

Taxonomic and Functional Composition of Soil Mycobiome of Two Agricultural Sites in Khartoum State, Sudan

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Abstract

Fungi are one of the most diverse groups of organisms and considered as one of the least-explored biodiversity resources. Soil fungal community was investigated in two agricultural sites in Khartoum state, Sudan, during two seasons. A total of 42 soil samples were collected, their physicochemical properties were determined, then subjected to metabarcoding and metagenomic analyses. fungal community composition, diversity and microbial trophic modes were determined utilizing R software packages. From both sites, a total of 15 different phyla were detected, out of them, 11 were the most abundant and frequent. Ascomycota was the dominant phylum (86.54% total abundance), followed by the Basidiomycota (8.29%). The dominant class was Sordariomycetes (41.02%), followed by Dothideomycetes (19.80%). *Aspergillus* (6.2%), *Curvularia* (6.0%), *Neurospora* (5.8%) and *Fusarium* (4.9%) were the most abundant genera. *Deniquelata* for the first time being recorded in Sudan. Apha diversity measures revealed sample richness ranging from 71 to 361 ASVs, and Shannon index ranging from 2.794 to 5.087. The two sites had significantly different alpha diversity. Land-use types were also significantly different in their diversity regardless of site. Season had no effect on alpha diversity of soil fungal communities. Beta diversity analysis indicated significant differences between the two sites and the different land-use types. No significant differences in the community structure recorded between the two seasons. The dominant trophic mode among the assigned ASVs in soil mycobiome was saprotroph mode (22.11%). Results of this study reveals that fungal community structure is affected by site and land-use type. It gives a comprehensive database for the mycobiome of the agricultural soil in Khartoum state.

1. Introduction

The soil represents one of the most dynamic and complex ecosystems in the world inhabited by a diverse group of microbial consortia in close association with roots of different plants and are responsible for occurrence of variety of biological and physiological processes (Choudhury and Jain 2012). It is highly needed to expand our knowledge regarding diversity and function of soil microbial community which is a necessary task to alleviate the harmful effects of soil degradation (Prasad et al. 2021). Also, most of the world's soils are known to lack the nutrients that needed for plant growth. Farmers tend to the extensive use of chemical fertilizers to meet the deficiency of nutrients. Therefore, there is an urgent need to explore the potential of soil microbes for proper nutrient recycling and to recognize alternative, sustainable, environment-friendly options for reducing the use and impacts of synthetic fertilizers (Malav et al. 2015; Prasad et al. 2021).

Fungi are one of the most diverse groups of organisms on earth and are integral ecosystem agents that play pivotal ecological roles as mycorrhizal partners of plants, saprotrophs, and agents of disease. The estimated number of fungal species on earth is about 1.5 million in 2001 and re-estimated to 3.8 million in 2017, whereas the number of described fungal species is 120,000 species only. They are widely distributed in all terrestrial ecosystems; however, distribution of fungal taxa or groups has been poorly documented (Tedersoo et al. 2014; Tedersoo and Nilsson 2016). Fungi are considered as one of the least-

explored biodiversity resources of our planet because most of them are uncultivable in the laboratory (Webster and Weber 2007). The number of cultured fungi is believed to be a small fraction of the total number of extant species (Rajendhran and Gunasekaran 2008). Therefore, rapid, and accurate rate of taxonomic and functional identification of fungi from complex environments such as soil, water, and tissues of plants and animals is of utmost importance (Tedersoo and Nilsson 2016). Hawksworth and Lücking(2017) in their article that estimating the number of fungi concluded that there were major sources for unrecognized fungal diversity, and the geographic areas and ecological habitats that are largely understudied, particularly in tropical regions and biodiversity hot spots are of the main sources.

Sudan, as many other African countries, also lacking information about fungal diversity particularly soil fungi. Although the importance of agriculture to Sudan, little is known about soil microbial community and microorganisms, particularly fungi. As far as we know, very few investigations on soil mycoflora have been conducted previously, with no study using the modern molecular culture-independent approaches. The first attempt was done very early by Nour (1955). In which investigated the microscopic soil fungi in ten alkaline soil samples from Khartoum state, Khartoum north with clay content ranging from 15–35 % using conventional culture-based methods. Other studies were conducted in different areas of Sudan viz Gezira, Sennar and White Nile state (Amin and Abdalla 1980; Abdel-Rahim et al. 1983; El-Amin and Saadabi 2007). Also, few studies were conducted to investigate arbuscular myorrhizal fungi (AMF) association with some crops. For instance, a survey was performed in 2009 in the White Nile state, Central Sudan, to assess AM root colonization and AMF spore densities and species richness in nine fields planted with 13 different important crop species (Abdelhalim et al. 2014).

In the last decade, advancement in high-throughput sequencing brought unprecedented growth in understanding the world of fungi through sequencing of targeted metabarcoding marker genes directly obtained from environmental samples. Metabarcoding uses universal PCR primers to mass-amplify a taxonomically informative gene (barcodes) from mass collections of organisms or from environmental DNA (Ji et al. 2013). The importance of metabarcoding in ecology is increasing dramatically, particularly in the ecology of microorganisms that are often difficult to be identified by other tools rather than molecular biology (Balint et al. 2014). In addition, metabarcoding is a reliable method for recovering diversity information from large-scale, field-collected data sets (Ji et al. 2013).

In fungal community ecology, metabarcoding is becoming a very necessary tool (Schmidt et al. 2013). The internal transcribed spacer (ITS) of nuclear DNA (nrDNA), that includes the ITS1 and ITS2 regions, separated by the 5.8S gene, was formally proposed as the primary fungal barcoding marker both for the identification of single taxon, that is particularly below the genus level, and mixed environmental templates (Bellemain et al. 2010; Nilsson et al. 2012; Schoch et al. 2012).

Therefore, the present study was aiming at investigating the fungal community diversity in different land-use soils, collected from two agricultural sites in Khartoum State, Sudan, using ITS metabarcoding; to contribute to the existing knowledge about diversity of soil fungal communities in Sudan and Africa.

2. Materials And Methods

2.1 Study area and sampling:

Khartoum State (31.5-34 E, 15-16 N) is located in the heart of Sudan at the confluence of the White Nile and the Blue Nile, where the two rivers unite to form the River Nile. Most of the state lies in the climatic semi-desert region, while northern areas lie in desert zones. The climate of the state is ranging from hot to very hot. According to Köppen climate classification the climate of the state is warm desert climate (Bwh). The weather is rainy in summers, cold and dry in winters. Average rainfall reaches 100-200 mm in the north-eastern areas and 300-200 mm in the north-western areas. Temperature ranges in summer between 25-40 degrees in the months from April to June, and 20-35 in the months from July to October. In winter, however, temperatures continue to decline between November to March from 25–15 degrees.

Two sites in Khartoum State with different soil types (clay and sandy textures) were chosen to study the soil fungal community. The first site is located in Shambat area, Khartoum North and the second site is located in Omdurman locality particularly west Omdurman. Four land-use types were considered to collect the soil samples. These were onion (*Allium cepa*), Mango (*Mangifera indica*), forage sorghum (*Sorghum bicolor* (L.) Moench var. *Abu Sabeen*) and *bare land* that have not been cultivated for several years.

At each site and for each land-use, soil samples were collected from the top 20 cm of the surface soil of three locations (points); five replicates for each location were taken. Samples of the five replicates were mixed and pooled to make a composite sample for each collection point. GPS coordinates for each sampling point were recorded. A representative sample of each collection (about 1 Kg) was put in a plastic bag to be used for determination of soil properties. Also, 20 grams of the sample was taken in zip- lock plastic bags and kept cooled till being transferred to the laboratory for DNA extraction.

The sampling was conducted in two seasons, winter, and summer, following the same sampling technique.

2.2 Sample preparation:

In the laboratory, roots and rocks were removed from the samples before sieving. The 1 kg-sized samples were left to air-dry at room temperature, whereas samples taken for DNA extraction were kept at -20 C° until processed.

2.3 Soil analyses:

2.3.1 Determination of soil physicochemical properties:

Soil physicochemical properties were determined using the standard recommended methods. These were: particle size (clay%, sand% and silt%), saturation percent (SP%), pH, electrical conductivity (ECe

in DS/m), calcium carbonate (CaCO₃%), total nitrogen (N%), phosphorus (ppm), organic carbon (O.C%), organic matter (O.M%), Carbon/ Nitrogen ratio (C/N%) was calculated from the obtained N% and C%.

2.3.2 Metabarcoding and metagenomic analysis:

Each soil sample (250 mg) was used to extract the DNA using Qiagen Dneasy® PowrSoil® DNA extraction kit (Qiagen) according to the manufacturer's instructions. The quality of the extracted DNA was checked using NanoDrop™ 2000c spectrophotometer (Thermo Fisher Scientific Inc, USA).

To study the soil fungal community, the ITS1 region was amplified using forward primer, ITS1FKYO2 (5'-TAGAGGAAGTAAAAGTCGTAA-3') and the reverse primer ITS2KYO2 (5' - TTYRCTRCGTTCTTCATC-3', Toju et al. 2012). The forward primer was linked with Ion Torrent specific adapters and Ion-Xpress barcodes to ease samples demultiplexing.

PCR reaction mixtures (20 µl each) contained: 10 µl of Q5® Hot Start High-Fidelity 2X Master Mix (New England Biolabs, UK, Ltd), 0.5 µl of each primer, 8.5 µl of ultra-pure water (Prepared by Millipore Direct-Q® 3 UV Water Purification System) and 0.5 µl of template DNA (1 to 10 µg / µl based on the checked concentrations). For each sample, PCR was performed in two replicates using BioRad T100™ Thermal Cycler (Bio-Rad Laboratories, Inc.).

The PCR program consisted of initial denaturation step at 98 °C for 30 seconds; 35 cycles of: 98 °C for 10 seconds, 53 °C for 30 seconds, 72 °C for 1 minute; then final extension at 72 °C for 2 minutes and incubation (infinite hold) at 12 °C. The PCR products were verified using 1.2 % agarose gel in 1% TAE Buffer. An aliquot of 3 µl of each PCR product sample was loaded after mixing with 1 µl of SYBR green dye and 0.5 µl of 6x loading buffer. Then samples were allowed to separate for 20 minutes using 100 volts in MUPID-EXU horizontal electrophoresis system (Gel Company, Inc.).

