarium (1.95%) and Aspergillus(0.27%)while the Logbakro field was grouped in 7 genera and dominated by *Fusarium* (1.13 %) and *Penicillim* (0.85 %). Thecore phytopathogenic fungi according to fields included *Fusarium, Penicillium, Trichoderma* and *Aspergillus*, themost abundant genus being *Fusarium.*

Table 1: Relative abundance of ASV count for each locality. Genus with zero count were removed

Genus	Relative abundance			Number of ASVs		
	Logbakro	**Seman**	**Zambakro**	**Logbakro**	**Seman**	**Zambakro**
Arthrobotrys	0.00422	0.0057	0.0742	1	1	7
Aspergillus	0.307	0.2743	0.797	24	61	47
Curvularia	0.106	0.005879	0.02297	8	5	5
Fusarium	1.138	1.9579	1.9	14	28	18
Magnaporthe	0.327	x	x	6	x	x
Penicillium	0.858	0.4839	0.10	33	30	24
Trichoderma	0.257	0.22	0.0731	9	16	8
Beauveria	x	0.000692	x	x	1	x
Colletotrichum	x	0.0006569	x	x	2	x

Exophiala	x	0.006612	0.0003	x	4	1
Lecanicillium	x	0.000218		x	1	x
Metarhizium	x	0.00193	0.01	x	2	4
Verticillium	x	0.0417	0.00131	x	3	1
Candida	x	x	0.0012	x	x	1
Clavispora	x	x	0.00073	x	x	1
Gaeumannomyces	x	x	0.0047	x	x	1

