ASSESSMENT OF DOMINANT SOIL BORNE FUNGAL PHYLA AND SPECIES FROM AMUKPE TOWN, DELTA STATE, USING NEXT GENERATION PROTOCOL

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Abstract: Fungal phyla associated with bulked soils from a commercially operated farm holding in Amukpe town, labeled as farmed soil and a nearby control site in Adavware village (control soil) both in Delta State, were ascertained with the aid of Next Generation Sequencing (NGS) procedures. The concentrations of the extracted DNA samples were 0.072 and 0.057 ng/µl for the control and farmed soils, respectively. Three (3) fungal phyla; *Ascomycota*, *Basidiomycota* and *Chytridiomycota* were detected in both compounded soils whilst the phylum *Zygomycota* was detected in the farmed soil. The ascomycete; *Knufia perforans* (SH 209750.06FU) (53%) was the predominant soil borne fungal species in both the control (53%) and farmed (48%) soils, respectively. Several soil borne ascomycetal and basidiomycetal classes such as; *Eurotiomycetes*, *Dothideomycetes*, *Sordariomycetes*, *Pucciniomycetes*, *Ustilaginomycetes*, *Agaricostilbomycetes* were detected in both compounded control and farmed soils, respectively.

Keywords: Amukpe, Ascomycetal, Basidiomycetal, Delta State, Herbicide, ITS1 region

Introduction

Soil quality has been defined as the inherent capacity of soil to keep unaltered key ecological functions, such as decomposition and formation of soil organic matter (Doran *et al*., 1996). Sofo *et al.* (2012) opined that soil quality can be based on chemical, physical or biological characteristics and the impairment of which can lead to a decline in agricultural production. Among the threats that can harm soil quality, xenobiotic compounds are noteworthy as these moieties can impact on soil quality by altering key ecological functions, with the consequent impairment of the natural environmental balance (Gianfreda and Rao, 2008). Among the xenobiotic compounds, herbicides are known to cause toxic effects on the living part of soil, even at root exudates, as the case of glyphosphate released from soybean roots (Zobiole *et al*., 2010). The authors also observed that herbicides can impact on the growth of rhizobacteria, indole-acetic acid (IAA) producers, enzymatic activities and soil respiration in addition to the known general detrimental effects on soil microbial biomass.

Herbicides are known to exhibit physiological effects on soil microorganisms and these effects include; a) causing changes in their biosynthetic mechanism (a change in the level of protein biosynthesis is indicated by the ratio of extracellular and intracellular enzymes); b) impacting on protein biosynthetic process (induction or repression of the synthesis of specific enzymes) and c) by affecting the integrity of cellular membranes (changes in transport and excretion processes) (Miloševiã and Govedarica, 2002).

Herbicide degradative processes in soil can be photochemical, chemical or microbial in nature (Miloševiã and Govedarica, 2002). While photochemical decomposition has been documented to predominate in air and water, only a small percentage of pesticides can be photochemically decomposed in the soil biome (Miloševiã and Govedarica, 2002). Chemical decomposition of soil borne herbicides is known to evolve through hydrolytic, non-hydrolytic transformative and oxidative processes. Microorganisms are known to be capable decomposers of aliphatic and hydroxyl compounds, but can degrade aromatic moieties at a slower rate (Miloševiã and Govedarica, 2002). Soil microbiological populations are known to be able to utilize herbicides and their metabolites as sources of biogenic elements (Radosevich *et al*., 1995). Hart and Brookes (1996) observed that long-term application (19 years) of glyphosate caused a reduction in the soil carbon biomass, but there was an increment in the rate of ammonification and nitrification in comparison with the untreated soil. Radosevich *et al*. (1995) listed several bacterial and fungal herbicide-degrading genera which included; *Arthrobacter*, *Pseudomonas*, *Bacillus*, *Actinomycetes*, *Mycoplana*, *Agrobacterium*, *Corynebacterium*, *Arthrobacter*, *Flavobacterium*, *Nocardia* and *Trichoderma*. Miloševiã and Govedarica, (2002) reported that the impacts of herbicides on the composition and morphology of soil microbial population is dependent not only on the composition and concentration of herbicide(s) applied but also on the kind of microorganisms present.

Large commercial farm holdings in both Northern and Southern Nigeria employ varying quantities of different types of herbicides in the course of their daily operations to eradicate weed growth in their farms. It is observed that the emphasis of most pollution effect centered studies reported by Nigerian researchers has been on the detrimental impact of both upstream and downstream petroleum exploration and transportation activities on both terrestrial and aquatic habitats. However, in view of the current sensitization efforts by the Nigerian Government at different tiers and levels in encouraging individuals to invest in commercial farming activities, there is an urgent need to assess the effect of regular herbicide usage on the health of non-target edaphic microbial biomass. These studies are essential, based on the documented critical roles these soil based microflora play in the biogeochemical cycling of macro elements such as carbon, nitrogen and phosphorus.

This research was aimed at evaluating the dominant fungal phyla associated with bulked herbicide impacted and control soils.