A molecular weight marker (0.1-20 kbp Gene Ladder Wide 2, NIPPON GENE CO., LTD) was also loaded with the samples. The gel was visualized in FujiFilm Luminescent Image Analyzer Model LAS-4000 (FUJIFILM CORPORATION).

To purify the PCR amplicon from the excess primers, nucleotides, salts and enzymes, amplicons were subjected to high-throughput purification using AGENCOURT® AMPURE XP PCR Purification system (Beckman Coulter, Inc., Brea, CA). This system utilizes an optimized buffer to selectively bind PCR amplicons 100 bp and larger to paramagnetic beads.

The purified PCR products were quantified using Invitrogen Qubit™ dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA, USA) as described by the manufacturer. Then the tubes were read in the Qubit® 2.0 fluorometer (Thermo Fisher Scientific).

According to the lowest concentration, the library was prepared by mixing different volumes of the purified PCR products in DNA LoBind tube (Eppendorf North America, Inc., USA). The size and the

quantity of the library were checked using Agilent High Sensitivity DNA Kit (Agilent Technologies) in Agilent 2100 Bioanalyzer integrated with 2100 Expert software.

Emulsion PCR was conducted using Ion PGM Hi-Q View OT2 kit 400 on the Ion OneTouch™ 2 system (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Sequencing was then performed using Ion Personal Genome Machine (Thermo Fisher Scientific, Waltham, Massachusetts, USA) with Ion PGM sequencing 400 Kit and Ion 316™ chip v2 BC (Life Technology, Inc.).

2.3.3 Quantification of the fungal biomass using qPCR:

The quantitative PCR (Real Time PCR) was used to determine the quantity of the fungi in the soil samples using LightCycler® 2.0 Instrument and the LightCycler® FastStart DNA MasterPLUS SYBR Green I Kit (Roche Diagnostics GmbH, Mannheim, Germany). The primers set for fungal rRNA genes was nu-SSU-1196F (5' -GGAACTCACCAGGTCCAGA-3') and nu-SSU-1536R (5' -ATTGCAATGCYCTATCCCCA-3', Borneman and Hartin 2000).

2.4 Metagenomic data processing

The ITS1 raw sequencing data were obtained from the Torrent Server-Torrent Suite™ as a demultiplexed FASTQ files. All analyses were done in QIIME2 version 2019.10.0 and 2020.6.0 (Bolyen et al. 2019), R software version 3.6.3 (**R Core Team 2020**) and RStudio version 1.2.5033 (RStudio Team 2019). Firstly, the primers were removed using QIIME2 Plugin 'cutadapt' version 2019.10.0 from package 'q2-cutadapt' version 2019.10.0 (Martin 2011; Bolyen et al. 2019).

The data were then analyzed using DADA2 pipeline through the R package DADA2 version 1.16.0 (Callahan et al. 2016). The quality of the reads in the FASTQ data files was inspected, then the data were filtered and trimmed using DADA2 standard filtering parameters.

Chimeras were removed from each filtered read using removeBimeraDenovo function. Then the Amplicon Sequence Variants (ASVs) table was created. The table records the number of times each exact amplicon sequence variant was observed for each sample. The taxonomy was assigned to ASVs using assignTaxonomy function of dada2 package. The UNITE general FASTA release for Fungi Version 04.02.2020 (Abarenkov et al. 2020) was used as a reference database for the fungal ITS1 sequences.

Samples were checked for total number of reads to be more than 5,000 reads. To enable comparisons, the ASVs counts were standardized by transformation to relative abundance and then multiplication by the median sample read depth using phyloseq package (McMurdie and Holmes 2013). The standardized data were merged at the lowest available taxonomic level or annotation using modified tax_glom.kv function of the same package. The merged taxa were filtered by removing taxa that are only present at very low numbers in a small minority of samples, that are present at least 10 counts in at least 20% of samples or that have a total relative abundance of at least 1% of the total number of reads (Lennard). The filtered data were used for further analyses.

2.5 Soil fungal community composition and diversity:

To study the fungal diversity within each soil community, the observed taxa (Richness) and Shannon indices were used as alpha diversity (Within-samples) measures and they were measured on unmerged standardized data. The `estimate_richness` function of `phyloseq` package was used for this purpose.

Beta diversity (between-samples) was examined on the merged filtered taxonomies using Bray-Curtis dissimilarity measure and the non-metric multidimensional scaling (NMDS) as ordination method using `phyloseq` package.

Heat maps for the top 50 most abundant taxa were created using unsupervised hierarchical clustering with Bray-Curtis distances for all samples.

2.6 Functional composition prediction:

The processed ASVs were used to predict functional communities of the samples. FUNGuild tool (v1.0 Beta) was used to taxonomically parse the fungal ASVs by ecological guild (Nguyen et al. 2016). The tool was used through the python script, provided by the tool developers, that has been run from the Ubuntu 16.04 command line.

2.7 Data analysis:

All analyses were performed in R software version 3.6.3 (R Core Team 2020).

The analysis of soil properties was conducted using one-way ANOVA to compare the properties for the different land-use in the same site and two-way ANOVA to compare the properties of land-use types in different sites.

Taxonomic bar plots to show taxonomic composition (at different levels) in all samples `phyloseq` package and for each site with the different land-use type were created using `phyloseq` R package. On the other hand, Tukey's 'Honest Significant Difference' (TukeyHSD) test was carried out to determine the statistical differences between different sites and land-use types' communities (alpha diversity measures). Then alpha diversity box plots were plotted using `amplicon` R package.

To examine how the composition of microbiome communities varies across different land-use types and the two sites, statistical test of significance on beta diversity was performed through permutational multivariate analysis of variance (PERMANOVA) using `adonis` function of `vegan` package version 2.5-6 (Oksanen et al. 2019).

The homogeneity of dispersion test was performed to estimate the homogeneity of each group regarding the taxonomic composition of their samples.

To determine the ASVs (taxa) that are significantly different between between the two sites, `super.fitZig.kv` function modified from `metagenomeSeq`'s `fitzig` and `mrfulltable` functions in R were used

The following parameters were used to determine significance: the ASV should have 0.2% presence across samples, and that to keep only ASVs where at least one of the sites have 20% of the samples positive for that ASV, had a fold change (beta coefficient) of 1.25 and had adjusted p -value of 0.5.

2.8 Effect of study factors on soil fungal biomass

To study the effect of different land-use types, site and the two seasons on the fungal biomass (ITS gene copy number), two-way ANOVA was performed in R. Before that, Levene's Test for Homogeneity of Variance for different groups was performed using Levene's Test function in car package.

3. Results

ITS1 amplicon sequence data generated by Ion Torrent sequencing platform were used to investigate which factor affects soil mycobiome the most. Two seasons (Winter and summer), different land-use types and four sites that have different soil properties in Khartoum state were included as the factors of the study.

3.1 Soil properties:

Soil physicochemical properties that were statistically different (P -value < 0.05) between the two sites were: EC, SP%, Clay%, Sand%, Silt%, OC%, OM% and N%. Whereas, the two sites had the same pH, CaCO₃, C/N and P (Table 3.1). Shambat had the highest SP, clay, silt, OC, OM and N values. Whereas Omdurman had the highest EC and Sand %. On the other hand, land use had an effect on Clay% and N% (Table 3.1). On analyzing each site separately, land use types in Omdurman indicated significant differences on EC while Shambat land-use types were significantly different in clay% and OM%.

3.2 Fungal biomass:

ANOVA test revealed that there were no significant differences (P -value =0.885109) between the two sites Shambat and Omdurman in the amount of the fungi. Also, there were no significant differences between the different land-use types of the two sites and the two seasons ((Pr(>F) 0.625927 and 0.446575, respectively). However, the interaction between land-use and site indicated highly significantly different result (Pr(>F) 0.000102).

3.3 Fungal community:

3.3.1 Metagenomic raw data and data processing:

The total number of raw reads in all 42 samples (Samples from the two sites, four land-use types and two seasons) was 1436320 reads decreased to 880097 after filtering and denoising and almost to the half (705829 reads) after removing chimeric sequences. Shambat had the highest number of reads, and

mango soils had the highest number of reads among all land-use types, followed by sorghum, bare land and onion. These reads were then classified into 4403 ASVs based on unite database and they were all classified as fungi. Among nonchimeric sequences, the minimum number of reads per sample was 7202 (Recorded for sample Omdurman_G2_L3 collected from Sorghum soil in Omdurman), and the maximum number was 45580 reads (Sample Shambat_G2_L1 collected from Sorghum soil in Shambat). With 16805.45 average reads number and median of 16661.5. All the samples were used in the analysis (after standardization) and no samples were excluded because of the low reads number. Merging the ASVs at the lowest available taxonomic annotation resulted in 677 ASVs, 231 out of them were remained after filtering (Present at 10 counts, in 20% of the samples or at 1% of the total abundance). These filtered data were used in all statistical tests.

3.2.2 Soil Fungal community structure and taxonomic composition:

In all soil samples, the taxonomic composition revealed 15 different fungal phyla that include a total of 39 classes, before merging and filtering. However, 1406 (31.9%) out of the 4403 ASVs could not be identified to phylum level. Among those identified to phylum level, 2017 ASVs did not assign to certain class. This represented 45.8% of the total number and 67.3% of the ASVs that assigned to certain phylum.

After merging ASVs that had similar taxonomy and then filtering based on abundance and/or frequency, there were 11 fungal phyla (Included 24 classes, 47 orders, 75 families, 123 genera and 117 Species), with number

Table 3.1 Soil properties (Means) for different land-use types and the twot sites.