X: not found

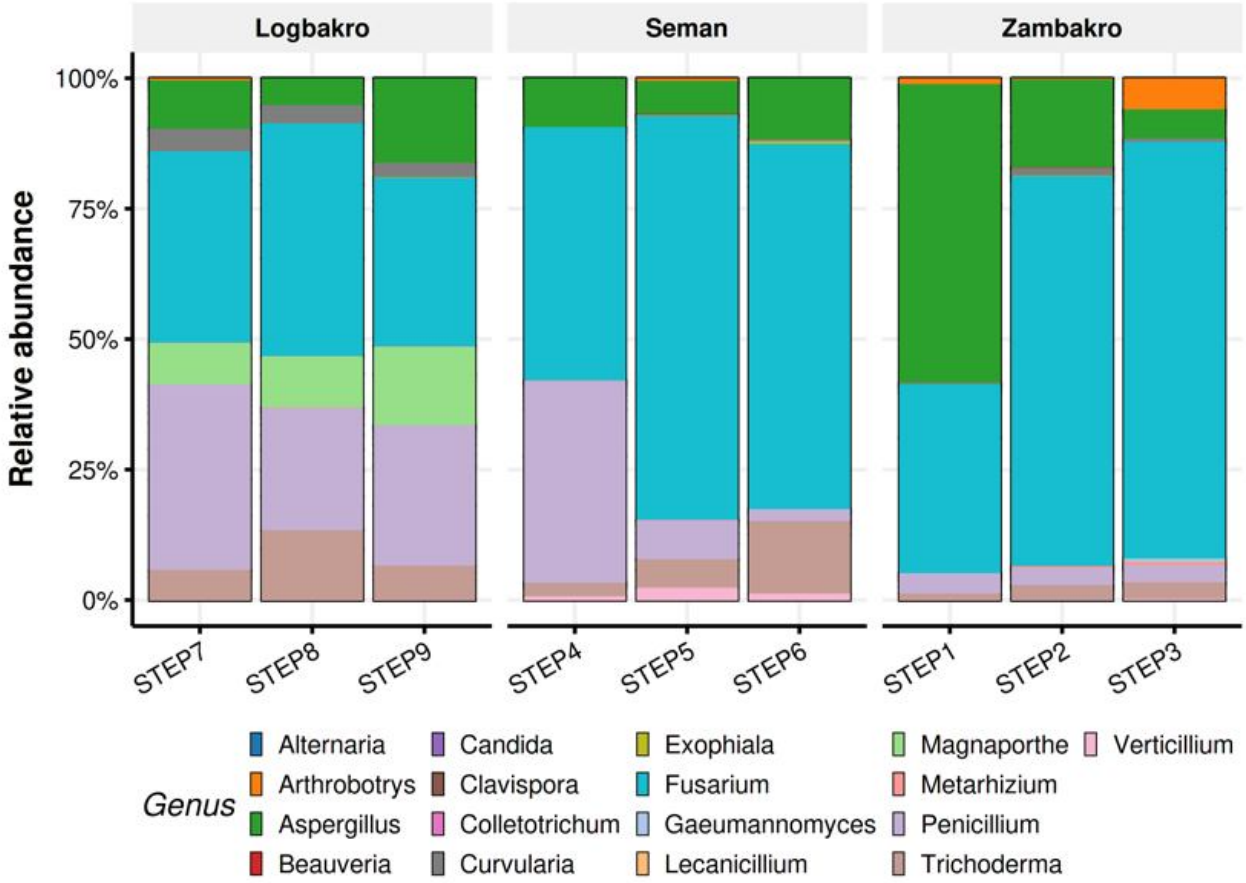

Figure 2: The relative abundance of the phytomycobiome in the yam sampled fields in Centre Côte d'Ivoire.

The distribution of ASV between the three sites (Figure 3) revealed that these three fields had 41 ASVs in common. The Zambakro and Logbakro fields were shown to have more ASVs in

common (17). However, each field had specific ASV (28, 19) for respectively both Logbakro, Zambakro and Seman. Of all the genera, some were not simultaneously present in all the three yam fields (Table 1 and Figure 2). The core genera in the yam fields regardless of the fields included Fusarium, Aspergillus, Penicillim and Trichoderma. These genera differed in abundance (Figure 2 and Table 1).