Materials and Methods

Description of study area

Sapele Local Government Area (LGA) of Delta State is bordered by Warri North and Uviwie LGA respectively. Sapele LGA has a plethora of farming communities which include; Adagbrasa, Amukpe, Adavware, Elume, Ogiedi, Ughorhen and Ikeresan. Several oil fields and flow stations are also located in some of these farming communities.

Amukpetown is located within Longitude E 5º 42' 55.76" and Latitude N 5º 51' 38.75" with elevation above sea level being 11 m. The vegetation is typical of the rainforest except for drainage streams where swampy areas exists. The commercial farm estate located in Amukpe, is an integrated farm estate patterned around the Songhai-Parakuo of the Republic of Benin.

Soil collection

Top soils were collected from an active farmland within the premises of the commercial farm holding in Amukpe town, Delta State. About 100 kg of the top soil was sourced with the aid of a sterile soil auger from the respective sampling stations at a depth of 0-15 cm. Another 100 kg of semi pristine fallow soil which have been left for an unknown period of time, utilized as the control were collected from Adavware community, near Amukpe. Geo referenced co-ordinates of all the sampled locations was obtained with the aid of an hand held GPS meter. These soil samples were placed in labeled sterile polyethylene bags and transported to the laboratory for further analyses.

Table 1: Sampling points and the GPS coordinates

Sampling Stations	GPS coordinates
Station one; watermelon and tomatoes farm	**N**05.84928º**E**005.74573º; **N**05.84928º**E**005.74573º; **N**05.84910º**E**005.74586º;**N**05.84901º**E**005.74594º;**N**05.84887º**E**005.74594º;**N**05.84888º**E**005.74607º; **N**05.84897º**E**005.74601º; **N**05.84907º**E**005.74597º; **N**05.84912º**E**005.7450º; **N**05.84918º**E**005.74594º
Station two; Maize farm	**N**05.84929º**E**005.74589º; **N**05.84941º**E**005.74578º; **N**05.84946º**E**005.74591º; **N**05.84938º**E**005.74598º; **N**05.84928º**E**005.74607º; **N**05.85104º**E**005.74635º; **N**05.85109º**E**005.74632º; **N**05.85115º**E**005.74627º; **N**05.85113º**E**005.74620º; **N**05.85106º**E**005.74627º
Station three; Cassava farm	**N**05.85097º**E**005.74631º; **N**05.85092º**E**005.74636º; **N**05.85903º**E**005.74629º; **N**05.85098º**E**005.74626º; **N**05.85103º**E**005.74621º; **N**05.85110º**E**005.74617º; **N**05.85100º**E**005.74614º; **N**05.85097º**E**005.74620º; **N**05.85091º**E**005.74624º; **N**05.85085º**E**005.74622º
Adavware community, near Amukpe	**N**05.80327 **E**005.77412

Fungal metagenomics analysis using next generation sequencing (NGS)

Total DNA was extracted from 2 g of each soil samples labeled "farmed" (for bulked soils from the commercial farm holding, Amukpe) and "control" (for compounded soils from Adavware community), using the NucleoSpin Soil Kit. The quantity and quality of the extracted DNA was assessed by NanoDrop Spectrophotometry and Qubit Fluorometry. PCR amplifications were performed for each DNA sample using the ITS1 primer set to amplify fungal DNA. PCR reaction products were evaluated via agarose gel electrophoresis. Each PCR product was cleaned using Agencourt AMPure XP beads. Unique index adapters were ligated to each PCR product to generate an ITS1 library for each sample. Libraries were evaluated by agarose gel electrophoresis. Each library was cleaned using Agencourt AMPure XP beds and subsequently evaluated by Qubit fluorometry and Agilent 2100 Bioanalyzer. ITS1 libraries were size-selected using BluePippin for a target fragment range of 250-600 bp. Final libraries were analyzed by Qubit fluorometry and Agilent 2100 Bioanalyzer. The final libraries were pooled with compatible libraries from other projects and loaded into the Illumina MiSeq to generate an average of at least 0.5M PE300 reads for each library. Raw Illumina data was converted into fastq format and de-multiplexed. Reads that are short (N<100) or poor quality (Q<20) were filtered out. The forward and reverse Illumina read for each cluster (amplicon molecule) were collapsed to generate a full sequence of the amplicon.

Results and Discussion

The amounts of the extracted DNA were 6.83 and 6.65 ng/µl for both the bulked control and farmed soils (Table 2). The Qubit fluorometric analyzed DNA concentrations were; 0.072 and 0.057 ng/µl for the control and farmed soils respectively (Table 3). The total DNA volume of the fluorometeric analyzed ITS1 libraries were; 120 and 190 ng/µl for the control ITS1 and farmed ITS1, respectively (Table 4). The total DNA volume of the fluorometeric analyzed ITS1 final sized-selected libraries were; 52 and 116 ng/µl for the control ITS1 and farmed ITS1 respectively (Table 5). The raw, trimmed and merged reads for the control ITS1 were; 504, 468, 471, 266 and 464,529 while for the farmed ITS1; the reads were; 502, 687, 483,302 and 476,051, respectively (Table 6).