Soil Properties	Land-use types				Sites	
	Bare Land	Mango	Onion	Sorghum	Omdurman	Shambat
SP%	47.267 a	43.267 a	40.333 a	46.733 a	28.9 b	59.9 a
pH	7.438 a	7.238 a	7.517 a	7.54 a	7.403 a	7.463 a
EC	1.0031 ab	1.221 a	0.904 ab	0.689 b	1.2012 a	0.7075 b
Clay%	34.465 b	43.215 a	41.383 a	40.24 ab	22.769 b	56.8825 a
Silt%	16.042 a	13.125 ab	9.792 b	14.167 ab	3.646 b	22.917 a
Sand%	49.493 a	43.66 a	48.825 a	45.593 a	73.585 a	20.201 b
CaCo3%	1.833 a	1.667 a	2.417 a	3.75 a	2.208 a	2.625 a
N%	0.045 a	0.075 a	0.03 a	0.055 a	0.03 b	0.0725 a
OC%	0.073 a	0.095 a	0.062 a	0.075 a	0.0508 b	0.1016 a
OM%	0.127 a	0.168 a	0.108 a	0.13 a	0.089 b	0.1775a
P(ppm)	1.683 a	1.367 a	1.483 a	1.383 a	1.425 a	1.533 a
C/N	2.159 a	1.930 a	2.031 a	1.385 a	1.1 a	1.753 a
Different letters in rows indicate significant differences between the types or sites based on TukeyHSD test (P value <0.05).						

of unidentified ASVs increases in the lower levels (Class 5, Order 12, Family 30, Genus 44, Species 110). Ascomycota was the dominant phylum in soil mycobiome. It represented 86.54 % of the total abundance and included 65.80% of the identified ASVs, followed by the phylum Basidiomycota (8.29% of total abundance and 17.75 of the total ASVs) (Table 3.2). The remaining nine phyla represented 5.17% of the total abundance and included 16.45% of the ASVs (**Fig. 3.1**). On the other hand, the dominant class of the phylum Ascomycota and among all classes was Sordariomycetes (41.02%), followed by Dothideomycetes (19.80%), while 12.41% of the total abundance recorded by ASVs that did not identified to class level (**Fig. 3.2**).

Pezizomycetes, Eurotiomycetes, Agaricomycetes and Mortierellomycetes were also recorded (8.35%, 6.99%, 6.67% and 1.29%, respectively).

At genus level, *Aspergillus* (6.2%), *Curvularia* (6.0%), *Neurospora* (5.8%), *Fusarium* (4.9%), *Deniquelata* (2.3%), *Nothophoma* (1.9%), *Preussia* (1.8%), *Acrophialophora* (1.7%) and *Thielavia* (1.7%) were the most abundant genera in the soil community with *Aspergillus* being frequent in all samples, *Curvularia* absent in only one sample (**Fig. 3.3**) and *Deniquelata* for the first time being recorded in Sudan.

The most abundant ten taxa belonged to the phylum Ascomycota; five of them to the class Sordariomycetes, and the other five belong to: Pezizomycetes (two), Dothideomycetes (one) and

Eurotiomycetes (one). The most abundant ASV among them represents sequences that identified to phylum level only and it was present in high abundance in all study samples.

Heat map of the top 50 abundant taxa (**Fig. 3.4**) showed the occurrence of those taxa in different samples was made.

The taxa that appear in hot colors indicates high abundance and those have blue colors indicates low level of abundance. It is clear that, most of these taxa not identified to genera and species levels.

3.3.2 Fungal community diversity (Alpha and beta):

Based on the observed number of taxa (ASVs), the minimum number of ASVs was 71 (recorded for sample Omdurman_G2_L3) and the highest one was 361 (recorded for sample Shambat_M2_L1). The minimum Shannon value was 2.794 (Omdurman_M1_L4) and the highest one was 5.087 recorded in sample (Shambat_R2_L3).

The effect of different land-use types (in one site) and seasonality on alpha diversity was checked by performing TukeyHSD statistical test. For Shambat site, the two alpha diversity measures indicated significant differences between the four land-use types. Based on observed measure, the types were also grouped in three groups ($\text{Pr}(> F)$ 0.00786). The first group consisted of mango and onion which had the highest diversity, the second one of bare land which had the lowest number of observed taxa. Sorghum was statistically not significant from the two previous groups. Mango, onion and sorghum had statistically similar Shannon index values (7.61×10^{-5}), while bare land was different in its diversity. Also, the two seasons had the same diversity based on the two diversity measures (Observed 0.208, Shannon 0.297; **Fig. 3.5**). The land-use types in Omdurman were highly significantly different ($\text{Pr}(> F)$ 0.00106) in the value of observed ASVs. However, the four land-use types had the same Shannon index ($\text{Pr}(> F)$ 0.262; **Fig 3.6**). Also, no significant difference was observed between the two seasons regarding their alpha diversity (Shannon $\text{Pr}(> F)$ 0.89, Observed $\text{Pr}(> F)$ 0.52).

Regardless of land-use types, the site had an obvious effect on alpha diversity. The two sites had different communities based on the two alpha diversity measures (Observed 4.64×10^{-5} , Shannon 0.000945). Shambat had the highest values of both diversity indices (Shannon and observed; **Fig 3.7**). Land-use types were also significantly different in their diversity regardless of site (Observed $\text{Pr}(> F)$ 4.92×10^{-5} , Shannon $\text{Pr}(> F)$ 0.000174). Onion and Mango had the highest diversity, bare land had the lowest Shannon index. Sorghum and bare land had the same observed number of taxa (**Fig 3.8**). Season had no effect on alpha diversity of soil fungal communities. Also, the interaction between site and land use had no effect on the diversity (Observed $\text{Pr}(> F)$ 0.258, Shannon $\text{Pr}(> F)$ 0.080834).

Table 3.2 The 11 phyla appeared in soil community and the corresponding number of ASVs, ASVs% from total ASVs, the abundance and abundance% for each phylum.

Phylum	No. of ASVs	ASVs%	Abundance	Abundance %
Ascomycota	152	65.80	518445	86.54
Basidiomycota	41	17.75	49680	8.29
Mortierellomycota	11	4.76	8114	1.35
Chytridiomycota	6	2.60	7742	1.29
Glomeromycota	7	3.03	4583	0.76
Blastocladiomycota	3	1.30	4080	0.68
Mucoromycota	4	1.73	3314	0.55
Rozellomycota	4	1.73	1042	0.17
Calcarisporiellomycota	1	0.43	880	0.15
Aphelidiomycota	1	0.43	758	0.13
Zoopagomycota	1	0.43	447	0.07

Beta diversity was studied using Bray-Curtis dissimilarity distance. NMDS (**Fig. 3.9**, Stress value 0.2116419) indicated that samples from each site tended to cluster together irrespectively of the land-use type except for one or two samples in each site. However, fungal communities according to different land-use types in Shambat were separated from each other's, particularly those from bare land soils which clustered apart from others and were grouped with Omdurman samples. The homogeneity of dispersion test revealed significant results of fungal composition between land-use groups ($F = 4.056$, $P = 0.019$) which indicates the heterogeneity of each land-use community. On the other hand, communities per sites and/or seasons test indicated homogeneous composition non-significant result ($F = 0.2697$, $P = 0.603$ and $F = 0.0918$, P value = 0.793, respectively). The PERMANOVA result indicated statistically significant differences in Bray-Curtis distance between the two sites and the different land-use types ($p = 0.001$; R^2 0.10587 and 0.20143, respectively). However, no significant differences ($P = 0.287$, R^2 0.01769) in the community structure recorded between the two seasons. On the other hand, significant differences ($P = 0.046$, R^2 0.04499) were recorded for interaction between season, land-use and site of collection. The interaction between the two factors, land-use and site, indicated significant differences ($P = 0.001$, R^2 0.14893). The differential abundance between the two sites revealed that there were 83 ASVs significantly different between the two sites that met threshold criteria mentioned previously (**Fig. 3.10**). 21 taxa were found only in Shambat, and only one taxon was found only in Omdurman.

3.2.4 Functional composition:

FunGuild tool made assignments on 156 ASVs out of 231 (75 ASVs were unassigned). The confidence of the assignments was varied. Of these 10.9% were highly probable to be assigned to the correct guild,

60.26% were probable, 28.85 % were possible to be assigned correctly.

There were six trophic modes determined for all samples, these are: Saprotroph (which included the highest number (48.7%) of the ASVs), Pathotroph-Saprotroph-Symbiotroph, Pathotroph, Saprotroph-Symbiotroph, Pathotroph- Saprotroph and Symbiotroph. The dominant trophic mode among the assigned ASVs in soil mycobiome was saprotroph mode (22.11% of the total reads), followed by Pathotroph-Saprotroph- Symbiotroph (11.45%), while 10.30% recorded for the pathotroph mode. The remaining three modes represented 7.35 %. About the half of fungal community (48.78%) represented by ASVs that failed to be assigned to trophic modes and ecological guilds.

The six modes included 32 ecological guilds (Table 3.3). A percent of 48.78 of the abundance was failed to be assigned to certain ecological guild. On the other hand, unidentified saprotroph was the most abundant guild which accounted for 15.59% of the total abundance. This was followed by plant pathogen (8.86%) and animal Pathogen-Endophyte-Lichen Parasite-Plant Pathogen-Soil Saprotroph-Wood Saprotroph (4.85%).

The most abundant saprotrophic taxa included: *Neurospora* (24.95%), *Deniquelata barringtoniae* (10.19%) , unidentified *_Ascobolaceae* (12.21%), *Thielavia subthermophila* (7.71%) and *Preussia* (7.27%). *Fusarium kyushuense* is the most abundant taxon that belonged to Pathotroph-Saprotroph-Symbiotroph mode (19.77% of the mode abundance and 2.26% of the total abundance), followed by *Fusarium solani* (12.64% and 1.448%) and *Alternaria tenuissima* (10.75% and 1.23%).

4. Discussion

We used metabarcoding approach to investigate the soil mycobiome of four land-use types in the two sites: Shambat and Omdurman in Khartoum State, Sudan. ITS1 was used as the fungal DNA barcode and sequencing was done using Ion Torrent platform. The total number of ASVs recorded in this study was 4403 ASVs which were represented 677 taxa merged at their lowest available taxonomic annotation. Of them 231 were abundant and/or frequent taxa. This result suggests high diversity in soil mycobiome of the study area compared to the number of investigated samples.

The significance was checked using the following parameters: perc = the ASV should have 0.2% percent presence across samples, and that to keep only ASVs where at least one of the sites have 20% of the samples positive for that ASV, had a fold change (beta coefficient) of 1.25 and had adjusted *p*-value of 0.5.