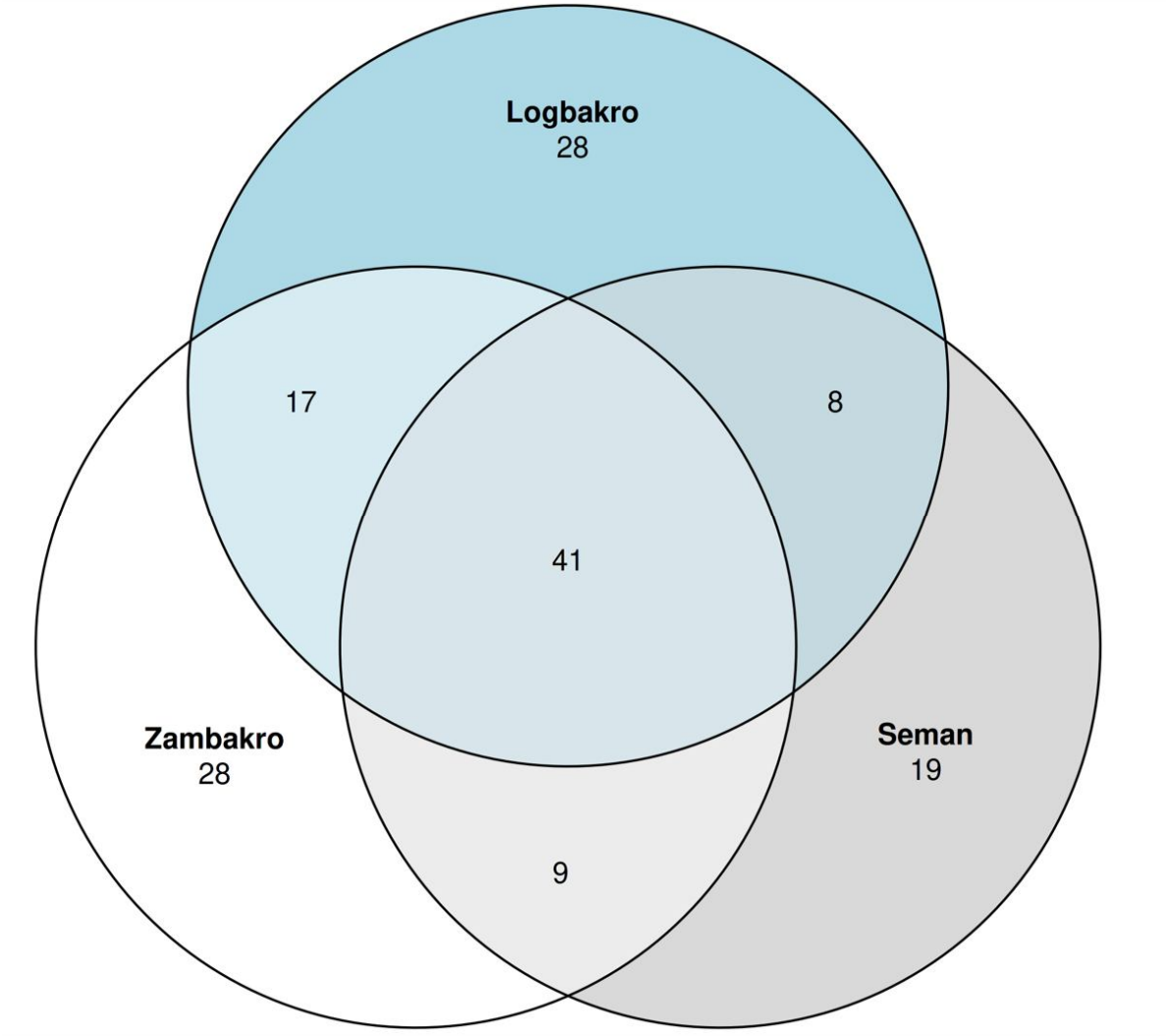

Figure 3: Venn diagram of ASV distribution in yam field soils in Centre Côte d'Ivoire

The observed and Shannon index were calculated for each field in order to evaluate rare ASV and give an estimate of the specific richness (Figure 4). The Shannon index was not significantly

different between Seman and Zambakro yam fields. However, the yam field in Logbakro had the highest reciprocal Shannon index, significantly different from both Seman and Zambakro while the observed index was different between the three sites. Overall, there was a variation of ASV alpha diversity supporting the fact that the beta diversity was significantly different between the Logbakro site and the other sites (Figure 5).