Table 2: Concentration and purity of extracted DNA from the control and farmed soils

Sample ID	Conc.	A_{260}	A_{280}	A_{260}/A_{280}	A_{260}/A_{230}
Control	6.83	0.137	0.087	1.57	0.53
Farmed	6.65	0.133	0.075	1.78	0.57

Table 3: Qubit fluorometry (ng/µl) analysis of extracted DNA from the control and farmed soils

Sample ID	Concentration	Volume (µl)	DNA (ng)
Control	0.072	50	3.6
Farmed	0.057	50	2.9

Table 4: Qubit fluorometry (ng/µl) analysis of ITS1 libraries from the control and farmed soils

Sample ID	Conc.	Volume (µl)	Total DNA (ng)
Control_ITS1	3.00	40	120
Farmed_ITS1	4.74	40	190

Table 5: Qubit fluorometry (ng/µl) analysis of final sized-selected libraries from the control and farmed soils

Sample ID	Conc.	Volume (µl)	Total DNA (ng)
Control_ITS1	1.31	40	52
Farmed_ITS1	2.90	40	116

Table 6: Summary of bioinformatics analysis from the control and farmed soils

Sample ID	Raw Reads	Trimmed Reads	Merged Reads
Control_ITS1	504, 468	471, 266	464,529
Farmed_ITS1	502, 687	483,302	476, 051

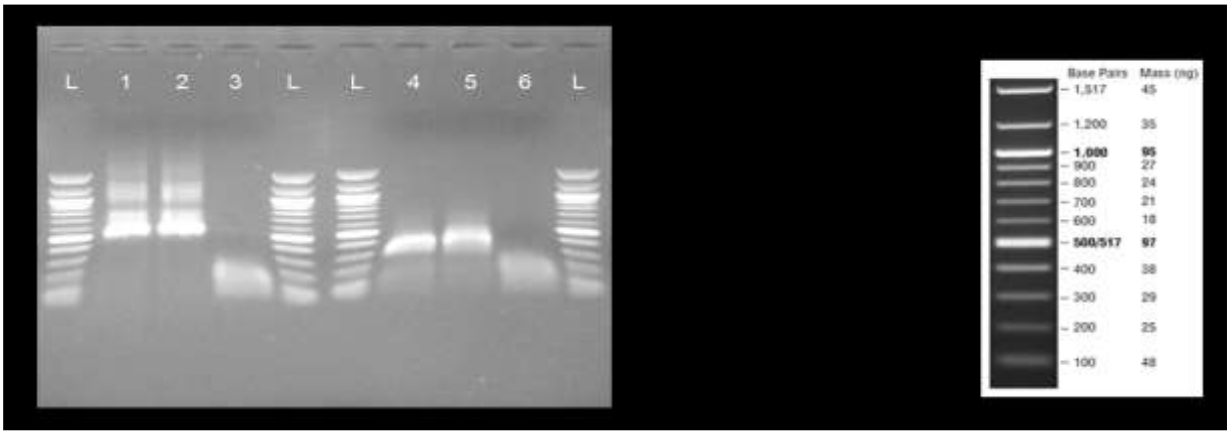

Fig. 1: Agarose gel electrophoresis of PCR-1 products of the extracted DNA from both the bulked control and farmed soils

The result of the agarose electrophoresis of the PCR-1 products of the extracted DNA from both the control and farmed soil samples employed for the next generation sequencing is presented in Fig. 1. The electropherograms of the initial and final size-selected Agilent 2100 bioanalyzer analysis of the bulked control and farmed soil samples at the _ITS1 region are shown in Figs. 2A to 2D.

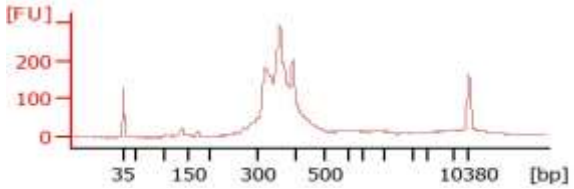

Fig. 2A: Agilent 2100 Bioanalyzer analysis of the _ITS1 region of the fungal DNA from the control soil

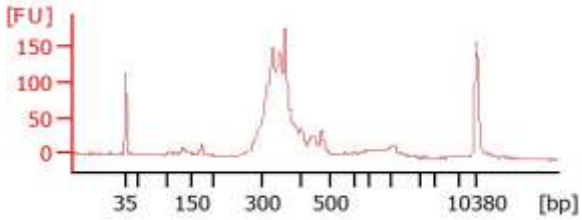

Fig. 2B: Agilent 2100 Bioanalyzer analysis of the _ITS1 region of the fungal DNA from the farmed soil