Table 3.3

The trophic modes and ecological guilds found in the soil mycobiome of the study with their corresponding number of ASVs, ASVs%, abundance and abundance%

Trophic mode	Guild	No. of ASVs	ASVs %	Abundance	Abundance%
Saprotroph	Undefined Saprotroph	42	18.18	93380	15.59
	Dung Saprotroph-Soil Saprotroph-Wood Saprotroph	2	0.87	18425	3.08
	Dung Saprotroph-Undefined Saprotroph	1	0.43	10208	1.70
	Dung Saprotroph	4	1.73	4651	0.78
	Dung Saprotroph-Plant Saprotroph	2	0.87	3260	0.54
Pathotroph-Saprotroph-Symbiotroph	Animal Pathogen-Endophyte-Lichen Parasite-Plant Pathogen-Soil Saprotroph-Wood Saprotroph	1	0.43	29074	4.85
	Bryophyte Parasite-Dung Saprotroph-Ectomycorrhizal-Fungal Parasite-Leaf Saprotroph-Plant Parasite- Undefined Saprotroph-Wood Saprotroph	15	6.49	12966	2.16
	Animal Pathogen-Endophyte-Plant Pathogen-Wood Saprotroph	1	0.43	7377	1.23
	Endomycorrhizal-Plant Pathogen-Undefined Saprotroph	4	1.73	5814	0.97
	Animal Pathogen-Endophyte-Fungal Parasite-Lichen Parasite-Plant Pathogen-Wood Saprotroph	2	0.87	4556	0.76
	Endophyte-Plant Pathogen-Wood Saprotroph	4	1.73	2780	0.46
	Animal Pathogen-Endophyte-Epiphyte-Plant Pathogen-Undefined Saprotroph	1	0.43	1910	0.32
	Animal Pathogen-Endophyte-Fungal Parasite-Plant Pathogen-Wood Saprotroph	8	3.46	1615	0.27
	Endophyte-Lichen Parasite-Plant Pathogen-Undefined Saprotroph	3	1.30	1577	0.26
	Animal Pathogen-Endophyte-Plant Pathogen-Undefined Saprotroph	1	0.43	955	0.16
Pathotroph	Plant Pathogen	18	7.79	53068	8.86

Trophic mode	Guild	No. of ASVs	ASVs %	Abundance	Abundance%
	Animal Pathogen	1	0.43	8629	1.44
Pathotroph-Saprotroph	Animal Pathogen-Plant Pathogen-Undefined Saprotroph	7	3.03	16049	2.68
	Plant Pathogen-Undefined Saprotroph	3	1.30	3079	0.51
	Insect Parasite-Undefined Saprotroph	1	0.43	2290	0.38
	Plant Pathogen-Plant Saprotroph	1	0.43	902	0.15
	Plant Pathogen-Undefined Parasite-Undefined Saprotroph	1	0.43	604	0.10
	Plant Pathogen-Wood Saprotroph	1	0.43	487	0.08
	Animal Pathogen-Fungal Parasite-Undefined Saprotroph	1	0.43	394	0.07
Saprotroph-Symbiotroph	Endophyte-Litter Saprotroph-Soil Saprotroph-Undefined Saprotroph	9	3.90	6951	1.16
	Dung Saprotroph-Ectomycorrhizal-Soil Saprotroph-Wood Saprotroph	2	0.87	2068	0.35
	Ectomycorrhizal-Undefined Saprotroph	3	1.30	2055	0.34
	Dung Saprotroph-Ectomycorrhizal-Litter Saprotroph-Undefined Saprotroph	1	0.43	367	0.06
Symbiotroph	Arbuscular Mycorrhizal	7	3.03	4583	0.76
	Ectomycorrhizal	6	2.60	3871	0.65
	Endophyte	1	0.43	339	0.06
Unassigned	Unassigned	75	32.47	292258	48.78

This study is the first to determine the whole mycobiome content in Sudan soils using the advanced metagenomic sequencing techniques. To the best of our knowledge and as mentioned above, very few mycological survey studies were conducted previously to survey soil fungi in Sudan as general and in Khartoum state in particular (Nour 1955; Amin and Abdalla 1980; Abdel-Rahim et al. 1983; El-Amin and Saadabi 2007). Therefore, little is known about the approximate number of fungal taxa in the soil. Nour (1955) studied 10 soil samples and recorded a total number of 35 species in a range of 8–29 species per sample. Amin and Abdalla (1980) isolated 112 species from 120 samples, collected from six localities in Sudan Gezira, that examined for soil mycoflora. Abdel-Rahim et al. (1983) conducted a study to

investigate mycoflora in two soil types of Clay and Sandy that collected from north west Sennar sugar cane plantation. They isolated 24 species from sugar cane rhizosphere, non-rhizosphere and root surfaces. Another study in White Nile state also, 23 species of 16 genera were recorded by El-Amin and Saadabi (2007) from sugarcane rhizosphere in twenty sites covering Kenana sugar Estate.

The number of recorded fungal taxa was varied between these studies and it was very low compared to the present study. Amin and Abdalla (1980) reported that Sudan's soils are generally poor in soil fungi based on the previous investigations. However, we assume that these low numbers of fungal taxa due to the method used in the investigation. As mentioned before, all previous studies were based on conventional culture-based methods that could only detect cultivable fungi which represents very small fraction of soil fungi. On the other hand, both cultivatable and uncultivable fungi could be detected by using metabarcoding approach which may be the reason behind the higher number of fungal taxa recorded in this study.

Also, the primers pair used in this study is characterized by high-coverage across diverse fungal taxa. It has been developed previously by Toju et al. (2012) to improve the coverage across fungi kingdom and they revealed that the designed primers covered 99% of fungal taxa at the species level. Furthermore, it could amplify the sequences of the two phyla Ascomycota and Basidiomycota) without significant taxonomic biases and could also cover an ecologically important clade of mycorrhizal fungi, Glomeromycota.

Of the whole ASVs, 31.9% identified as Fungi only and did not assigned to certain phylum. Also, at class level, 45.8% of the total number of sequences could not be identified to class and 67.3% of the ASVs that assigned to phylum failed to be identified to class as well. The percentage of unidentified ASVs is increasing toward the lowest taxonomic rank such as genera and species. The high percentage of unidentified fungi in the soil mycobiome at different levels confirmed that there is a large number of fungi that still not explored. For example, the most abundant ASV (after merging) accounted for 10.67% which represented by unknown sequences that belong to Ascomycota, and they may include different taxa of different importance. Therefore, more studies should be conducted to explore these unknown fungi.

Results of this study also indicated that the dominant fungal phylum in soil community is Ascomycota which represented 86.54% of the community and 65.8% of the recorded taxa belonged to Ascomycota, followed by Basidiomycota. This result is consistent with most previous studies investigating soil fungi in other geographical regions (Egidi et al. 2019; Al-Sadi et al. 2017; Tedersoo et al. 2014). It has been recorded that ascomycetes and basidiomycetes comprise the vast majority of fungal diversity (Blackwell 2011). The phylum Ascomycota (ascomycetes) is by far the largest group of fungi. There is a very wide range of lifestyles. Some ascomycetes are saprotrophs, others necrotrophic or biotrophic parasites of plants and animals, including humans (Webster and Weber 2007). The Basidiomycota (basidiomycetes) are also a large group of fungi with over 30,000 species. Many are saprotrophic and are involved in litter and wood decay, but there are also pathogens of trees such as the honey fungus, *Armillaria*. According to Dictionary of Fungi (2008), the phylum Ascomycota includes 23 classes; eight of them were recorded in

this study. Basidiomycota containing 20 class; only three were recorded in this study. With several sequences that failed to be assigned to certain class in each case.

Two studies, surveying the global distribution of soil fungi (Tederso et al. 2014; Egidi et al. 2019), reported similar results in term of dominant fungal taxa. The recent one that conducted by Egidi et al. (2019) surveyed soils from 18 countries and 8 continents. The authors stated that Ascomycota was the dominant phylum in soils worldwide. They recorded 83 fungal taxa that were dominant worldwide, 81 of them were belonging to Ascomycota. Although some Ascomycota are more dominant than other Ascomycota and Ascomycota are more dominant than Basidiomycota. Egidi et al. (2019) have also reported that the dominant fungi in arid grasslands and shrublands are members of the Ascomycota phylum. Ascomycota fungi are important drivers in carbon and nitrogen cycling in arid ecosystems and they play important roles in soil stability, plant biomass decomposition, and endophytic interactions with plants. They may also form symbiotic associations with biocrust components or be latent saprotrophs or pathogens that live on plant tissues.

However, in the study conducted by Tedersoo et al. (2014) Basidiomycota was the most abundant and accounted for (55.7%), followed by Ascomycota (31.3%), Mortierellomycotina (6.3%), and Mucoromycotina (4.4%). Whereas the most OTU-rich phyla were the Ascomycota (48.7%) and Basidiomycota (41.8%). Their global study revealed representatives of all major phyla and classes of Fungi. They reported that richness of Ascomycota—in particular, that of Archaeorhizomycetes, Dothideomycetes, Eurotiomycetes, Orbiliomycetes, and Sordariomycetes—peaked in tropical ecosystems. This is somehow consistent with our results where Sordariomycetes and Dothideomycetes were the most dominant classes and Eurotiomycetes was also among the dominant classes.

On the other hand, Amin and Abdalla (1980) mentioned that apart from the perfect state of the genera *Aspergillus* and *Penicillium*, the Ascomycetes were poorly represented in the Gezira soils. Another study analyzed fungal diversity in soil trapped behind dams in three arid regions of Oman using Culture-based techniques revealed that Ascomycota was the most dominant phyla in all samples under the study, contributing to 89% of the total species. Eurotiomycetes, Sordariomycetes and Dothideomycetes were the most common classes in dam soils. Zygomycetes, Pezizomycetes and Oomycetes were also detected, but at lower levels (Al-Sadi et al. 2017).

At genus level, *Alternaria*, *Cladosporium*, *Fusarium* and *Chaetomium* were among the dominant genera recorded from drylands. Although, the study had limited representation of tropical systems (Egidi et al. 2019). However, all local studies revealed that *Aspergillus* is the most abundant genus in Sudanese soils. El-Amin and Saadabi (2007) recorded that *Aspergillus* representing the most abundant genus followed by *penicillium*, *Alternaria*, *Rhizopus*, *Curvularia* and *Fusarium*. Amin and Abdalla (1980) also reported *Aspergillus* as the dominant genus. *Rhizopus stolonifer*, *Aspergillus nidulans*, *A. niger*, *A. flavus*, *Curvularia lunata*, *Alternaria tenuis*, and *Fusarium solani* have been the most frequent fungi isolated by Nour (1955). Fifty fungal taxa were recovered from the three dam soils, with *Aspergillus*, *Penicillium* and *Trichoderma* being the most common genera (Al-Sadi et al. 2017). In general, fungal communities had

lower diversity than did bacterial communities (Tian et al. 2017). Furthermore, Africa had lower fungal richness compared with other tropical regions independent of biome type, but generally, the overall richness of soil fungi increased toward the equator (Tedersoo et al. 2014).