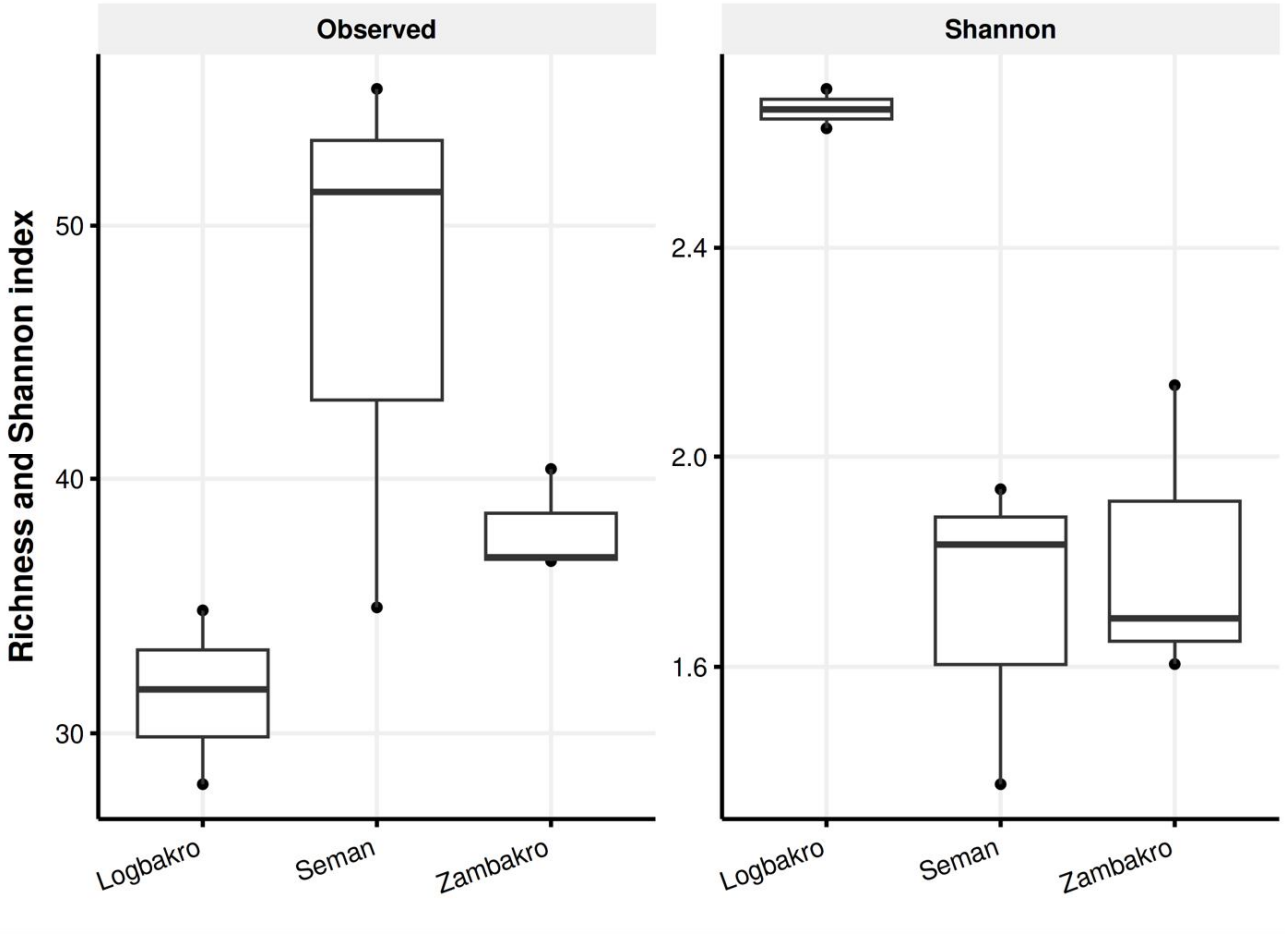

Figure 4: alpha diversity per yam field sites comparison

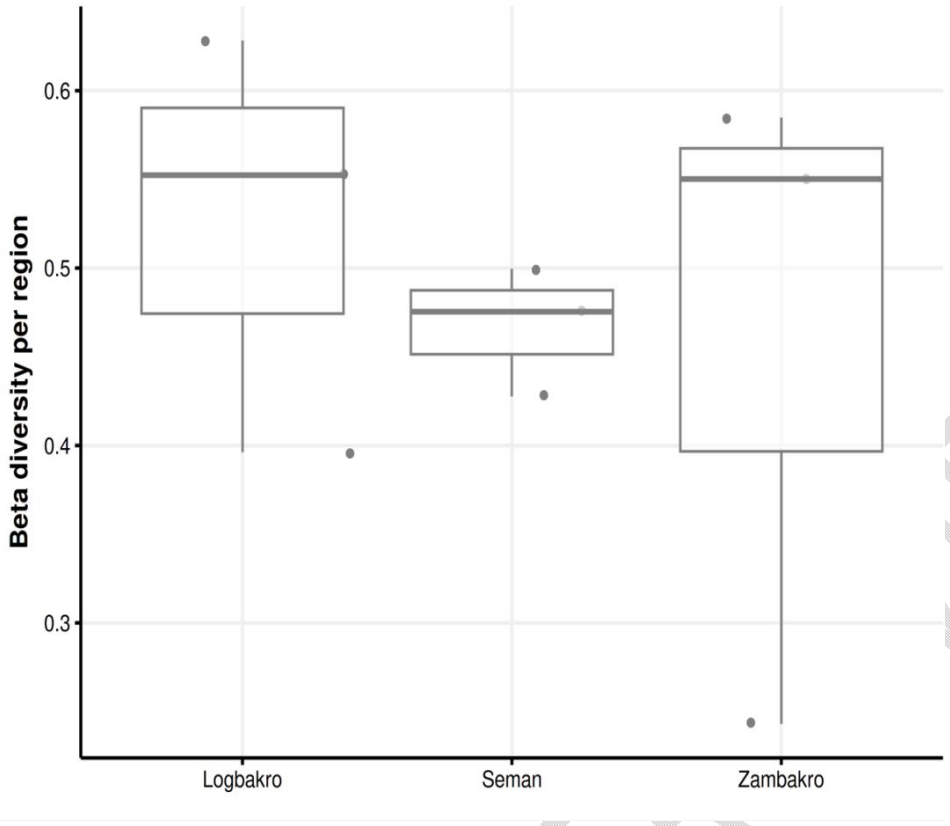

Figure 5: Beta diversity per yam field sites comparison

Soil factors that shape the phytomycobiome in the contrasted yam fields

The interaction between the phytomycobiome diversity and soil characteristics in the three fields were analysed by Principal Coordinate Analysis (PCoA). The Logbakro community coordinate was opposed to those of Seman and Zambakro (Figure 6). Clearly the F1 axis opposed the soils and diversity of Zambakro and Seman rich in Assimilable P, clay, Na, sand, Ca2, CEC, K, N, Total P and MO to this of (pH) to to those of Logbakro yam field rich only in Mg2, C, N and silt.