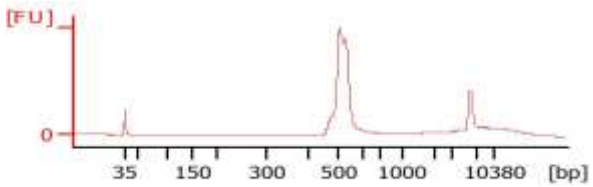

Fig. 2C: Agilent 2100 Bioanalyzer analysis of Final size-selected _ITS1 region of the fungal DNA from the control soil

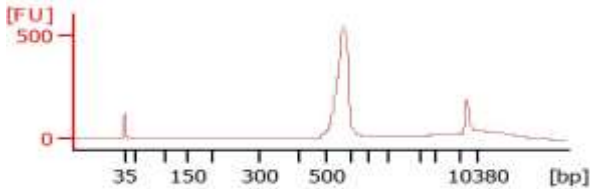

Fig. 2D: Agilent 2100 Bioanalyzer analysis of Final size-selected _ITS1 region of the fungal DNA from the farmed soil

Table 7: Fungal phyla distribution in the control and bulked soils

Phyla	Fungi	
	Control soil	Farmed soil
Ascomycota	+	+
Basidiomycota	+	+
Chytridiomycota	+	+
Zygomycota	-	+

Table 7 revealed the distribution of fungi phyla isolated from both the bulked control and farmed soils. Ascomycota, Basidiomycota and Chytridiomycota were detected in both soils samples while Zygomycota was detected only in the farmed soil. Three (3) representative fungal phyla; Ascomycota, Basidiomycota and Chytridiomycota were identified in the bulked control soil (Table 7, Fig. 3). The ascomycete; *Knufia perforans* (SH 209750.06FU) (53%) was the predominant soil borne fungal specie (Figs. 4 and 5). Three (3) ascomycetal classes; *Eurotiomycetes*, *Dothideomycetes* and *Sordariomycetes* were identified and among the Basidiomycetous fungi, *Ramaria rubribrunnescens* (SH221766.06FU) (52%) was the most dominant specie (Fig. 5). Two (2) basidiomycetal classes; *Agaricomycetes* and *Tremellomycetes* were detected in the control soil (Fig. 6). The chytrid; *Olpidium bornovanus* SH229162.06FU (97%) was the most dominant species among the chytridiomycetes detected (Fig. 7). The usage of next generation sequencing (NGS) procedures revealed the presence of a thriving fungal community in both the bulked control and farmed soils. Comparatively there were slight differences between the fungal community structure of both the control and the farmed soils. The farmed soil had a higher diversity as four (4) representative fungal phyla were detected as against three (3) phyla for the control soil. It is difficult to suggest a particular reason that would explain this trend, as a wide variety of factors aside from anthropogenic land conversion pattern, such as fungistatis can affect the structure of soil fungal microbiome.

Four (4) representative fungal phyla; *Ascomycota*, *Basidiomycota*, *Zygomycota* and *Chytridiomycota* were identified in the bulked farmed soil (Fig. 7, Table 7), and the ascomycete; *Knufia perforans* (SH 209750.06FU) (48%) was the predominant soil borne fungal specie (Figs. 7 and 8). The Ascomycota phylum was the most abundant in both the control and the experimental soils. Castaneda and Barbosa (2017) observed similar trends in respect of soil borne microbial communities present in Chilean vineyards and surrounding native forests. Shary *et al.* (2007) reported that members of this phylum possess the ability to degrade plant polymers such as cellulose and hemicellulose present in

woody plant litter which may account for their abundance in a wide variety of soils from around the world.

Eleven (11) ascomycetal classes; *Eurotiomycetes*, *Dothideomycetes*, *Leotiomycetes*, *Lecanoromycetes*, *Saccharomycetes*, *Schizosaccharomycetes*, *Pezizomycetes*, *Orbiliomycetes*, A*rchaeorhizomycetes*, *Taphrinomycete* and *Sordariomycetes* were identified (Fig. 8) and among the *Zygomycetous* fungi; *Mortierella ambigua* (SH232483.06FU) (23%) was the most dominant specie (Fig. 9). Nine (9) basidiomycetal classes; *Agaricomycetes*, *Microbotryomycetes*, *Basidiomycota* unidentified, *Pucciniomycetes*, *Ustilaginomycetes*, *Agaricostilbomycetes*, *Exobasidiomycetes Tremellomycetes* and Incertae sedis 4 were detected in the bulked farmed soil (Fig. 10) and *Rhodotorula lamellibrachiae* (SH227552.06FU) (10%) was the most dominant specie (Fig. 10). Among the Chytridiomycota, the dominant specie was *Chytridiomycota* sp. (SH233268.06FU) (Fig. 11).