It can be revealed from the results of this study that Shambat soil has the highest alpha diversity in terms of number of species (Richness) in soil communities as well as Shannon index. This result suggests that the diversity is due to different soil properties between the two sites. For example, Shambat soil had high clay and silt content, SP%, nitrogen, organic carbon and organic matter. They could be the main reason behind the higher diversity of Shambat soil. El-Amin and Saadabi (2007) showed that organic matter, pH, N, P, and K in soil have an effect on the population of fungi in soil. Organic matter increases the complexity and activity of mycoflora. Significant correlation between OM% and colonies isolated was obtained. However, number of fungi slightly correlated with other factors.

Also, different land-use types had different diversity. Onion and Mango were similar in their alpha diversity based on the observed number of taxa; they had the highest number of recorded taxa (Richness). Whereas sorghum and bare land had smaller number of taxa and they were statistically different from mango and onion. The four land-use types had similar soil properties except for EC, clay% and silt%. However, our results suggest that land-use type does not affect most of soil properties. Although, other studies reported different results. For example, Tian et al. (2017) found that vegetated land had advantages in soil chemical properties over bare land and that the natural forest land had advantages over plantation forests and shrub land.

Mango had highest and significantly different clay content which means high nutrients and minerals. Therefore, high diversity of mango community may be due to the high clay content. Bare land is lacking nutrients and other factors that related to plant cover such as root exudate which may enhance the microbial growth. Also, and although it is not significant from the others, mango and sorghum recorded the highest organic matter content. This may refer to the continuous falling leaves in mango land and the animal feces in sorghum. Other factors related to each land-use type such as root exudates may be responsible for the difference in their diversity.

Beta diversity analysis result revealed that both land-use and site affect the fungal community structure with land-use having the greatest effect that responsible for 20.14% of the total variance, while 10.59% of the variance due to site. The two seasons had similar fungal community structure. Also, the interaction between the two factors site and land-use had affected the fungal community structure as well as interaction between the three factors. This is consistent with our hypothesis that different land-use types and different soil types or properties have different soil communities and consistent with previous studies (Tian et al. 2017). Also, previous studies reported that seasonality had no effect or had small effect on fungal community diversity (Tian et al. 2017).

The quantity and composition of the microbial biomass depend on soil characteristics and the abundance of carbon (C) for energy and cell metabolism. Soil organic carbon (SOC) is the backbone of organic matter, which is the source of energy for most of the soil biota (Hesham et al. 2021). However, it

has been reported previously that the quantity and composition of the microbial biomass depend on soil characteristics and the abundance of carbon (C) for energy and cell metabolism (Prasad et al. 2021).

The fungal biomass was statistically not significant in the two sites and the different land-use types. But Shambat had higher value than Omdurman, and sorghum and mango had also higher values than other land-use types. This is obviously related to the organic matter which is highly needed for microbial growth. Shambat had high organic matter and carbon. Also, sorghum land receives organic matter from grazing in sorghum after cut. While Mango, the permanent land-use type received the organic matter from the dead leaves.

Many global studies reported that dominant fungi opposite to bacteria are not influenced by soil properties and elements or edaphic factors (Egidi et al. 2019; Tian et al. 2017; Tedersoos et al. 2014). Egidi et al. (2019) reported that soil properties (e.g pH) were poor indicators of relative abundance of dominant fungal taxa. Tedersoos et al. (2014) also suggested that edaphic characteristics had minor effect on fungi except specific groups such as AMF. On the other hand, climate has been reported as the most important environmental factor predicting fungal community composition. Tian et al. (2017) reported that there was no correlation with fungal community diversity and the soil chemical properties. In contrast to the bacterial communities, the fungal communities were mainly driven by soil P content. This further suggests that bacterial and fungal communities respond to different soil chemical properties factors.

5. Conclusion

Based on soil samples collected from 42 representative sites in two agricultural sites within Khartoum State, Sudan, this study demonstrated the high diversity of soil fungal communities in these ecosystems. We found that variations in soil fungal community diversity and composition were mainly regulated by soil composition and land-use while season had no effect. Different sites and different land-use types had different alpha diversity, whereas the two seasons had the same diversity.

Declarations

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Code availability: Not applicable.

Authors' contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Marwa H. E. Elnaiem. The research project and the draft

of the manuscript were designed by Marmar A. El Siddig and revised by Takeshi Taniguchi. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Consent to participate: Not applicable.

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Figure

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Figures

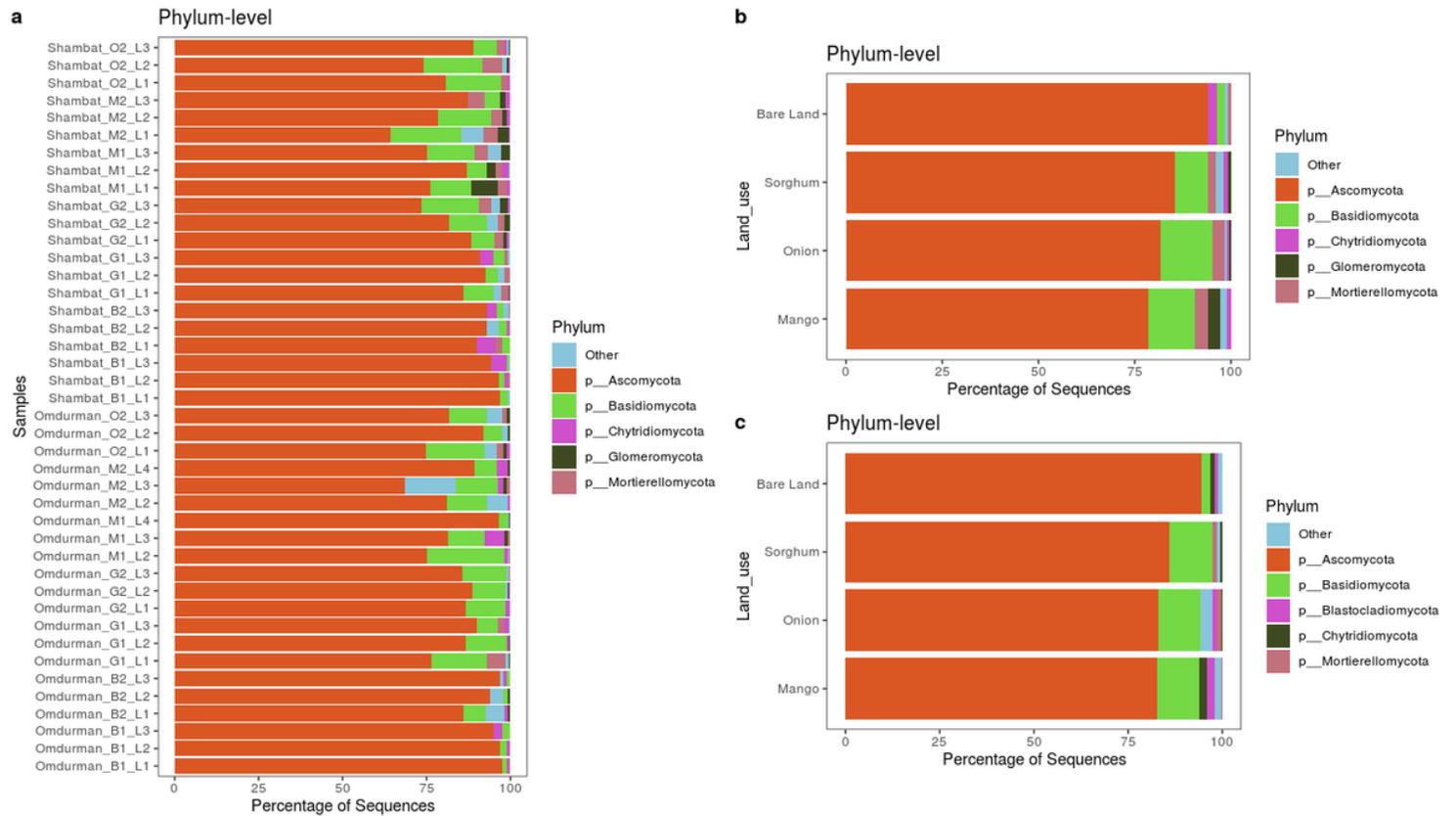


Figure 1

3.1 Relative abundances of the five most abundant phyla in soil mycobiome of study area, a: In each individual sample, b: In different land-use types in Shambat, c: In different land-use types in Omdurman. Other represents the remaining least abundant phyla