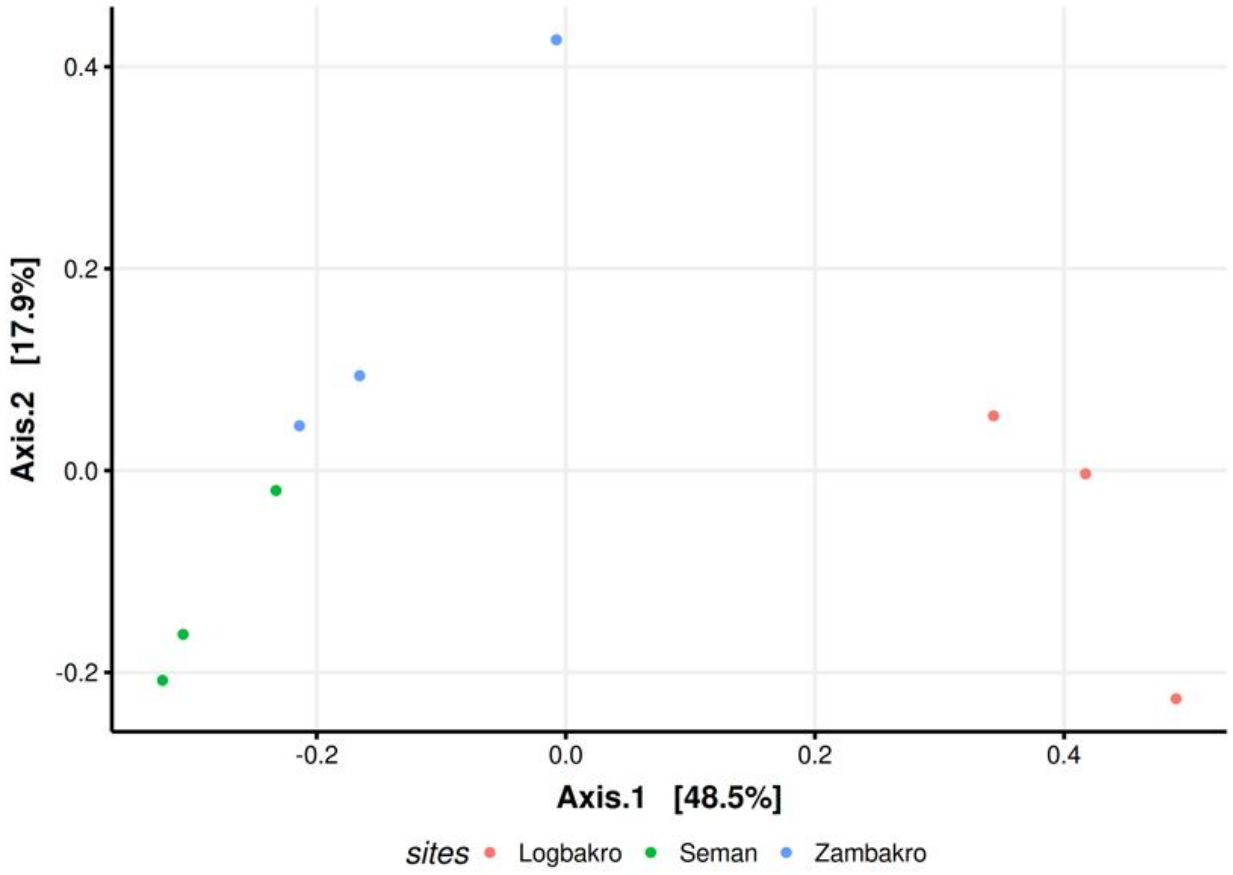

Figure 6: Principal Coordinate Analysis (PCoA) of the pathogenic fungi community composition in the three yam field sites

The impact of these soil characteristics were specifically evaluated on the dominant phytopathogenic genera including *Fusarium, Aspergillus, Magnaporthe, Penicillium* and *Trichoderma* that were encountered within the three yam sites. It was shown that the range of total explained variance in these dominant genera diversity varied from 80% for *Fusarium* to 100% for *Magnaporthe* (Figure 7). When examining the impact of each field soil physicochemical characteristics, it was observed that the distribution of each genus was influenced by five parameters. Based on their cumulative influence on major genera, the drivers could be ranged in the order Na >CEC>N>Silt>Total P. Indeed, Na was the main driver that explained the diversity variation of the major genera,followed by CEC and N.