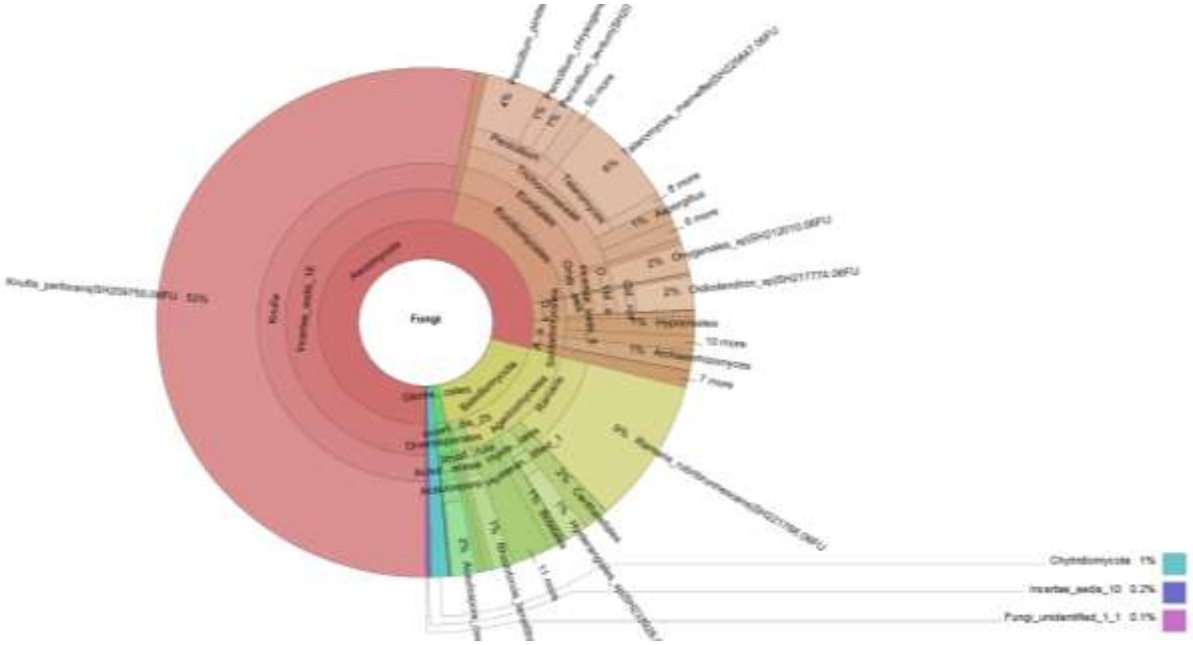

Fig. 3: Percentage occurrence of the fungal domain present in the bulked control soil

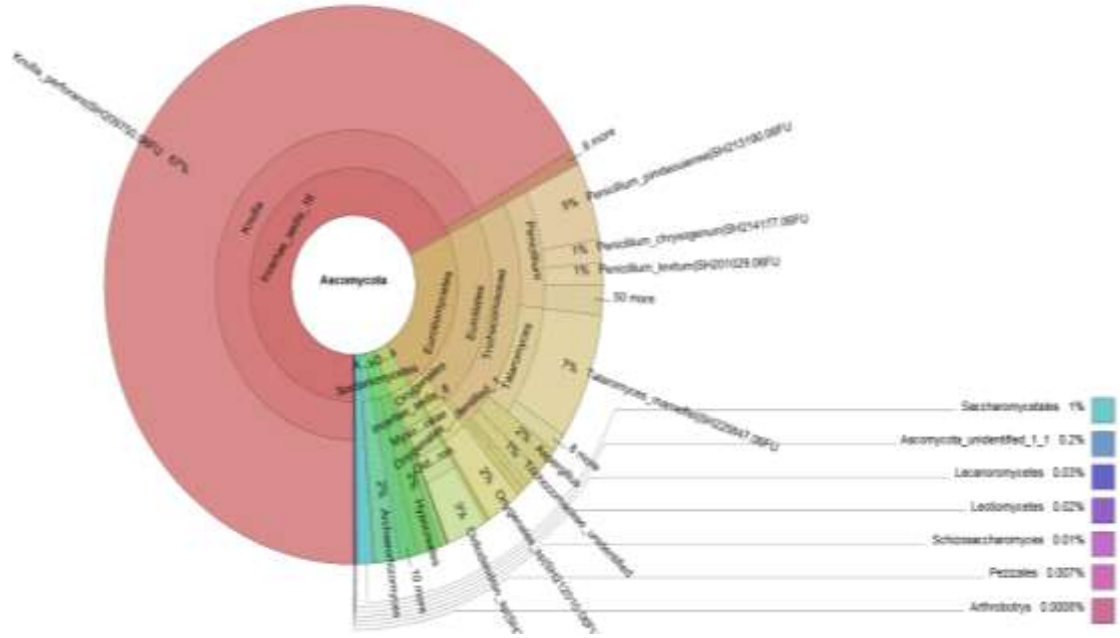

Fig. 4: Percentage occurrence of the phylum *Ascomycota* present in the bulked control soil