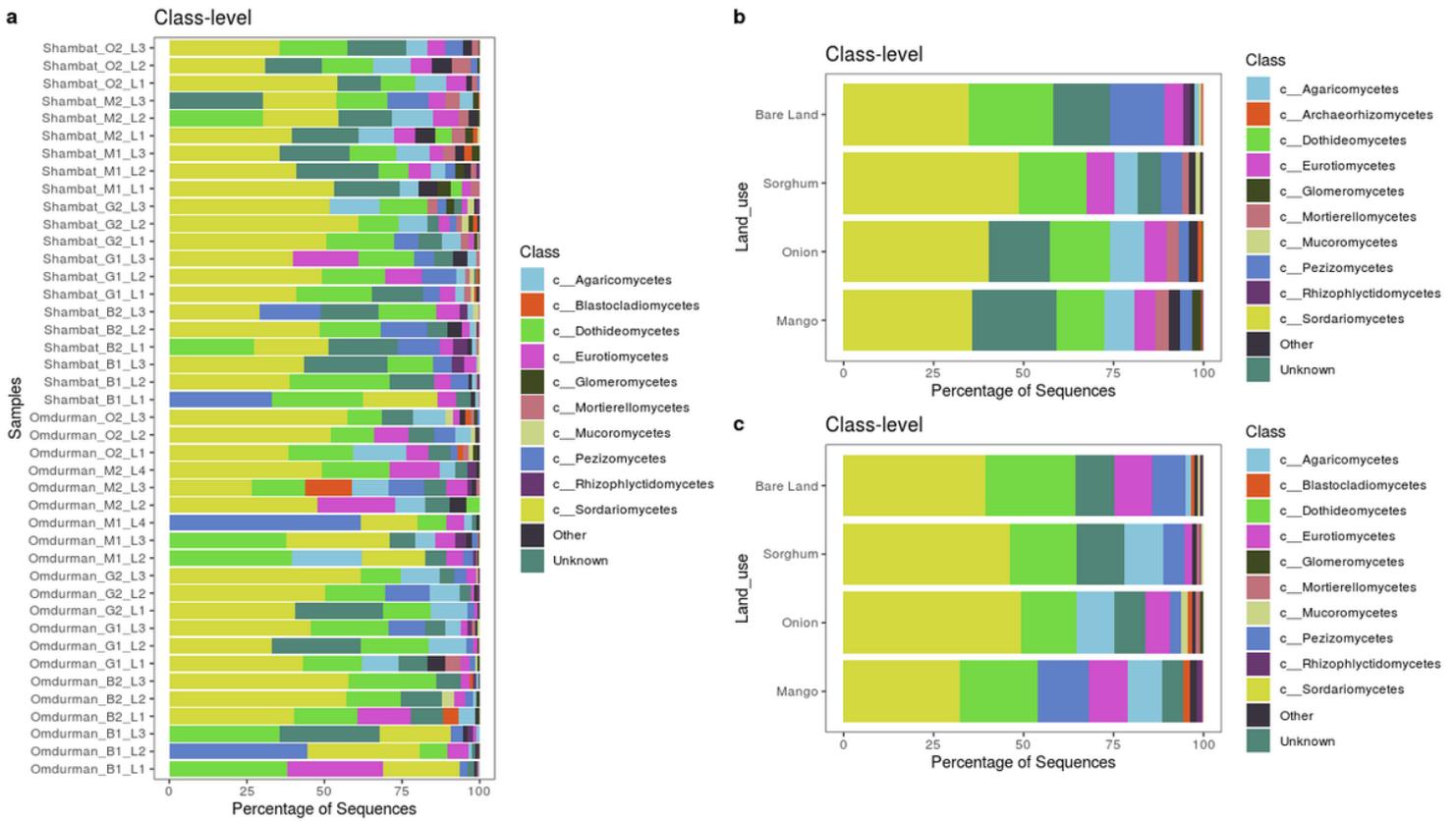


Figure 2

3.2 Relative abundances of the most dominant classes in study soil community. 1: a: In each individual sample, b: In different land-use types in Shambat, c: In different land-use types in Omdurman. Other represents the remaining classes. Unknown: ASVs that not identified to class

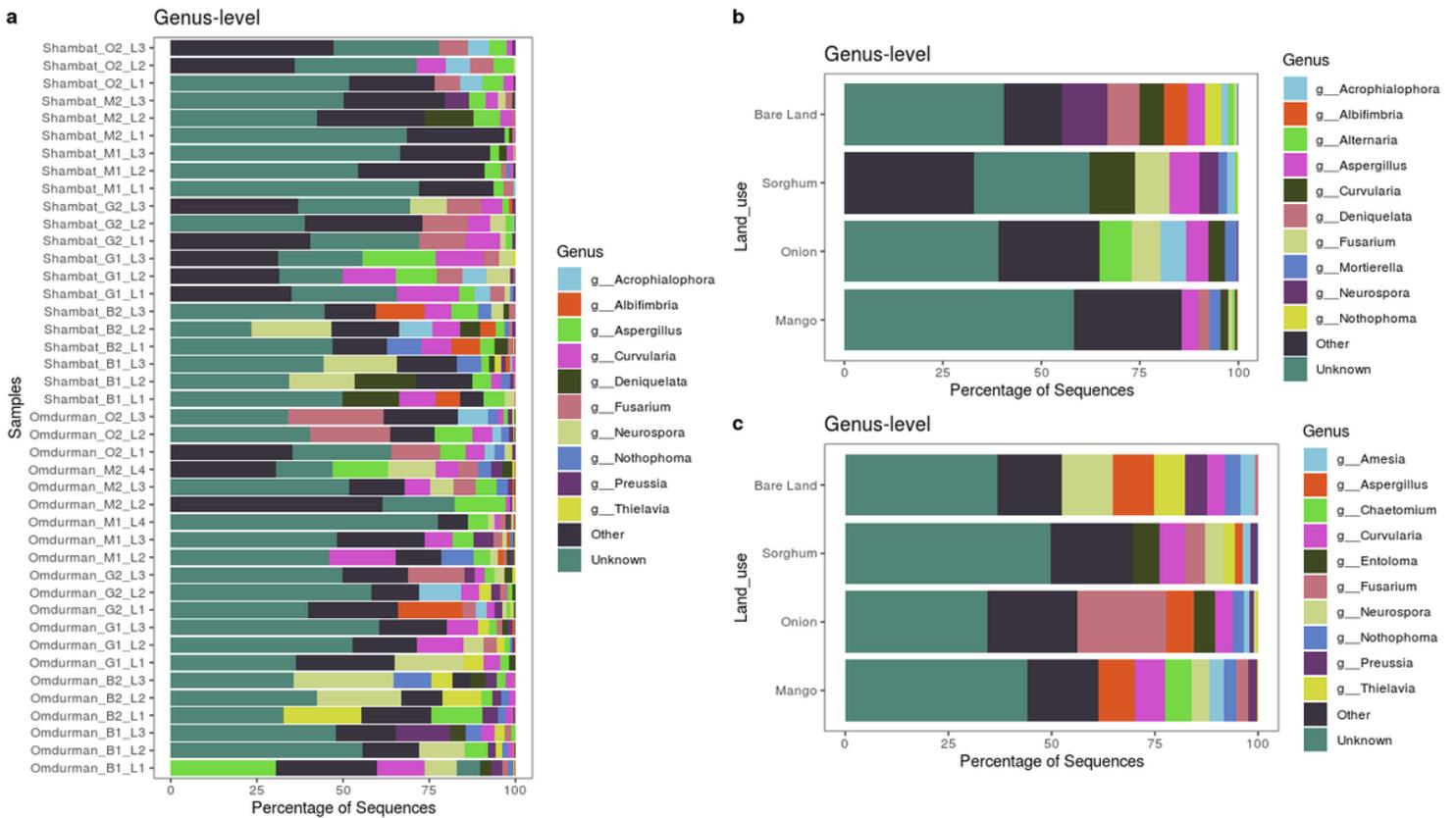


Figure 3

3.3 Relative abundances of the most dominant genera in study soil community. a: In each individual sample, b: In different land-use types in Shambat, c: In different land-use types in Omdurman. Other represents all remaining genera Unknown: ASVs that not identified to genus

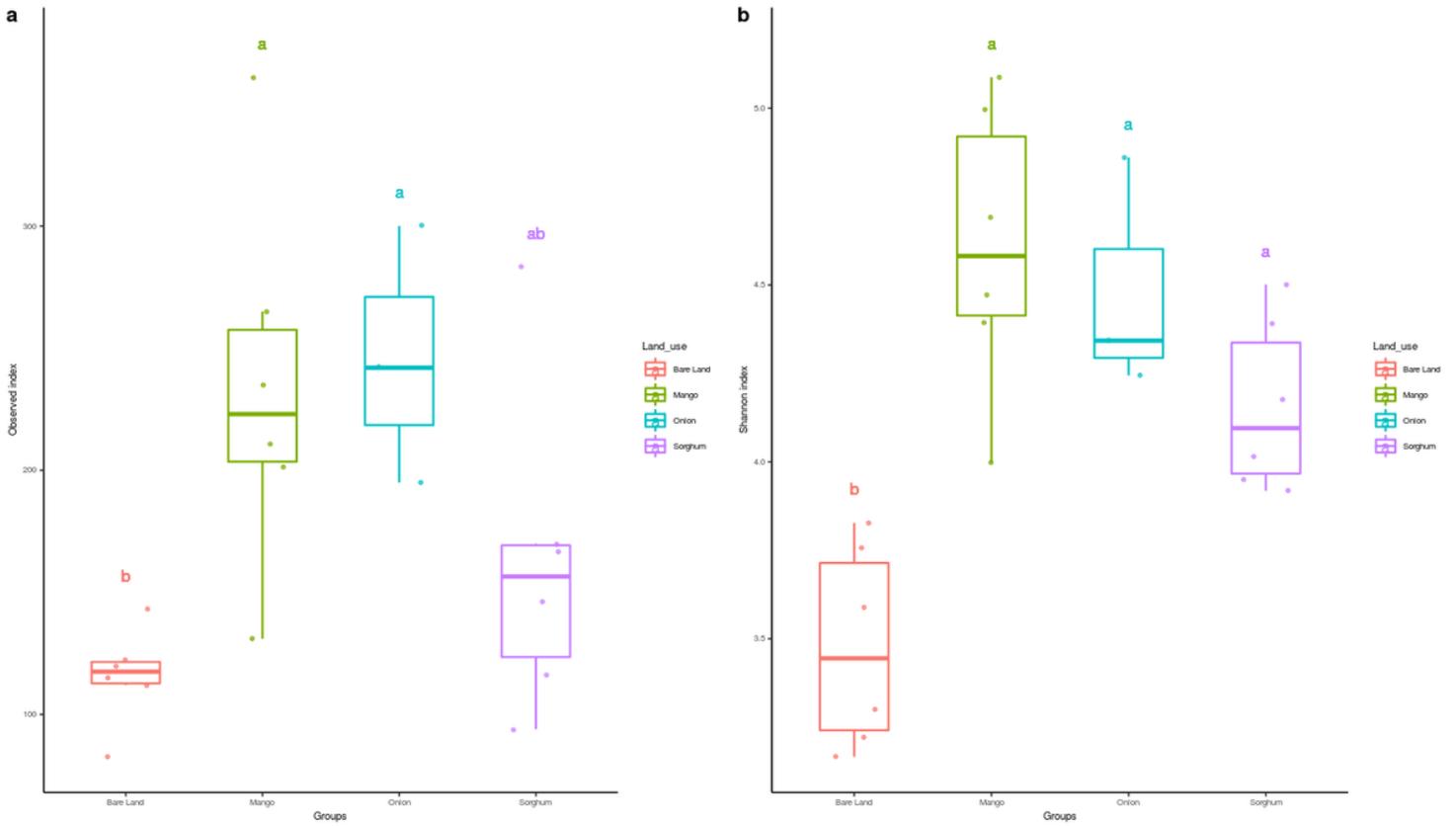


Figure 5

3.5 Box plots of alpha diversity measures in the different land-use types in Shambat (a: Observed number of taxa, b: Shannon index)