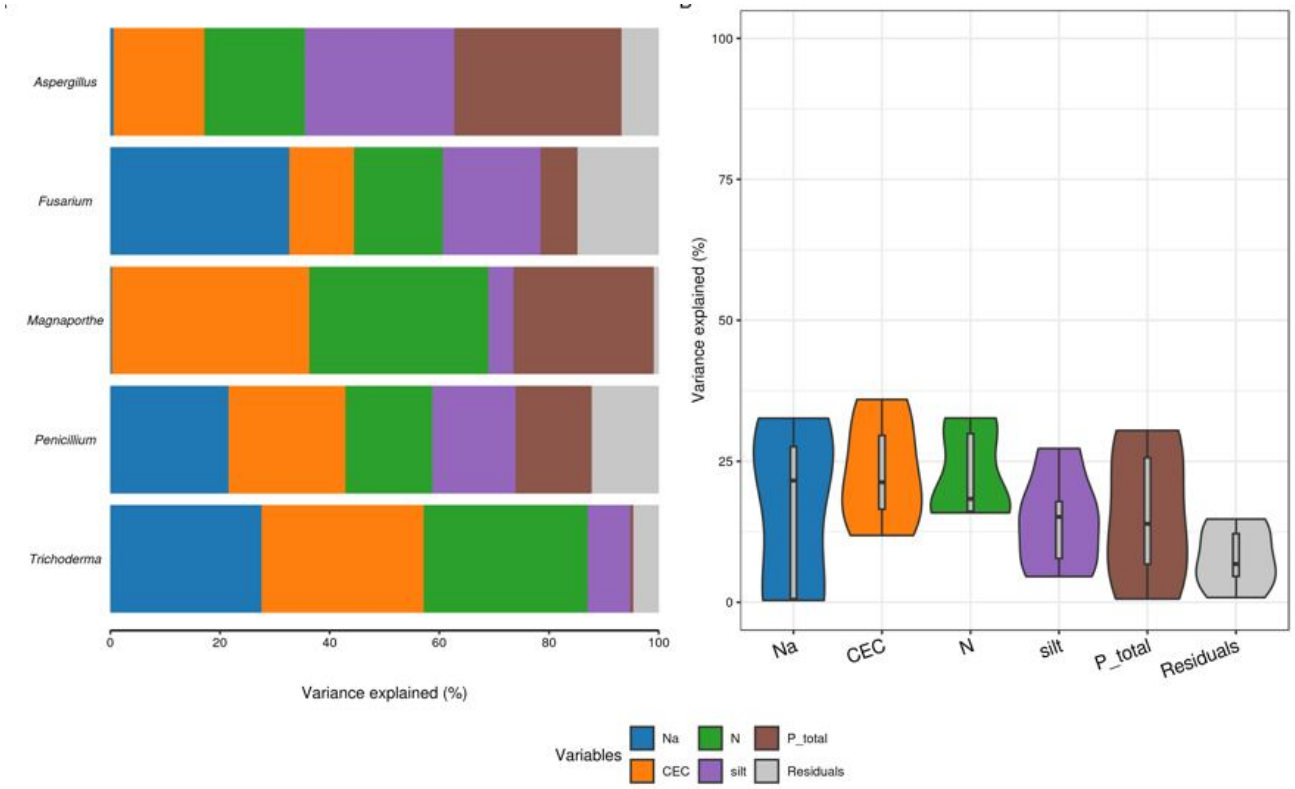

Figure 7. Variance partitioning of major pathogenic fungi genera in the yam fields. (A) Total variance for each genus is partitioned into the fraction due to soil properties. (B) Violin and box plot of percent variation in genera diversity explained by each variable.