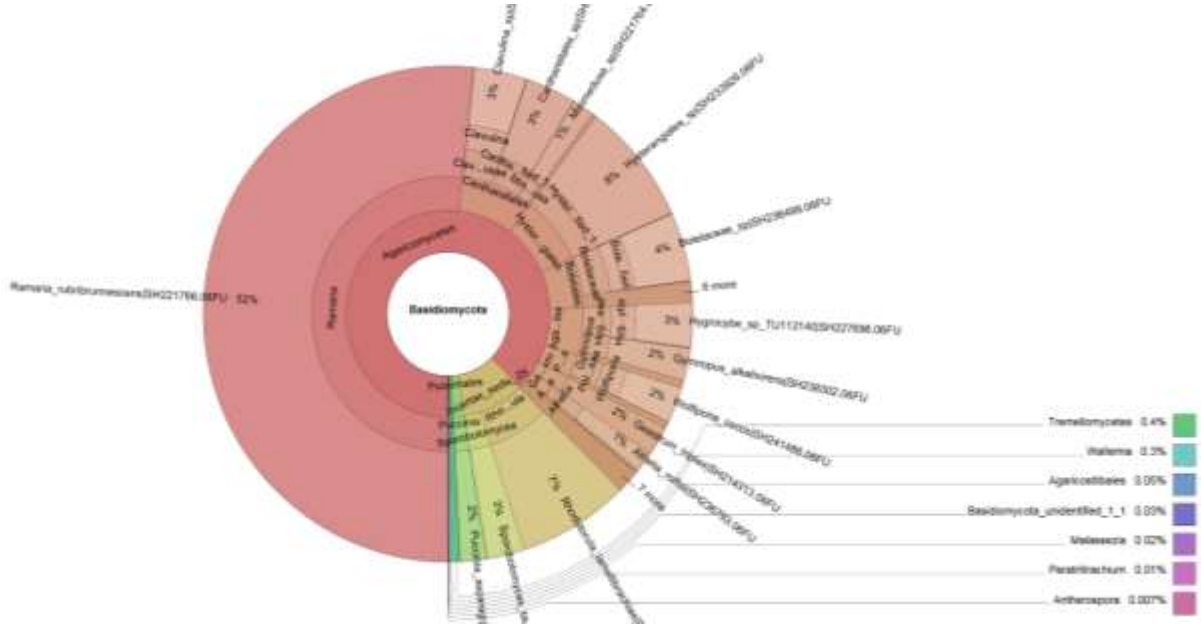

Fig. 5: Percentage occurrence of the phylum *Basidiomycota* present in the bulked control soil

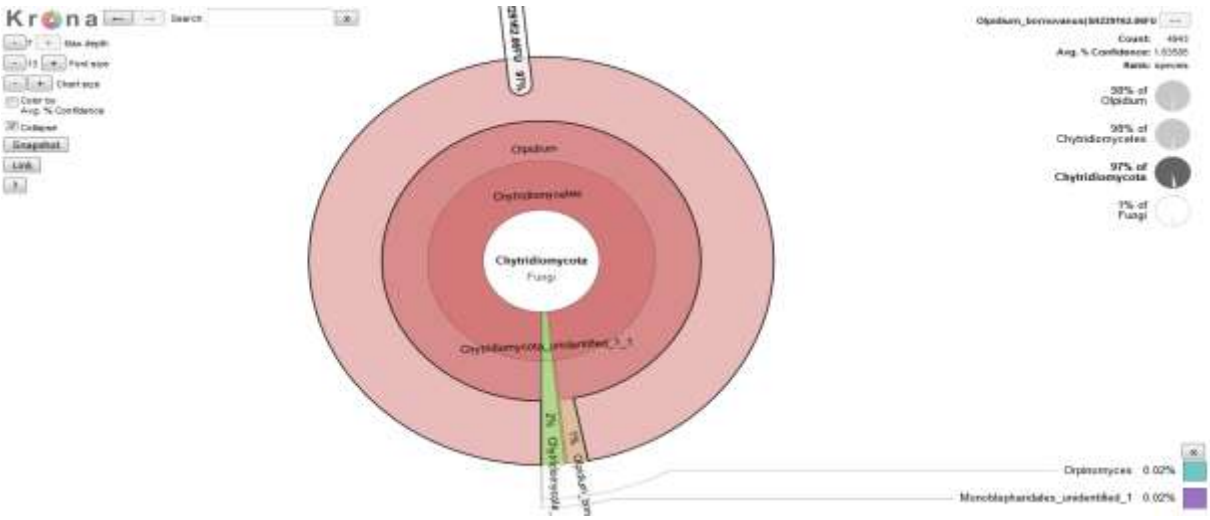

Fig. 6: Percentage occurrence of the phylum *Chytridiomycota* present in the bulked control soil