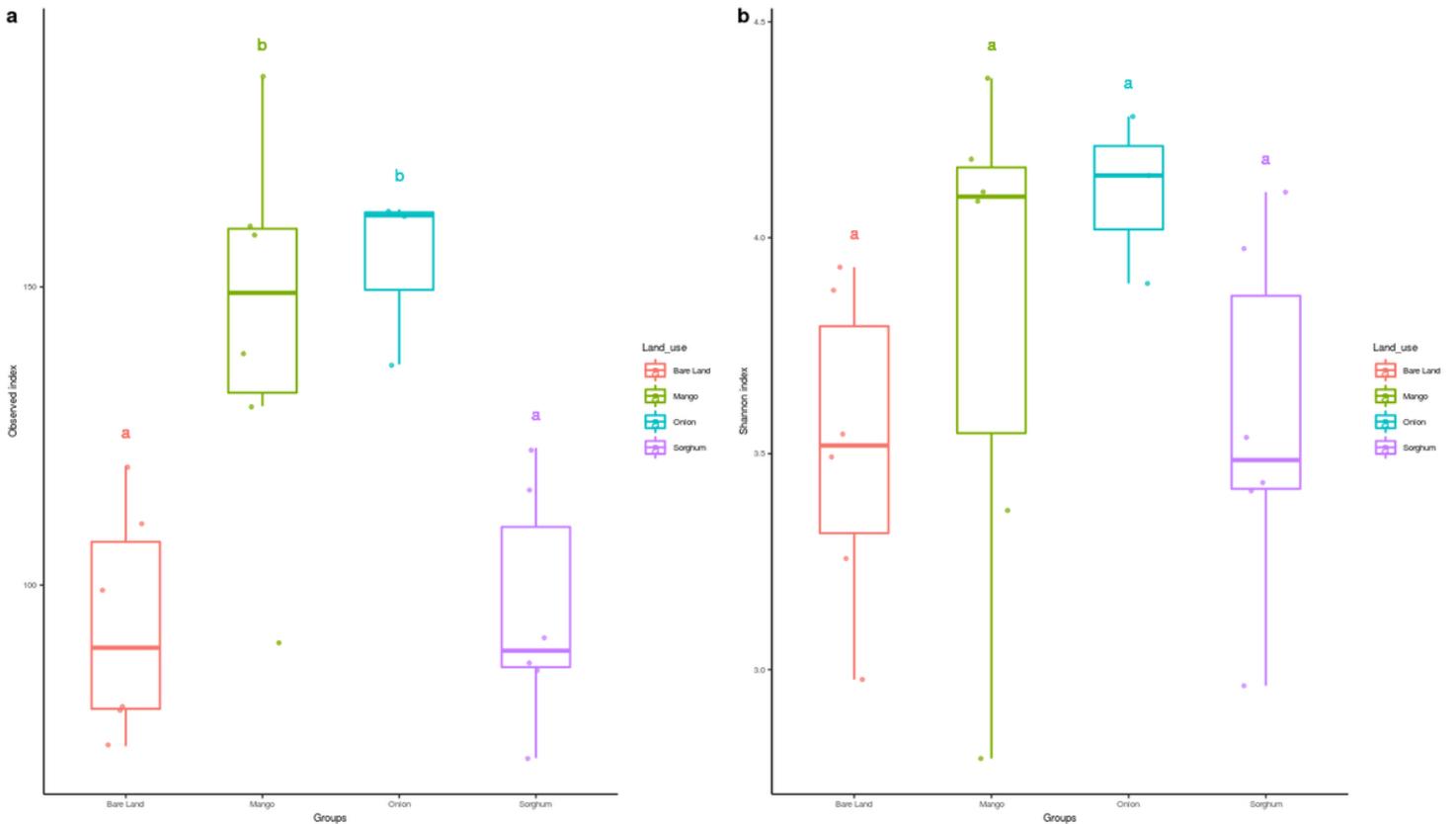


Figure 6

3.6 Box plots of alpha diversity measures in the different land-use types in Omdurman (a: Observed number of taxa, b: Shannon index)