Discussion

Yam field soil pathogenicfungi constitute a real threat to yam productivity and conservation since they can continue spreading even after harvesting, during yam storage (Morse, 2020). It means that information on soil pathogenic fungi is a very important step if we were to efficiently manage field and storage diseases due to pathogenic fungi (Amusa et al 2003). This study is the first report of pathogenic fungi communities diversity and structure in contrasted yam fields. Indeed, despite pH values expected from tropical regions as alreay reported for soils in Côte d'Ivoire (Amon et al. 2023), the yam field soils were contrasted in terms of physicochemecial contents and could be divided in two groups.The pathogenic fungi communities in these contrasted yam soils were characterized using the ITS region Illumina Miseq technology. Overall, the diversity of these pathogenic fungi communities accounted for 7.33 % of all the total. This is comparable to the account of of the phytogen communities recently found in agricultural

soils when the ITS region was used (Du et al. 2022). Our study identified in the yam soils several dominant genera including *Fusarium*, *Penicillium*, *Trichoderma* and *Aspergillus*, the most abundant genus being *Fusarium*. Fusarium was also found to be dominant in different agricultural soils (Du et al. 2022).These genera have the potential to cause yam diseases. Indeed the genus *Fusarium* which is ubiquitous in agricultural soils can affect yam plant (Dongzhen et al. 2020). The genera Fusarium and Aspergillus were also reported to be pathogenic to yam crop in Côte d'Ivoire (Souleymane et al. 2023). Some Penicillium species have also been recently reported to be pathogenic to yam crop (Uy et al. 2022).

Despite the fact that the soils of the three sites were contrasted in terms of physicochemical characteristics, the CEC, Na+ and N were not significantly different. However, it was found that CEC, Na+ and N were the main drivers of major genera relative abundance variation within the yam soils. Recently it has been shown that soil organic carbon (SOC), total carbon (TC), total sulfur (TS) as well as climate conditions (Delgado-Baquerizo et al. 2020 , Delavaux et al. 2021) and agricultural managements could influence soil pathogenic fungi communities (Du et al. 2022). It has been reported in previous studies that CEC and Na+ are drivers of diversities and functionalities within soil fungal communities (Canini et al. 2020). Moreover in agricultural systems it has reported that nutrient such as nitrogen and phosphorus fertilization can favor pathogenic fungi in grassland soils (Lekberg et al. 2021). While the soils in Logbakro on one hand in the ones in Ceman and Zambakro had the same CEC, the soils in Zambakro were loamy while the others were sandy clay. It is well known that the amount of clay in soil can influence soils ability to retain water and nutrients and give favourable conditions for microbial activities in Zambakro and Ceman. The CEC had a profound influence on the composition of the total fungal community and of some taxonomic and functional groups studied, as well as on their richness and relative abundance. Due to traditional practice conditions for yam cultivation in Côte d'Ivoire, the duration of natural fallows is greatly reduced and consequently impacts the soil fertility in yam fields. However, the C/N ratio in Logbakro soils were higher. This may imply a good mineralization of organic matter and good availability of nitrogen in Logbakro soils than in Seman and Zambakro (Javeed et al. 2023). While soil C and N are known to affect fungal richness and C/N is a major predictor of fungal abundance and gene function on global and regional scales (Bahram et al., 2018), in this study they did not influence the abundance and community composition of the fungal pathogenic communities in contrasted yam fields.

Although soil pH was among the main drivers of fungal diversity and distribution at the global scale (Tedersoo et al., 2014; Bahram et al., 2018), it did not show a significant effect on the fungal community abudance and diversity in this study.

Conclusions

This study is the first to provide novel information into the prevalence and diversity of fungal phytopathogenic genera in 03 contrasted yam field soils in Yamoussokro. The results have shown that despite the fact that the three sites exhibited contrasted soils grouped in two types according to physicochemical parameters, four ubiquitous genera including *Fusarium*, *Penicillium*, *Aspergillu*s and *Trichoderma* were identified within pathogenic fungi communities in yam field soils, with Fusarium as the most dominant core genus.Soil type determined the distribution of pathogenic fungi communities in yam field soils, and this effect was attributed to soil properties related to yam soil cation exchange capacity (CEC) and silt content as well as three micronutrients including Na, N and total phosphorus.

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