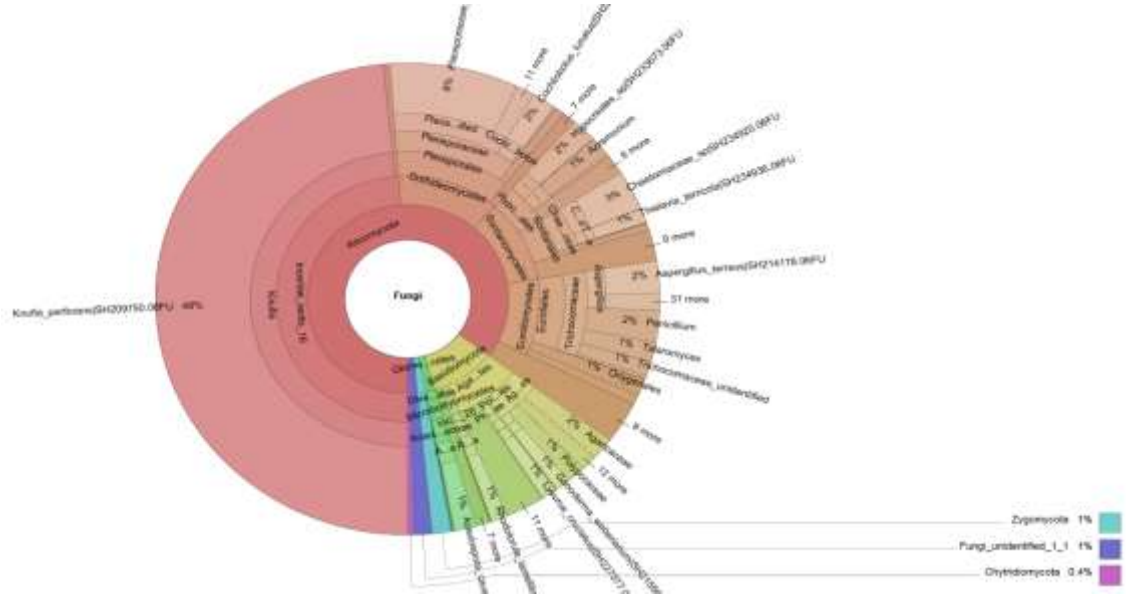

Fig. 7: Percentage occurrence of the fungal domain present in the bulked farmed soil

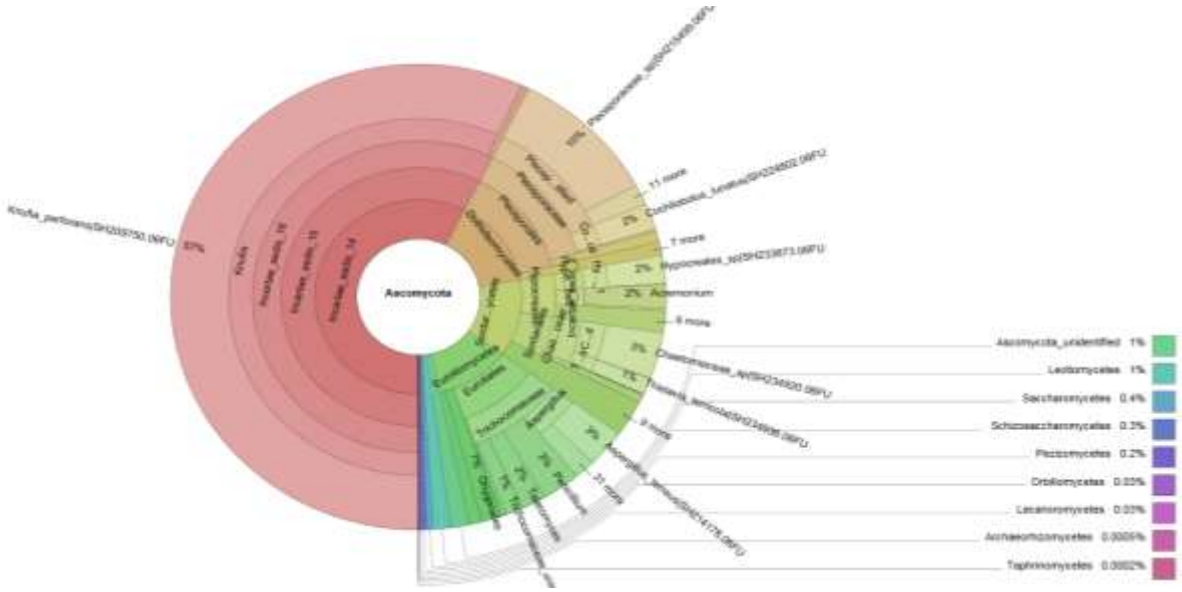

Fig. 8: Percentage occurrence of the phylum *Ascomycota* present in the bulked farmed soil