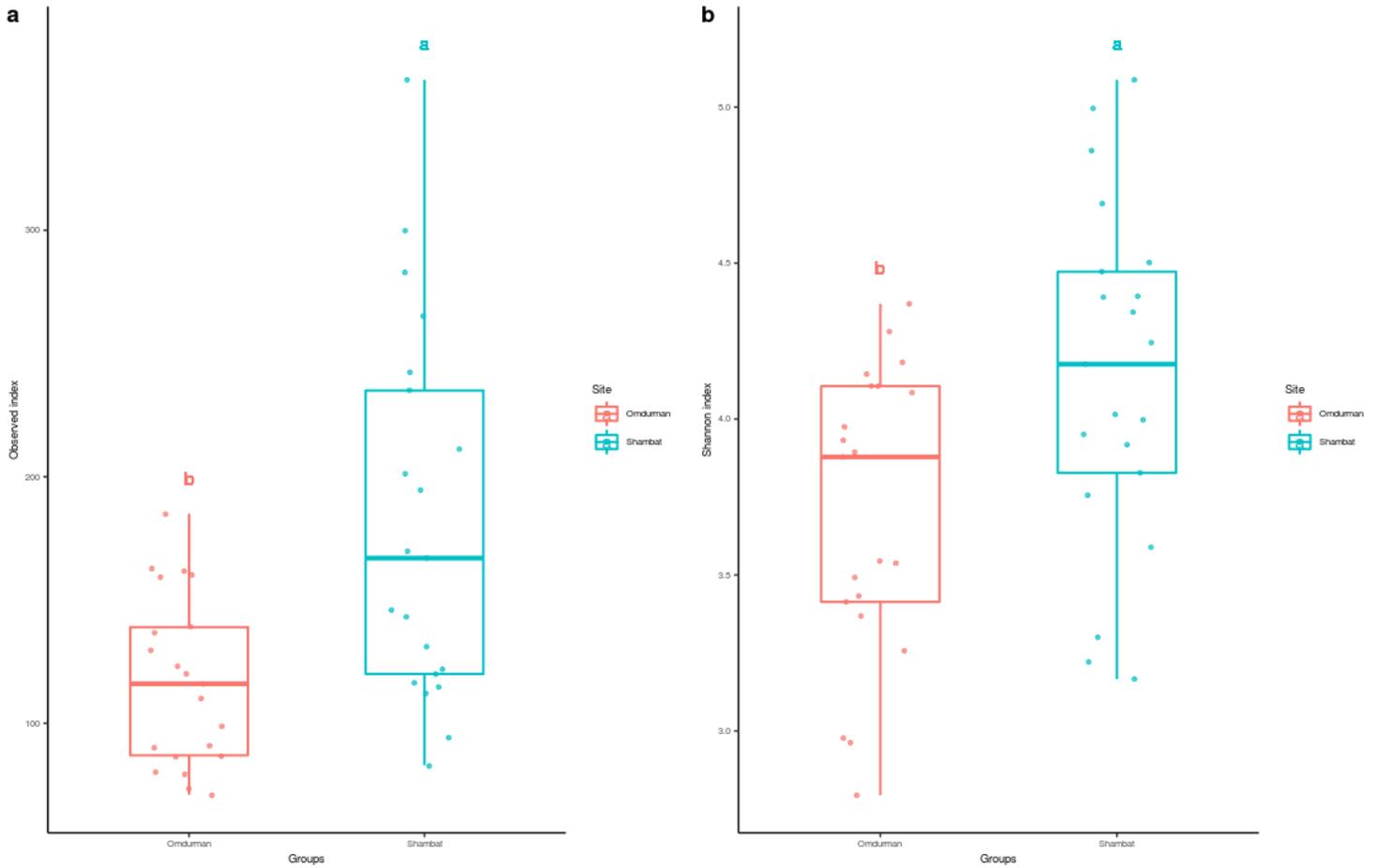


Figure 7

3.7 Box plots of alpha diversity measures in the two sites (a: Observed number of taxa, b: Shannon index)

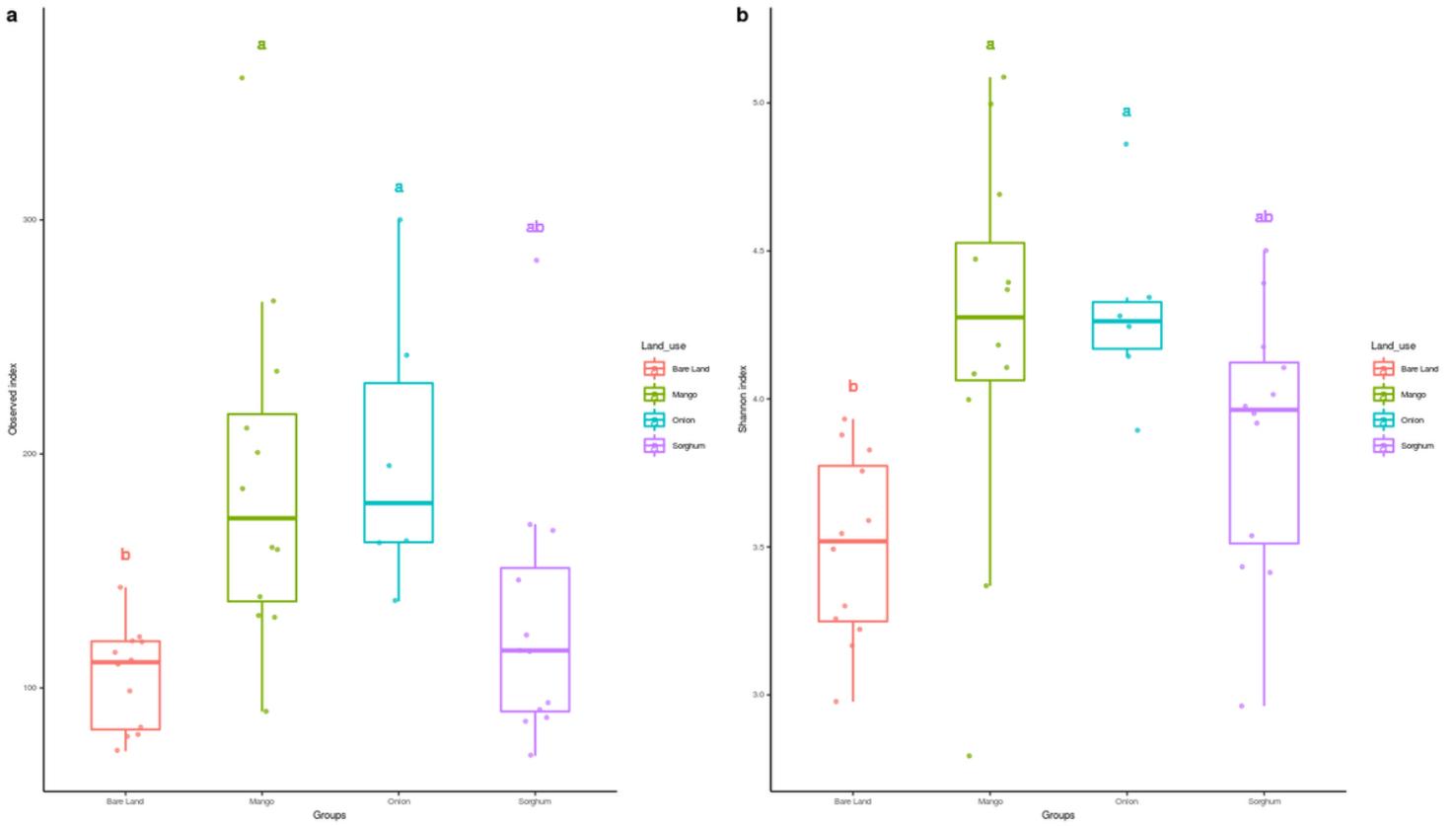


Figure 8

3.8 Box plots of alpha diversity measures in the different land-use types (a: Observed number of taxa, b: Shannon index)

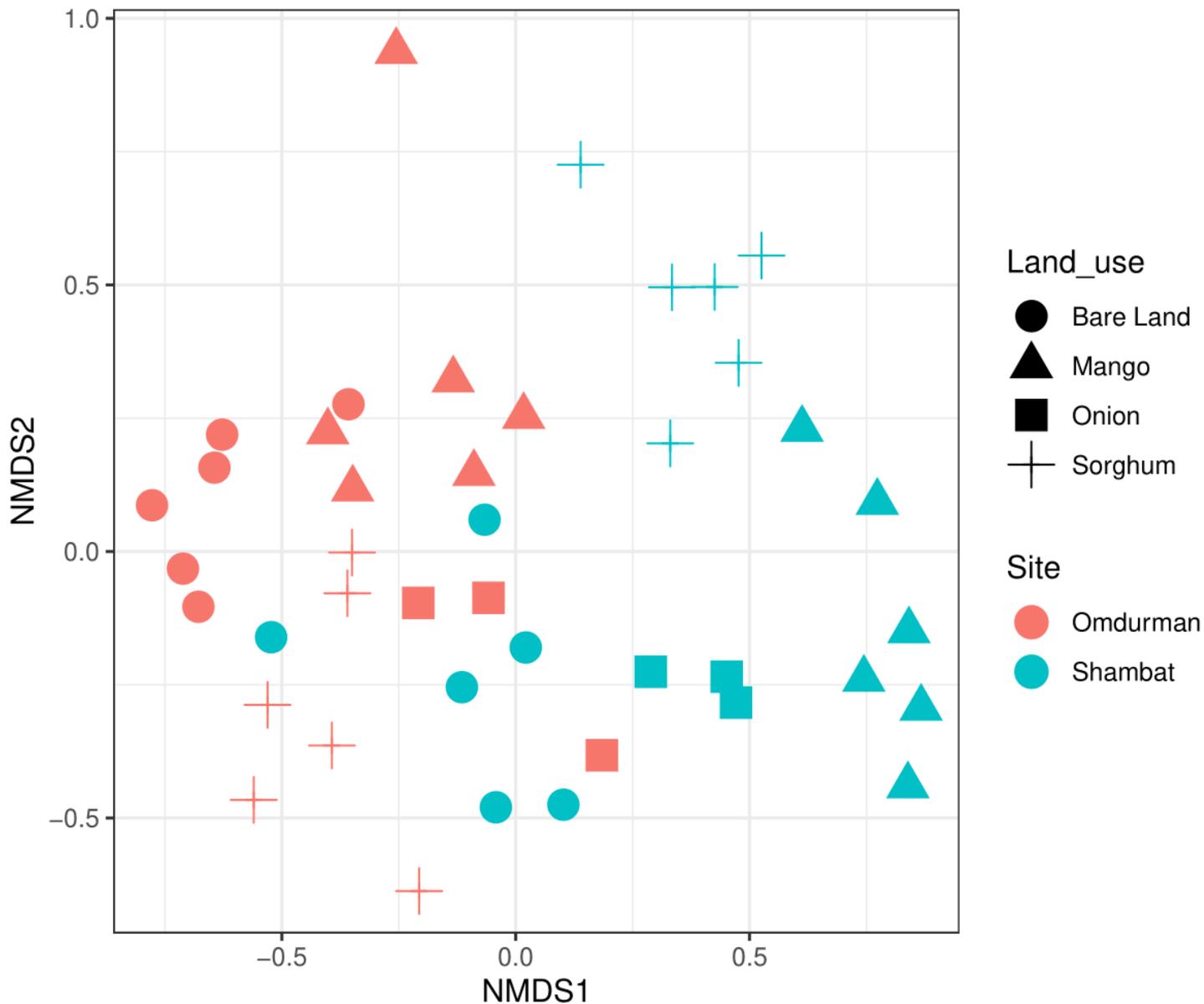


Figure 9

3.9 Beta diversity was studied using Bray-Curtis dissimilarity distance. NMDS (Fig. 3.9, Stress value 0.2116419) indicated that samples from each site tended to cluster together irrespectively of the land-use type except for one or two samples in each site. However, fungal communities according to different land-use types in Shambat were separated from each other's, particularly those from bare land soils which clustered apart from others and were grouped with Omdurman samples. The homogeneity of dispersion test revealed significant results of fungal composition between land-use groups ($F = 4.056$, $P = 0.019$) which indicates the heterogeneity of each land-use community.

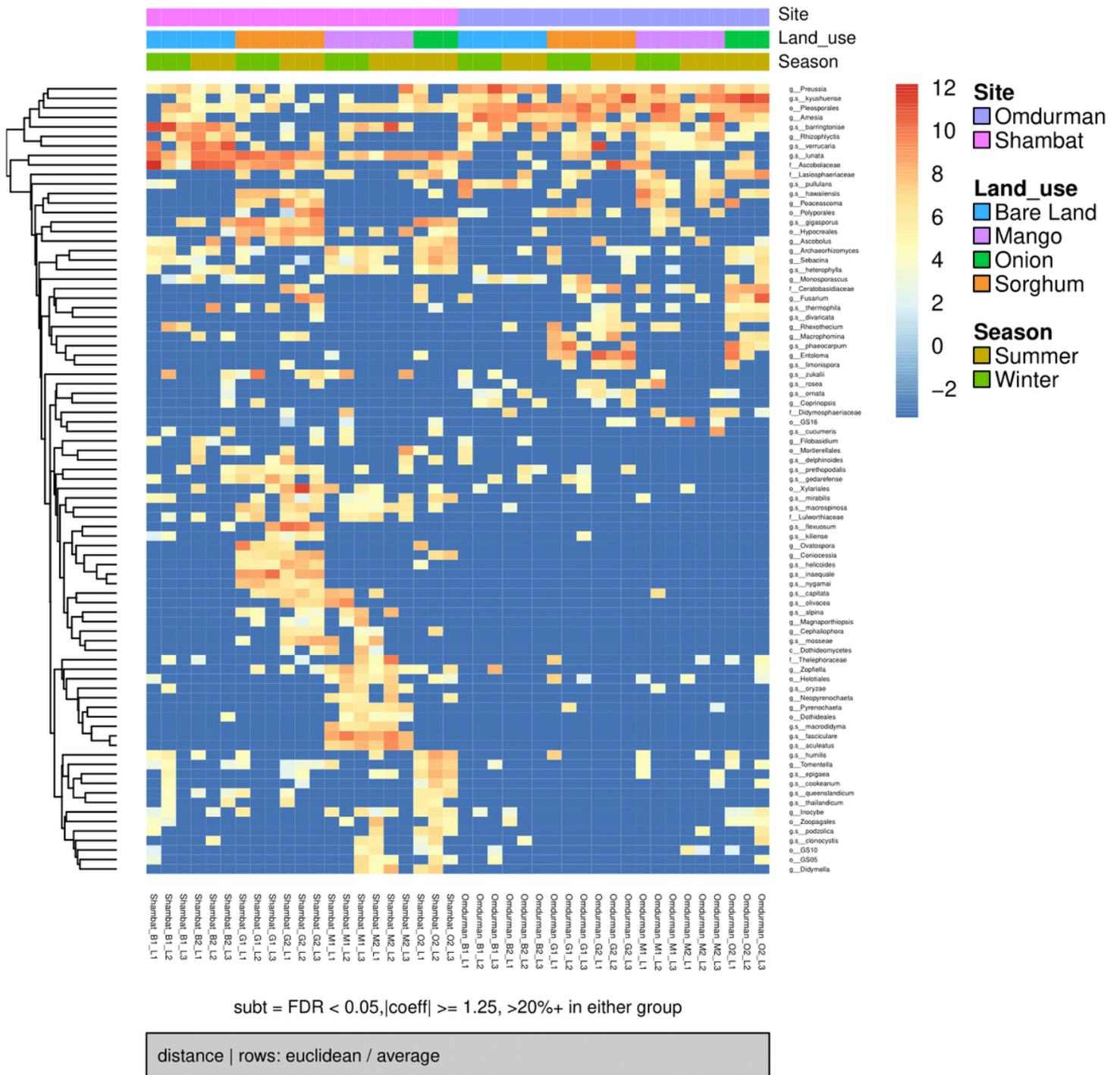


Figure 10

3.10 The interaction between the two factors, land-use and site, indicated significant differences ($P=0.001$, R^2 0.14893). The differential abundance between the two sites revealed that there were 83 ASVs significantly different between the two sites that met threshold criteria mentioned previously (Fig. 3.10). 21 taxa were found only in Shambat, and only one taxon was found only in Omdurman.