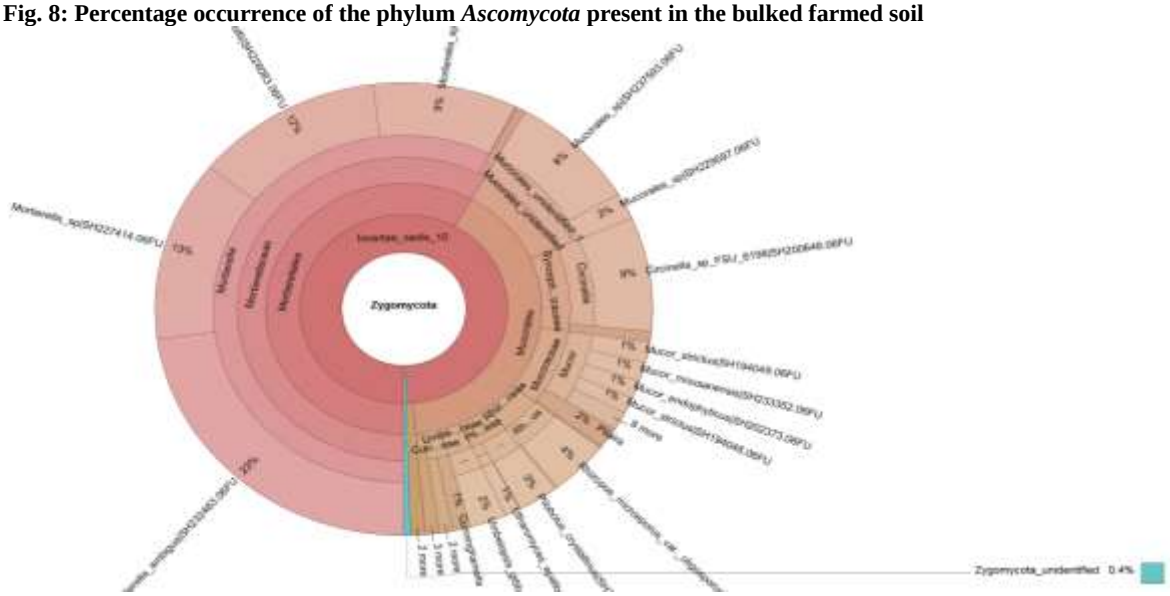

Fig. 9: Percentage occurrence of the phylum *Zygomycota* present in the bulked farmed soil

Fig. 10: Percentage occurrence of the phylum *Basidiomycota* present in the bulked farmed soil

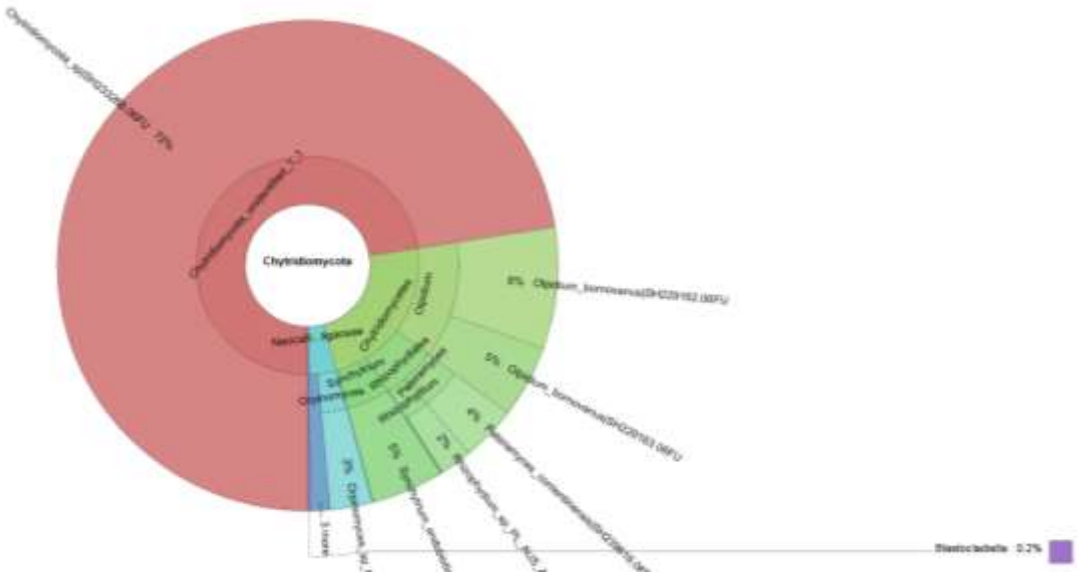

Fig. 11: Percentage occurrence of the phylum *Chytridiomycota* present in the bulked farmed soil

During the collection of top soils from the premises of the commercial farm estate, the farm manager was interviewed and disclosed that herbicides and pesticides were utilized in the course of farming operations for weed and pest control. He further stated that the herbicide utilized were mainly those of the glyphosate group sourced from the open market. Glyphosate is a non- selective, broad spectrum herbicide and crop desiccant. It is an organophosphorus compound, specifically a phosphonate, used to kill weeds especially annual broadleaf weeds and grasses that are known to compete with crops. Glyphosate ($C_3H_8NO_5P$) has a water solubility of 1.01 g/100ml and acidity pKa <2, 2.6, 5.6, 10.5 and traded under the several names which include; Roundup® and Rodeo® respectively. A major limitation of this study was the inability to investigate the physiological functions of the identified existing soil borne fungal phyla. Appropriate studies aimed at elucidating the metabolic functionalities of the fungal phyla in active agricultural soils utilizing available metaproteomics, metatranscriptomics and proteogenomics protocols is hereby recommended.

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Conflict of Interest

Authors declare that there is no conflict of interest.

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